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Motor cortical and corticospinal function differ during an isometric squat compared to isometric knee extension.

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NEW FINDINGS

What is the central question of this study?

In order to discern information about testing modalities when assessing neuroplastic responses to squat resistance training, the present study investigated whether corticospinal and intracortical function was different between a joint-angle matched isometric squat, and isometric knee extension.

What is the main finding and its importance?

The present data shows poor agreement of corticospinal and intracortical function between the isometric squat and isometric knee extension. The data reinforces the notion that task specificity is of utmost important for assessing neuroplasticity.

ABSTRACT

It has been suggested that task-specific changes in neurophysiological function (neuroplasticity), should be assessed using testing modalities that replicate the characteristics of the intervention. The squat is a commonly prescribed resistance exercise that has been shown to elicit changes in central nervous system (CNS) function. However, previous studies

have assessed squat-induced neuroplasticity using isometric knee extension, potentially confounding the results. The present study aimed to assess the agreement between corticospinal and intracortical activity relating to the knee extensors during isometric knee extension compared to an isometric squat task. Eleven males completed a neurophysiological assessment in an isometric squat (IS), and knee extension (KE) task matched for joint-angles (hip, knee, and ankle). Single- and paired-pulse transcranial magnetic stimulation (TMS) were delivered during isometric contractions at a range of intensities to assess short-interval cortical inhibition (SICI) and corticospinal excitability. Group mean values for SICI (70 ± 14% vs. 63 ± 12% of unconditioned MEP during IS and KE, respectively) and corticospinal excitability (mean differences 2-5% of M_{max} at 25, 50, 75 and 100% MVC between the IS and KE) were not different between the two tasks ($P > 0.05$) in the *vastus lateralis* (VL). However, limits of agreement were wide, with poor-to-moderate average ICCs (SICI: $ICC_{3,1}$ = 0.15, corticospinal excitability: average $ICC_{3,1}$ range = 0.0-0.63), indicating disparate corticospinal and intracortical activity between the IS and KE. These data highlight the importance of task-specificity when assessing the modulation of corticospinal excitability and SICI in response to interventions resulting in neuroplastic changes.

INTRODUCTION

In recent years, there has been an increase in the number of studies applying transcranial magnetic stimulation (TMS) in sport and exercise and movement sciences to assess intracortical and corticospinal activity in response to various interventions (Weier & Kidgell, 2012; Brownstein *et al.*, 2017; Thomas *et al.*, 2017b). Single-pulse TMS permits the quantitative assessment of corticospinal excitability through the size of the compound electromyography (EMG) response, while paired-pulse TMS separated by 2-5 ms and 10-15

ms can be used to examine intracortical inhibitory (termed short-interval intracortical inhibition; SICI) and facilitatory circuits (termed intracortical facilitation; ICF), respectively (Kujirai *et al.*, 1993). Single and paired-pulse TMS paradigms have been used as tools to investigate responses to exercise such as fatiguing isometric single-limb contractions (Hunter *et al.*, 2016; Kennedy *et al.*, 2016; Goodall *et al.*, 2018) and locomotor exercise (Sidhu *et al.*, 2012; Brownstein *et al.*, 2017; Thomas *et al.*, 2017a), mechanisms of locomotion (Sidhu *et al.*, 2013b) and neural adaptations to strength training (Weier & Kidgell, 2012).

While many studies have used TMS to assess neural responses to whole-body, dynamic exercise, a common feature amongst these studies was that responses were assessed in a single-limb, isometric model (Weier & Kidgell, 2012; Brownstein *et al.*, 2017). As such, a discrepancy exists between the neuromechanics of the intervention and the testing modality used to detect changes in intracortical and corticospinal activity in response to the interventions. The discrepancy between intervention and testing modality has been highlighted previously by Sidhu *et al.* (2013a), Avela and Gruber (2010), and, more recently, by Kalmar (2018), who suggested that future studies utilising TMS to assess neuromuscular responses to whole-body exercise should employ testing modalities that more closely replicate the characteristics of the intervention. In support of this supposition, considerable evidence suggests that when assessing neuroplasticity following an intervention, the motor task performed for testing should mirror the motor task(s) performed during the intervention. For instance, Schubert *et al.* (2008) and Beck *et al.* (2007) found that intracortical, corticospinal and spinal adaptations to two separate motor training tasks (four weeks of stability or ballistic training) were constrained to the trained task and were not apparent when performing the non-trained motor task. More recently, Giboin *et al.* (2018) compared neuroplasticity responses to two different modalities of isometric strength training (maximal isometric explosive or slow sustained knee extension), and displayed that corticospinal

adaptations were evident when responses were measured during the trained task, but not for the untrained task. These findings corroborate numerous other studies that have found plasticity of the CNS is specific to the task trained (Liepert *et al.*, 1998; Muellbacher *et al.*, 2001; Jensen *et al.*, 2005). Additionally, postural differences between motor tasks can have large effects on evoked responses (Baudry *et al.*, 2015; Nuzzo *et al.*, 2016), adding further support to the notion that testing posture and contraction type should be specific to the trained task.

During a period of strength training, it is well documented that improvements in force production in the first ~4 weeks precede significant structural adaptations (Carroll *et al.*, 2001; Gabriel *et al.*, 2006), indicating that adaptations within the CNS are the primary explanatory factor for strength improvements. One common training modality for improving lower limb strength is the squat. Previous studies have employed the squat in both chronic training (Weier & Kidgell, 2012) and acute bout scenarios (Thomas *et al.*, 2017b), assessing neurophysiological function pre- and post-intervention. While Weier and Kidgell (2012) and Weier *et al*. (2012) both showed alterations in CNS function following four weeks of heavy-load squat training, Thomas *et al*. (2017b) found no changes in corticospinal or intracortical activity when assessing the neuromuscular basis of acute performance enhancement in the minutes following a heavy-resistance squat protocol, despite inducing an increase in jump performance. However, much like the issues highlighted by Sidhu *et al.* (2013a) and Avela and Gruber (2010), the evoked CNS responses in the aforementioned studies were recorded in single-limb isometric knee extension, rather than the motor task (squat) performed during the intervention. If strength can be mediated by a neuroplastic response to a training stimulus, then the optimal method to assess the alterations in corticospinal and intracortical mechanisms of neuroplasticity might be during the motor task performed throughout the intervention. Thus, it is unclear whether, given the importance of testing specificity,

intracortical and corticospinal adaptations in response to squat interventions could be masked if assessments are conducted using testing modalities which are dissimilar to the imposed intervention.

In order to elucidate the appropriateness of using isometric knee extension to assess adaptations to squat exercise, it is first important to identify whether differences exist in intracortical and corticospinal activity between knee extension exercise and a testing modality that more closely replicates the characteristics of a squat exercise (i.e. a bilateral, multi-joint movement comprising axial loading). The present study aimed to investigate and compare intracortical and corticospinal responses to single- and paired-pulse TMS in the 'traditional' isometric knee extension (KE) set up, and a joint angle-matched equivalent isometric squat (IS) set up. Given the differences in the biomechanical characteristics of KE and IS exercise, we hypothesised that there would be limited agreement between corticospinal excitability, short interval cortical inhibition (SICI), and intracortical facilitation (ICF) during the two motor tasks.

METHODS

Ethical Approval

The study received ethical approval from the Northumbria University Faculty of Health & Life Sciences Ethics committee (HLSCB251115) in accordance with the ethical standards established in the *Declaration of Helsinki*, with the exception of registration in a database.

Participants

Eleven young male adults (age: 27 ± 4 years; stature: 181 ± 7 cm; mass: 86.6 ± 15.6 kg) gave written informed consent to take part in the study. Participants were recreationally-active, resistance trained males and reported squatting at least once a week, were free of any cardiorespiratory, neurological or neuromuscular health disorders, had no metal plates in the head/brain, and were not taking any medication that might have interfered with the nervous system. All participants completed a TMS safety screening questionnaire prior to the data collection procedure (Keel *et al.*, 2001). Participants were required to refrain from alcohol consumption and strenuous physical activity in the 24-hours prior to data collection, and to abstain from caffeine consumption for the 12 hours prior to each experimental visit.

Design

Participants visited the laboratory on one occasion, and performed a series of submaximal and maximal isometric contractions in two exercise modalities: unilateral isometric knee extension (KE) and bilateral isometric squat (IS), with both conditions matched for hip and knee angle (90°), to avoid muscle length-related differences in neural recruitment (Behrens, 2017; Doguet *et al.*, 2017). Participants were familiarised with the study procedures immediately prior to data collection, including habituation with performing IS and KE exercise, and receiving TMS during submaximal contractions. Furthermore, all participants had previously taken part in studies in our laboratory involving measures of TMS recorded in the knee extensors, and were thus familiar with performing maximal voluntary contractions (MVCs) and receiving TMS during submaximal contractions. The conditions were pseudorandomised, with a 30-minute rest given between the two conditions in order to minimize the influence of fatigue. During both conditions, participants received single- and

paired-pulse TMS and electrical stimulation of the femoral nerve whilst performing submaximal and maximal isometric contractions. Corticospinal excitability, SICI and ICF, the maximal compound muscle action potential (M_{max}) and EMG/force relationship were measured in the *vastus lateralis* (VL) and *rectus femoris* (RF) using surface electromyography (EMG). These variables were then compared between the two conditions.

Procedures

Isometric knee extension

A calibrated load cell (MuscleLab force sensor 300, Ergotest technology, Norway) was used to measure isometric knee extensor force (N). The load cell was fixed to a custom built chair and strapped with a non-compliant cuff to the participant's right leg, superior to the ankle malleoli. During contractions, participants were instructed to grasp the handles on the side of the chair for support during maximal voluntary contractions (MVC). Participants were instructed to maintain hip and knee angle at 90° flexion during contractions, with these joint angles measured using a goniometer at the beginning of the trial, and visually inspected by the investigators during contractions to ensure no change in joint angle occurred. Three MVCs were performed prior to the trial, with 60 s between each contraction. In order to control for any learning effect on performing MVCs during KE, participants were asked to perform an additional MVC if there was a > 5% increase in force during successive MVCs. This was performed until three consecutive MVCs were performed with force values within 5% of each other. Three participants were required to perform one additional MVC, and one participant was required to perform two additional MVCs. The maximum force from the MVCs was recorded in order to calculate the submaximal contraction values. The force trace was displayed on a computer screen directly in front of participants in order to assist in

providing maximal efforts during MVCs and to provide the target force during submaximal contractions. Target forces were set using guidelines and real-time force feedback (Spike2, CED, Cambridge, UK).

Isometric squat

Isometric squat force (N) was measured using a force plate placed directly under the right foot (Type 9286B, Kistler Group, Winterthur, Switzerland). In order to provide support during isometric contractions, participants were seated on a bench directly under a fixed barbell, with knee and hip angle maintained at 90° flexion measured using a goniometer (Figure 1). This procedure was implemented after pilot testing revealed that when participants were unsupported, rather than being seated on a bench, the contraction intensity and level of EMG activity required to support their own body weight whilst maintaining knee and hip angle at 90° flexion was too high to allow the measurement of SICI (Ortu et al., 2008). The barbell height was adjusted at the beginning of each trial based on the participants torso length and was positioned on the shoulders (high-bar position). The participants' feet were positioned hip width apart with toes pointing forwards, with foot position determined at the beginning of the trial and marked to ensure consistent placement throughout the trial. Participants held the barbell during contractions and were given freedom to choose their hand position, which was maintained throughout the trial. During contractions, participants were instructed to exert force upwards against the bar using their whole body (Bishop *et al.*, 2017). The investigators visually inspected hip and knee angle during contractions to ensure no change in joint angle occurred. Three MVCs were performed prior to the trial, with 60 s between each contraction. In order to control for any learning effect on performing MVCs during IS exercise, participants were asked to perform an additional MVC if there was a >

5% increase in force during successive MVCs. This was performed until three consecutive MVCs were performed with force values within 5% of each other. Six participants were required to perform one additional MVC. The maximum force from the MVCs was recorded in order to calculate the submaximal contraction values. The force trace was displayed on a computer screen directly in front of participants in order to assist in providing maximal efforts during MVCs and to provide the target force during submaximal contractions.

Isometric contraction protocol

During assessment of corticospinal excitability in KE and IS trials, seven sets of brief (~3 s) isometric contractions were performed at 25, 50, 75 and 100% MVC. Contraction intensities were randomized, and participants given 60 s rest between each contraction and 3 minutes between each set to avoid the potential influence of fatigue on MEP properties. Two electrical nerve stimuli and five TMS pulses were delivered at each contraction intensity. For assessment of SICI and ICF, 40 stimuli (20 single- and 20 paired-pulses) were delivered in six sets of six and one set of four during a 10% MVC, with 30 s between each set (see below for details).

Instrumentation

Electromyography recordings

EMG activity was recorded from RF, VL and *biceps femoris* (BF), with a reference electrode placed on the patella; the areas underneath were cleaned and shaved prior to electrode

placement. Surface electrodes (Ag/AgCl; Kendall H87PG/F, Covidien, Mansfield, MA, USA) were placed 2 cm apart over the muscle belly. The electrodes recorded electrical activity in the VL, RF and BF, with the signal processed to permit analysis of the root-mean-square (RMS) amplitude for voluntary contractions, the compound muscle action potential (M-wave) elicited by electrical stimulation of the femoral nerve, and the motor evoked potential (MEP) elicited by TMS. Signals were amplified: gain ×1000 for EMG and ×300 for KE force (CED 1902; Cambridge Electronic Design, Cambridge, UK), band-pass filtered (EMG only: 20-2000 Hz), digitized (4 kHz; CED 1401, Cambridge Electronic Design) and analysed offline. Further details on these methods are provided below.

Percutaneous nerve stimulation

Percutaneous stimulation of the right femoral nerve was administered using square wave pulses (200 μs) via a constant-current stimulator (DS7AH, Digitimer Ltd., Hertfordshire, UK) using self-adhesive surface electrodes (CF3200, Nidd Valley Medical Ltd., North Yorkshire, UK). The cathode was placed over the femoral nerve high in the femoral triangle, and the anode between the greater trochanter and iliac crest. Cathode placement was adjusted to elicit the greatest M_{max} amplitude in the VL. Stimulations were delivered in 20 mA step-wise increments beginning at 20 mA until the maximum quadriceps twitch amplitude (Q_{tw}, N) and muscle compound action potential (M_{max}, mV) in the VL were elicited. The resulting intensity was then increased by 30% in order to ensure the stimulation intensity was supramaximal. This procedure was conducted during both KE and IS exercise to ensure the stimulation intensity was supramaximal under both modalities, with stimulation intensities of 229 ± 119 mA during KE, and 260 ± 100 mA during IS.

Transcranial magnetic stimulation

Single- and paired-pulse TMS were delivered over the motor cortex via a concave double cone coil using a BiStim unit and two Magstim 200^2 stimulators (The Magstim Company Ltd, Whitland, UK). The junction of the double cone coil was aligned tangentially to the sagittal plane, with its centre 1-2 cm to the left of the vertex. The optimal coil placement was determined at the start of each trial as the position that elicited the largest MEP in the VL muscle at 50% stimulator output during a 10% MVC contraction. This procedure was conducted separately during both KE and IS exercise to ensure optimal coil placement during both modalities. The position was then marked with indelible ink to ensure consistent placement throughout the trial. The stimulator intensity was based on active motor threshold (AMT) during a 10% MVC during each condition. AMT was defined as the intensity that elicited a MEP amplitude of >200 μV in 3 out of 5 stimulations in the VL (Weier & Kidgell, 2012). We believed it was more appropriate to base AMT and stimulator output on responses in the VL rather than the RF, which has a bi-articular make up and is involved in both hip and knee extension, potentially influencing the level of recruitment during the IS and KE and thereby confounding intracortical and corticospinal responses. For single-pulse TMS, the stimulus intensity was set at 120% AMT. The configuration used during paired-pulse TMS consisted of a conditioning stimulus intensity of 70% AMT with an inter-stimulus interval (ISI) of 2 ms for SICI, and a conditioning stimulus intensity of 60% AMT with an ISI of 10 ms for ICF. The suprathreshold test pulse intensity was maintained at 120% AMT for both SICI and ICF. Pilot work from our laboratory has identified this configuration as eliciting the highest degree of SICI and ICF in the active knee extensors, while twenty single- and twenty paired-pulse TMS stimuli were identified as the minimum number required to obtain an accurate estimate of SICI and ICF.

Data analysis

The peak-to-peak amplitude of the EMG responses to motor nerve stimuli and TMS were analysed offline. The root mean square EMG amplitude (RMS_{EMG}) and average force were calculated in the 500 ms prior to each TMS stimulus to ensure a similar level of background muscle activity during each stimulation when assessing SICI and ICF, and to assess the EMG/force relationship at different contraction intensities. For the latter, RMS_{EMG} at a given contraction intensity was normalised to RMS_{EMG} during the mode specific 100% MVC. To quantify SICI and ICF, the ratio of the average conditioned paired-pulse MEP amplitude was expressed relative to the average unconditioned MEP amplitude at 120% AMT. A conditioned vs. unconditioned ratio < 100% indicates inhibition, and a ratio > 100% indicates facilitation. If the ratio for SICI was > 100%, or the ratio for ICF was < 100%, the data from the corresponding participant was removed from the analysis. In order to assess corticospinal excitability and EMG activity at different contraction intensities, MEP amplitude and RMS_{EMG} were averaged across the five TMS pulses and normalised to the M_{max} assessed at each contraction intensity (MEP/M_{max} and RMS/M_{max}, respectively).

Statistical analyses

SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. All data are presented as means ± standard deviation unless stated otherwise. Significance was set at an alpha level of 0.05. Normality of data was assessed using the Shapiro-Wilk test. If the data were not normal, transformations were performed using common logarithm or square root. In order to demonstrate there were no learning or fatigue effects associated with performing multiple MVCs during IS and KE exercise, a repeated measures ANOVA was performed to assess for differences in MVC values obtained throughout the contraction

protocol. Analysis revealed no significant main effect of time on MVC scores in either KE ($F_{6,60}$ = 2.736, P = 0.084, η_p^2 = 0.215) or IS ($F_{6,60}$ = 2.539, P = 0.086, η_p^2 = 0.215). Furthermore, reliability analysis revealed excellent reliability for both IS ($ICC_{3,1}$ = 0.96, 95% confidence interval; CI 0.90 – 0.99) and KE ($ICC_{3,1}$= 0.96, 95% CI 0.90 – 0.99), with low coefficients of variation for both modalities (2.9 and 1.9% for IS and KE, respectively). A paired sample t-test was performed to determine the difference in SICI between the IS and KE. Agreement between modalities was assessed graphically using Bland-Altman plots, with lines indicating the mean difference and the 95% limits of agreement (mean difference ± 2SD of the difference). Absolute agreement between contraction modalities was assessed using two-way mixed-effect intraclass correlation coefficients ($ICC_{3,1}$) with 95% CIs. As per the guidelines recommended by Koo and Li (2016), ICCs between 0.5 and 0.75 were considered as moderate agreement, values between 0.75 and 0.9 were considered as good agreement, and values above 0.9 considered as excellent agreement. Sphericity was assessed using Mauchly's test. In the case of a violation, Greenhouse-Geisser correction was employed. A 2 × 2 repeated measures ANOVA (2 types of stimuli – single- and paired-pulse, and 2 modalities – IS and KE) was used to assess differences in RMS during investigation of SICI. A 4 × 2 repeated measures ANOVA (4 contraction intensities – 25, 50, 75 and 100%, and 2 modalities – IS and KE) was used to assess differences between the modalities in M_{max}, RMS/M_{max}, MEP/M_{max}, and BF EMG during different contraction intensities. In the case of significant main effects, analysis was continued using pairwise comparisons with Bonferroni correction. Partial eta squared (η_p^2) was reported as a measure of effect size. An interaction was only reported whenever it was found to be statistically significant. The EMG-force relationship was estimated by linear regression, with the determination coefficient considered acceptable at $r^2 > 0.95$ and $P < 0.05$.

RESULTS

Short interval intracortical inhibition and facilitation

We were unable to induce SICI during the IS trial for one subject (conditioned vs. unconditioned ratio > 100%); as such, this participant was excluded from further analysis. On average, SICI was similar in the IS compared to KE in both VL (70 ± 14% vs. 63 ± 12%; t_9 = 1.330, P = 0.216) and RF (58 ± 19% vs. 71 ± 19%; t_9 = −1.577, P = 0.149; Figure 2A). No difference was found in pre-stimulation EMG activity during KE and IS at 10% MVC between single and paired pulse stimulation in the RF (IS: 0.030 ± 0.005 mV vs. 0.030 ± 0.005 mV; KE: 0.040 ± 0.014 mV vs. 0.040 ± 0.011 mV; $F_{1, 9}$ = 0.322, P = 0.584, η_p^2 = 0.035) or VL (IS: 0.044 ± 0.016 mV vs. 0.045 ± 0.016; KE: 0.081 ± 0.128 vs. 0.081 ± 0.128; $F_{1, 9}$ = 0.606, P = 0.456, η_p^2 = 0.063). Despite this, the agreement between modalities was poor-to-moderate; $ICCs_{3,1}$ for SICI was 0.15 (95% CI 0.00-0.67) in the VL and 0.09 (95% CI 0.00-0.63) in the RF (Figure 2B). Limits of agreement were −25% to 39% and −62% to 37%, systemic bias: 7% and −13%, in the VL and RF respectively (Figure 2C).

We were able to induce ICF during both the IS and KE in only 5 and 2 subjects in RF and VL, respectively (conditioned vs. unconditioned ratio > 100%). In all other subjects, we were unable to elicit ICF in either modality, with conditioned vs. unconditioned ratios < 100%. Due to the small number of valid cases, no statistical analyses were performed for ICF.

Maximal compound action potential

On average, M_{max} was similar in KE and the IS in both VL (5.5 ± 1.8 vs. 5.0 ± 1.5 mV; $F_{1, 10}$ = 2.106, P = 0.177, η_p^2 = 0.174) and RF (5.8 ± 2.2 vs. 5.9 ± 2.6 mV; $F_{1, 10}$ = 0.013, P =

0.911, η_p^2 = 0.001. However, agreement between modalities varied at different contraction intensities, with $ICC_{3,1}$ values ranging between poor-to-moderate and moderate-to-excellent (Table 1). M_{max} was also similar across different contraction intensities in VL ($F_{1.4,\ 14.3}$ = 1.106, *P* = 0.337, η_p^2 = 0.100) as well as RF ($F_{2.1,\ 21.1}$ = 0.907, P = 0.424, η_p^2 = 0.083).

Motor evoked potentials

On average, MEP/M_{max} was similar between IS and KE in both VL ($F_{1,\ 10}$ = 1.062, *P* = 0.327, η_p^2 = 0.096) and RF ($F_{1,\ 10}$ = 2.407, *P* = 0.152, η_p^2 = 0.194; Figure 3A). However, the agreement between the modalities varied between MEP/M_{max} measured at different contraction intensities, with average $ICCs_{3,1}$ values ≥ 0.0 and ≤ 0.63 (Table 1; Figure 3B). Bland-Altman plots with limits of agreement and systematic bias are displayed in Figures 3C-F. In both modalities, MEP/M_{max} was modulated by contraction intensity in both VL ($F_{3,\ 30}$ = 14.826, *P* < 0.001, η_p^2 = 0.597) and RF ($F_{3,\ 30}$ = 11.153, *P* < 0.001, η_p^2 = 0.527; Figure 3A) such that MEP/M_{max} was smaller at 25% MVC compared to higher contraction strength in both muscles (*P* ≤ 0.025). In RF, there was also a statistically significant modality × contraction intensity interaction for MEP/M_{max} ($F_{3,\ 30}$ = 3.267, *P* = 0.035, η_p^2 = 0.246). Specifically, post hoc test revealed MEP/M_{max} was smaller during IS compared to KE at 25% MVC (24 ± 23% vs. 47 ± 20%; p = 0.004).

Electromyography and force-EMG relationship

In VL, RMS/M_{max} was similar in both modalities on average ($F_{1,\ 10}$ = 2.695, *P* = 0.132, η_p^2 = 0.212; Figure 4A), but the agreement between them was generally poor, with $ICC_{3,1}$ values ranging from poor to moderate-to-good (Table 1; Figure 4B). However, RMS/M_{max} in VL

was influenced by contraction intensity ($F_{3, 30}$ = 111.389, P < 0.001, η_p^2 = 0.918) in that it was greater with increased contraction strength (P < 0.005; Figure 4A). There was also statistically significant modality × contraction intensity interaction for RMS/M_{max} in VL ($F_{1.4, 14.1}$ = 10.242, P = 0.004, η_p^2 = 0.506). Post hoc testing showed RMS/M_{max} was greater during KE compared to IS at 50% MVC (4 ± 1% vs. 2 ± 1%; p = 0.013).

In RF, RMS/M_{max} was higher on average during KE compared to the IS ($F_{1, 10}$ = 10.688, P = 0.008, η_p^2 = 0.517; Figure 4A). Furthermore, agreement for RMS/M_{max} between the IS and KE in RF ranged from poor to poor-to-moderate at different contraction intensities (Table 1; Figure 4B). Both modalities were also modulated by contraction intensity ($F_{3, 30}$ = 174.329, P < 0.001, η_p^2 = 0.946) such that RMS/M_{max} increased with greater contraction intensity (P < 0.005; Figure 4A).

The determination coefficient of linear regression was significant in both the VL and RF for both modalities, suggesting the force – EMG relationship was linear in all cases (see Figure 5). On average, the antagonist EMG activity was similar between KE and IS (0.06 ± 0.05 vs. 0.05 ± 0.02 mV; $F_{1, 10}$ = 1.722, P = 0.219, η_p^2 = 0.147), but it was affected by contraction intensity ($F_{1.2, 12.1}$ = 24.179, P < 0.001, η_p^2 = 0.707) insofar as BF EMG activity increased stepwise from 25% to 100% MVC (KE: 0.04 ± 0.01, 0.05 ± 0.02, 0.08 ± 0.06, 0.09 ± 0.08 mV; IS: 0.04 ± 0.01, 0.04 ± 0.01, 0.05 ± 0.01, 0.06 ± 0.02 mV; P < 0.05).

DISCUSSION

The aim of the present study was to compare corticospinal and intracortical responses to single- and paired-pulse TMS during an IS and KE exercise. The key finding from the study was that the two motor tasks resulted in disparate corticospinal and intracortical activity, with a poor level of agreement between the two exercise modalities. Specifically, despite a similar

level of background EMG during measurements of SICI and a comparable response on a group level, absolute agreement assessed through ICCs in the VL and RF were poor-to-moderate, and limits of agreement were wide, indicating disparate activity of inhibitory interneurons during the tasks. Similarly, comparable responses were observed at a group level between normalised MEP amplitude in response to single-pulse TMS delivered at a range of contraction intensities in the VL and RF, but agreement between the tasks was generally poor, with ICCs ranging from poor to poor-to-good, and wide limits of agreement at most contraction intensities. Collectively, these results highlight the task specific nature of corticospinal and intracortical activity and could have implications regarding the requirement for testing specificity when assessing CNS responses.

Differential intracortical and corticospinal activity during IS and KE exercise. Previous work has displayed that SICI is a task dependent, highly specific phenomenon, which is differentially modulated by the requirements of the motor task (Liepert *et al.*, 1998; Devanne *et al.*, 2002). In the present study, ICCs revealed a poor-to-moderate level of agreement between SICI measured during KE and IS squat exercise. Previous work has displayed moderate-to-excellent within-day reliability of corticospinal excitability and SICI when measured in the knee extensors, suggesting that the poor agreement between the modalities is not simply a result of variability in the measures (O'Leary *et al.*, 2015). Furthermore, limits of agreement for SICI were ± 32% and ± 50% in the VL and RF, respectively. These limits of agreement are wide in the context of previously observed changes in SICI measured in the knee extensors as a consequence of strength training. For example, studies have reported statistically significant changes in SICI ranging between 22% and 35% in response to strength training interventions (Weier & Kidgell, 2012; Weier *et al.*, 2012). Given that the ICCs for SICI measured during KE and IS in the present study were lower than has

previously been reported during isometric knee extension (O'Leary *et al*., 2015), and that limits of agreement were wider than the magnitude of previously observed changes in SICI in response to strength training interventions (Weier & Kidgell, 2012; Weier *et al*., 2012), this implies that the agreement between the two modalities was poor, indicating differences in the activity of intracortical inhibitory interneurons during the tasks.

While voluntary contraction strength has been shown to influence the degree of SICI (Ridding *et al.*, 1995; Ortu *et al.*, 2008), the similar relative contraction intensity and background pre-stimulation EMG in the VL and RF during measurements of SICI in the present study suggests that the disparity between SICI measured in the two modalities was not due to differences in the level of motor drive to the muscle. Instead, it is plausible that the differences in the neuromechanics of the IS and KE could have contributed to the lack of agreement between SICI measured in the two conditions. Specifically, the bilateral versus unilateral nature of the IS and KE, respectively, could have influenced the level of SICI in the VL and RF. Indeed, it has previously been reported that there are differences in voluntary control of unilateral versus bilateral contractions that could be mediated through alterations in intracortical inhibition (Ferbert *et al.*, 1992; Skarabot *et al.*, 2016). For example, during bilateral contractions, it has been suggested that inhibition is modulated through interhemispheric interactions between homologous muscle representations of the primary motor cortex acting to produce a coordinated movement of the two limbs (Oda & Moritani, 1995). Another integral difference between the two conditions which might have contributed to the lack of agreement in SICI is that the KE is a single-joint exercise, in which the *quadriceps femoris* muscle group is the sole contributor to force production, while the IS is a multi-joint exercise, in which additional agonist and synergist muscle groups, including the hip extensors, are activated. It has been speculated that SICI could be involved in the 'fractionation' of muscular activity, such that inhibitory influences are reduced on the

contracting muscle whilst maintaining or increasing inhibition in the non-contracting muscles (Zoghi *et al.*, 2003; Ortu *et al.*, 2008). Although there were no differences in SICI on a group level, the concurrent activation of agonist and synergist muscle groups during the IS could influence the degree of inhibition measured in the knee extensors. In support of this supposition, previous studies have found that concurrent activation of synergist muscles influences the magnitude of SICI measured in a target muscle (Devanne *et al.*, 2002; Kouchtir-Devanne *et al.*, 2012), possibly due to interactions between muscle representations within the motor cortex (Capaday *et al.*, 2013). Thus, differences in the neuromechanics of muscle recruitment between the IS and KE could have contributed to the lack of agreement in SICI between the two motor tasks.

Similar to measures of SICI, ICCs showed generally poor agreement between corticospinal excitability measured during the KE and IS at a range of contraction intensities. Furthermore, limits of agreement between corticospinal excitability measured during KE and IS ranged from ± 42% and ± 53% in the RF and ± 28% and ± 44% in the VL across different contraction intensities. These limits of agreement are wider than much of the previously reported changes in corticospinal excitability measured in the knee extensors in response to locomotor exercise. For example, Thomas *et al* (2017a) reported a statistically significant 5% decrease in corticospinal excitability 24 h following competitive soccer match-play. Similarly, both Goodall *et al* (2018) and Jubeau *et al* (2014) reported a ~15% increase in corticospinal excitability in response to fatiguing isometric and locomotor exercise, respectively. Given that responses were normalised to M_{max}, these differences between the tasks could not have been related to differences in neuromuscular transmission at the sarcolemma. It should be noted that at certain contraction intensities, there were differences in the EMG activity in the VL and/or RF muscles between the two modalities. In particular, EMG in the RF was higher in the KE compared with the IS at all contraction intensities

above 25% MVC, and higher in the VL during KE at 50% MVC. However, the increased EMG activity at these contraction intensities was not synonymous with an increase in corticospinal excitability. This can likely be explained by the plateau in MEP amplitude observed above 50% MVC, which has previously been observed in work conducted in the knee extensor musculature (Goodall *et al.*, 2009; Sidhu *et al.*, 2009). This observation is likely due to a decline in motoneuron output in response to the stimulus arising from an inability of some motoneurons to fire in response to excitatory input (Todd *et al.*, 2003; Goodall *et al.*, 2014). Nevertheless, it is possible that the differences in the level of muscle activity could have contributed to the lack of agreement between corticospinal excitability measured during the two modalities.

During multi-joint muscle contractions, the motor cortex and corticospinal tract work as a dynamic and integrated neural network in order to execute the required movement (Devanne *et al.*, 2002; Capaday *et al.*, 2013; Mason *et al.*, 2017). Rather than each muscle group involved in the movement being controlled singly and separately by distinct territories within the motor cortex, cortical points are interconnected by intrinsic collaterals which function to control muscle synergies in an integrated manner (Capaday *et al.*, 2013). For example, cortical mapping experiments examining the topography of muscle representations within the motor cortex have shown that the areal representations of task-related proximal and distal muscles of the upper limbs overlap considerably, despite differences in the location of their optimal points (Devanne *et al.*, 2006). In the case of the IS, the *quadriceps femoris* muscles act as the primary agonist muscle group during contraction, but are subserved by other agonist and synergist muscles such as the hip extensors. Given the overlapping and intertwined nature of muscle representations in the motor cortex and corticospinal tract, it is plausible that the activation of synergist muscles during the IS could have contributed to the lack of agreement between corticospinal excitability measured in the KE and IS. In support of

this, Devanne *et al* (2002) reported differences in corticospinal excitability during a finger pointing task involving co-activation of multiple muscle groups in the upper limb compared to an isolated contraction of each muscle and suggested that interactions between muscle representations within the motor cortex were responsible for the differential modulation of corticospinal excitability.

While interactions between muscle representations within the motor cortex could have contributed to the divergence in corticospinal excitability between the two tasks, given that MEP amplitude depends on the level of excitation of the motor cortex and spinal motor neurons, the possibility that there might have been a contribution at the spinal level cannot be ruled out. For example, differences in "recruitment gain" of the motoneuron pool, whereby the range of thresholds for different motoneurons within the pool can be compressed or expanded depending on the nature of the motor task (Kernell & Hultborn, 1990; Vestergaard & Berg, 2015), could have influenced corticospinal excitability measured in the knee extensors. Further insight into the potential spinal contribution during the two motor tasks could be gained from the stimulation at the cervicomedullary junction (Taylor & Gandevia, 2004). Although a contribution of spinal factors cannot be ruled out, the lack of agreement in SICI during the motor tasks suggest that intracortical mechanisms at least partially contributed to the results of the present study.

In addition to SICI, the present study also attempted to measure and compare ICF during the KE and IS. In an attempt to induce the maximum level of facilitation, we implemented paired-pulse stimulus variables (ISI and conditioning stimulus intensity) which have previously been optimised during pilot work in our laboratory when assessing ICF in the *rectus femoris* . Despite this, we were able to induce facilitation during both the IS and KE in only a limited number of participants, and consequently were unable to make a valid comparison between the two modalities. In particular, we were unable to induce ICF in the

VL in most participants during the IS or KE, despite AMT being based on responses in the VL. Previous work has similarly shown that ICF demonstrates significant inter-subject variability in the knee extensors, such that some individuals do not exhibit facilitation using paired-pulse paradigms previously shown to elicit ICF (O'Leary *et al*., 2015; Kujirai *et al*., 1993). For example, when attempting to assess the reliability of ICF in the active *vastus lateralis*, O'Leary *et al* (2015) displayed an average ratio of conditioned/unconditioned MEP amplitude below 1.0 in a cohort of 16 participants. While it is suggested that ICF reflects the excitability of glutamate mediated N-methyl-D-aspartate excitatory interneurons (Liepert *et al.*, 1997; Nakamura *et al.*, 1997), this still remains unclear (Ni & Chen, 2011). These results question the validity and applicability of measuring ICF in the *vastus lateralis*.

Limitations. While the present study opens up an interesting area for future research concerning CNS adaptations to squat based exercise, it is important to acknowledge the study's limitations. Namely, although the set-up employed during the IS exercise was designed to more closely replicate the characteristics of the squat exercise, there are a number of differences between the IS set-up utilised in the present study compared with a conventional dynamic squat, such as the contraction mode, being supported versus unsupported, and potential differences in joint angles. Nevertheless, our aim was to employ a testing modality that more closely replicates the characteristics of the squat exercise whilst also allowing us to compare responses with the conventional method used to assess neuroplasticity in response to squat interventions, i.e. isometric knee extension with hip and knee angles of 90° (Weier *et al*., 2012; Weier & Kidgell, 2012). Using an experimental set-up which precisely replicated that of normal squat exercise, i.e. dynamic, unsupported

movement under load with self-selected hip and knee angles, would have had obvious methodological impracticalities which would have precluded us from taking neurophysiological measures under such conditions. However, given the closer biomechanical similarities between the IS and normal squat exercise compared to that of KE, the IS set-up used in the present study has the potential to provide a more valid means of assessing neuroplasticity in response to squat based interventions, providing an intriguing avenue for future investigations.

CONCLUSION

The present study found disparate corticospinal and intracortical responses to single- and paired-pulse transcranial magnetic stimulation in the *vastus lateralis* and *rectus femoris* during joint-angle specific isometric squat and knee extension exercise, despite similar levels of background EMG during the two modalities. The lack of agreement noted between corticospinal excitability and SICI could have been a consequence of the differences in the characteristics of the tasks, such as the bilateral, multi-joint contraction implicated during the isometric squat compared with the unilateral, single-joint contraction involved during isometric knee extension. The results highlight the task specific nature of corticospinal and intracortical activity and emphasise the requirement for testing specificity when assessing CNS responses. Future studies should assess differences in the sensitivity of the IS compared with isometric KE in detecting changes in CNS function in response to interventions involving the squat.

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Competing Interests

The authors have no competing interests to declare, financial or otherwise.

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Author Contributions

Experiments were performed in the Integrated Physiology Laboratory at Northumbria University. CB, PA, JS, SG, and KT designed the study protocol; CB, PA, and JS acquired the data; CB, PA, JS, AF, DK, GH, SG, and KT analysed and interpreted the data; CB, PA, JS, AF, DK, GH, SG, and KT drafted or revised the final manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons listed qualify for authorship.

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Table and Figure Legends

Table 1. Intraclass correlation coefficients ($ICC_{3,1}$ with 95% confidence intervals) displaying the level of agreement between the isometric squat and knee extension in the *vastus lateralis* and *rectus femoris* during different contraction intensities for M_{max}, MEP/M and RMS/M (n = 11).

		Vastus lateralis	**Rectus femoris**
M_{max}	*25% MVC*	0.92 (0.72 – 0.98)	0.33 (0.00 – 0.77)
	50% MVC	0.64 (0.08 – 0.89)	0.08 (0.00 – 0.55)
	75% MVC	0.36 (0.00 – 0.77)	0.33 (0.00 – 0.77)
	100% MVC	0.42 (0.00 – 0.79)	0.25 (0.00 – 0.73)
MEP/M_{max}	*25% MVC*	0.15 (0.00 – 0.68)	0.34 (0.00 – 0.74)
	50% MVC	0.59 (0.00 – 0.87)	0.32 (0.00 – 0.76)
	75% MVC	0.63 (0.08 – 0.88)	0.22 (0.00 – 0.45)
	100% MVC	0.41 (0.00 – 0.79)	0.00 (0.00 – 0.24)
RMS/M_{max}	*25% MVC*	0.54 (0.00 – 0.85)	0.12 (0.00 – 0.52)
	50% MVC	0.00 (0.00 – 0.35)	0.01 (0.00 – 0.45)
	75% MVC	0.31 (0.00 – 0.76)	0.07 (0.00 – 0.53)
	100% MVC	0.41 (0.00 – 0.80)	0.00 (0.00 – 0.44)

Figure 1. Experimental setup for eliciting and recording electromyographic responses via transcranial magnetic and percutaneous nerve stimulation during an isometric squat.

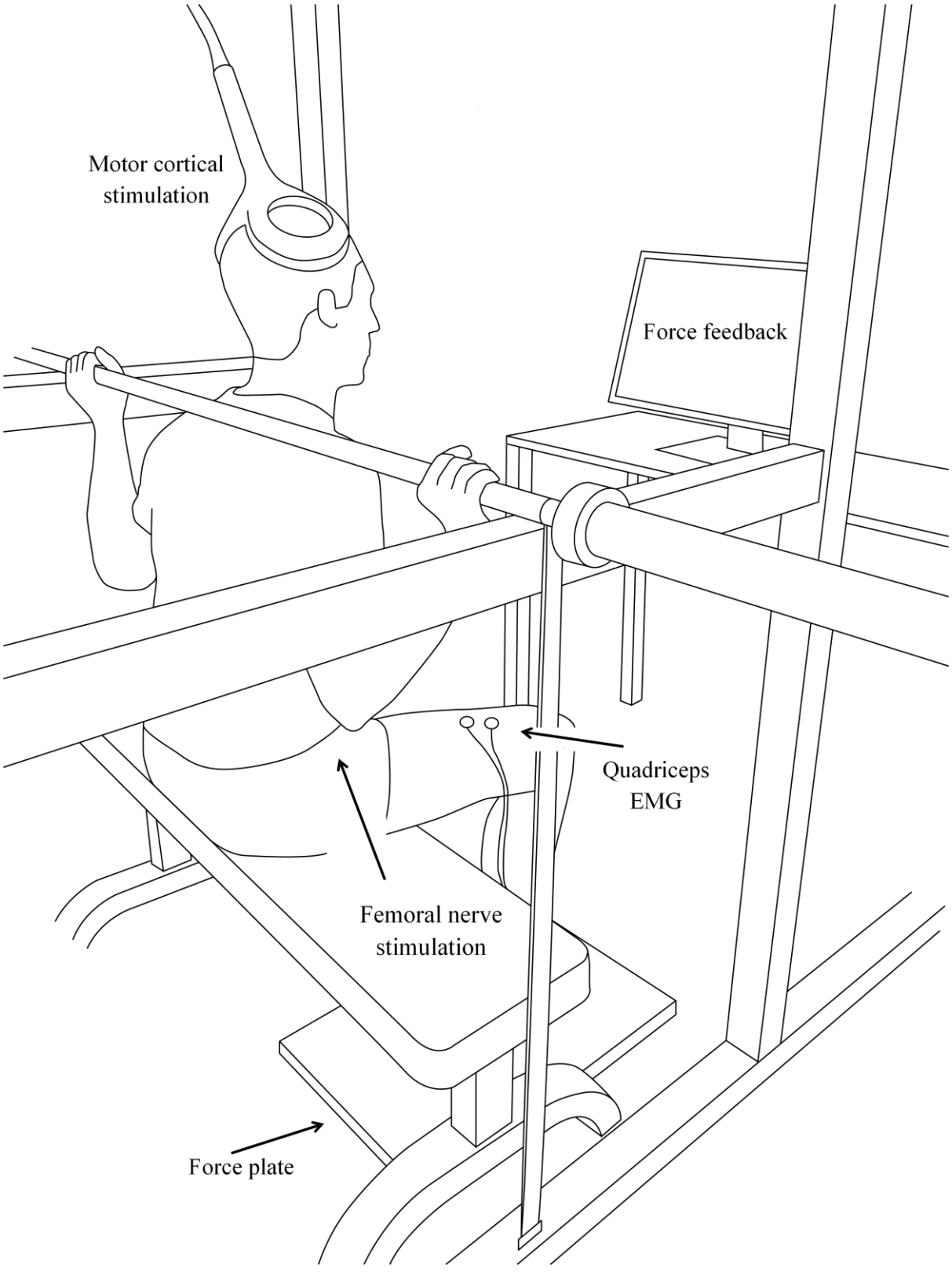

Figure 2. Short interval intracortical inhibition during the isometric squat and knee extension measured in the *rectus femoris* and *vastus lateralis,* with values displayed on a group level (A) as mean ± SD (filled bars = isometric squat; unfilled bars = knee extension), as individual data points (B) during the isometric squat relative to knee extension (filled circles = *vastus lateralis*; open circles = *rectus femoris*), with the dashed line representing the line of agreement (n = 11), and Bland-Altman plots (C) with systemic bias (continuous lines) and 95% limits of agreement (dashed lines) showing agreement between the modalities.

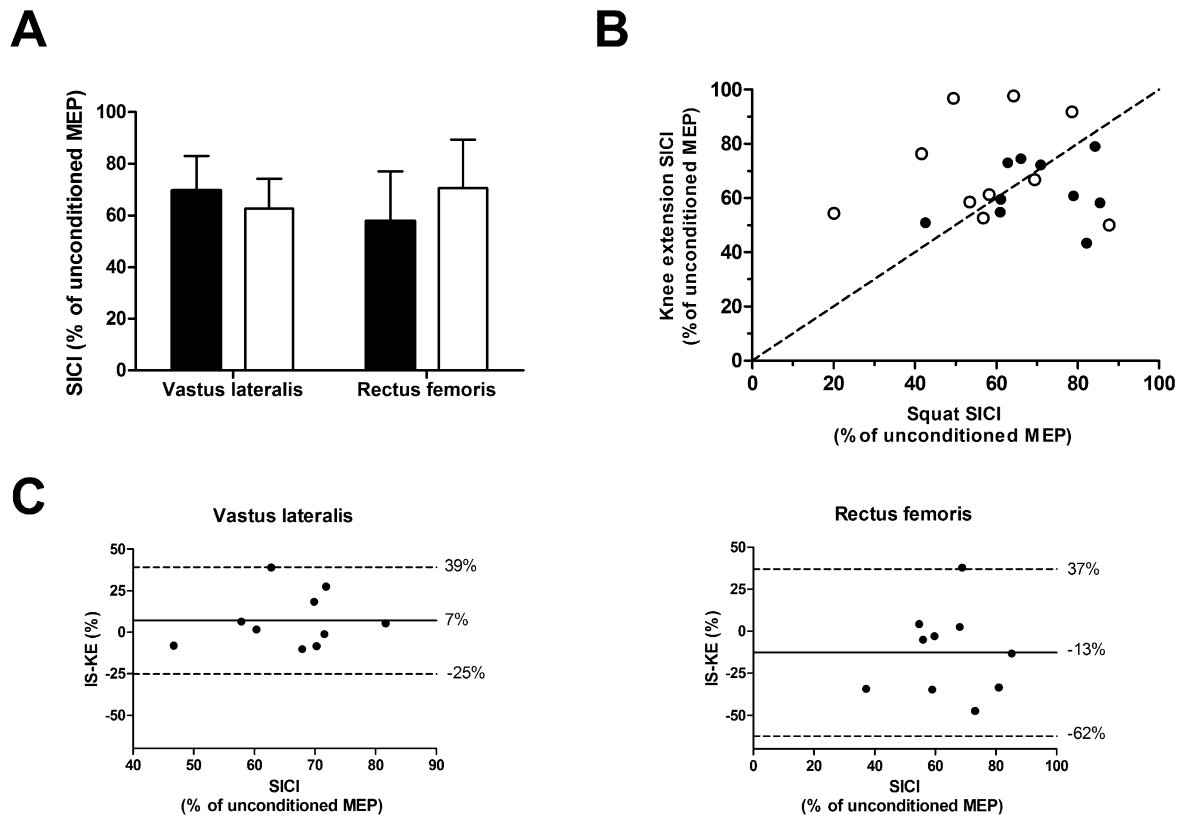

Figure 3. Motor evoked potentials normalised to maximal compound action potential at the same contraction intensity measured in the *vastus lateralis* and *rectus femoris* during isometric squat and knee extension at different contraction intensities expressed as percentage of MVC (n = 11). Values are displayed on a group level (A) as mean ± SD, and as individual data points during the isometric squat relative to knee extension (B), with the dashed line representing the line of agreement, and Bland-Altman plots with systemic bias (continuous lines) and 95% limits of agreement (dashed lines) at 25 (C), 50 (D), 75 (E) and 100% MVC (F). $^{*}P \leq 0.025$ compared to other intensities in both modalities, $^{\#}P = 0.004$ compared to the other modality.

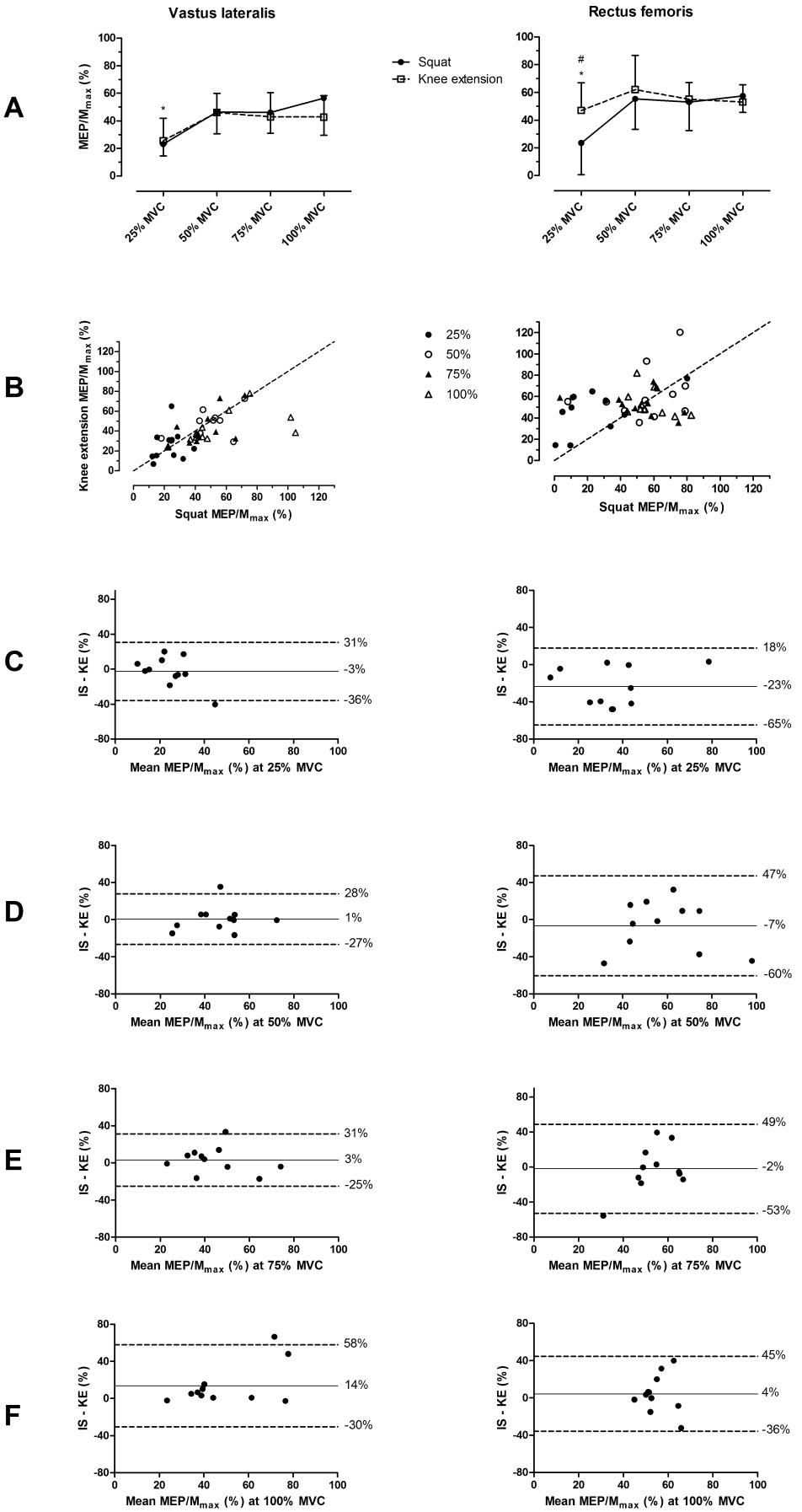
A
Vastus lateralis
Rectus femoris
Squat
Knee extension
MEP/Mmax (%)
25% MVC
50% MVC
75% MVC
100% MVC
B
Knee extension MEP/Mmax (%)
Squat MEP/Mmax (%)
25%
50%
75%
100%
C
D
E
F
IS - KE (%)
Mean MEP/Mmax (%) at 25% MVC
Mean MEP/Mmax (%) at 50% MVC
Mean MEP/Mmax (%) at 75% MVC
Mean MEP/Mmax (%) at 100% MVC
31%
-3%
-36%
18%
-23%
-65%
28%
1%
-27%
47%
-7%
-60%
31%
3%
-25%
49%
-2%
-53%
58%
14%
-30%
45%
4%
-36%

Figure 4. Root mean square EMG activity at different contraction intensities normalised to maximal compound action potential at the same contraction intensity in *vastus lateralis* and *rectus femoris* during the isometric squat and knee extension at different contraction intensities expressed as percentage of MVC (n = 11). Values are displayed on a group level (A) as mean ± SD, and as individual data point during the isometric squat relative to knee extension (B), with the dashed line representing the line of agreement, and Bland-Altman plots with systemic bias (continuous line) and 95% limits of agreement (dashed lines) at 25 (C), 50 (D), 75 (E) and 100% MVC (F). $^{*}P < 0.005$ compared to higher contraction intensities, $^{\#}P < 0.015$ compared to the other modality.

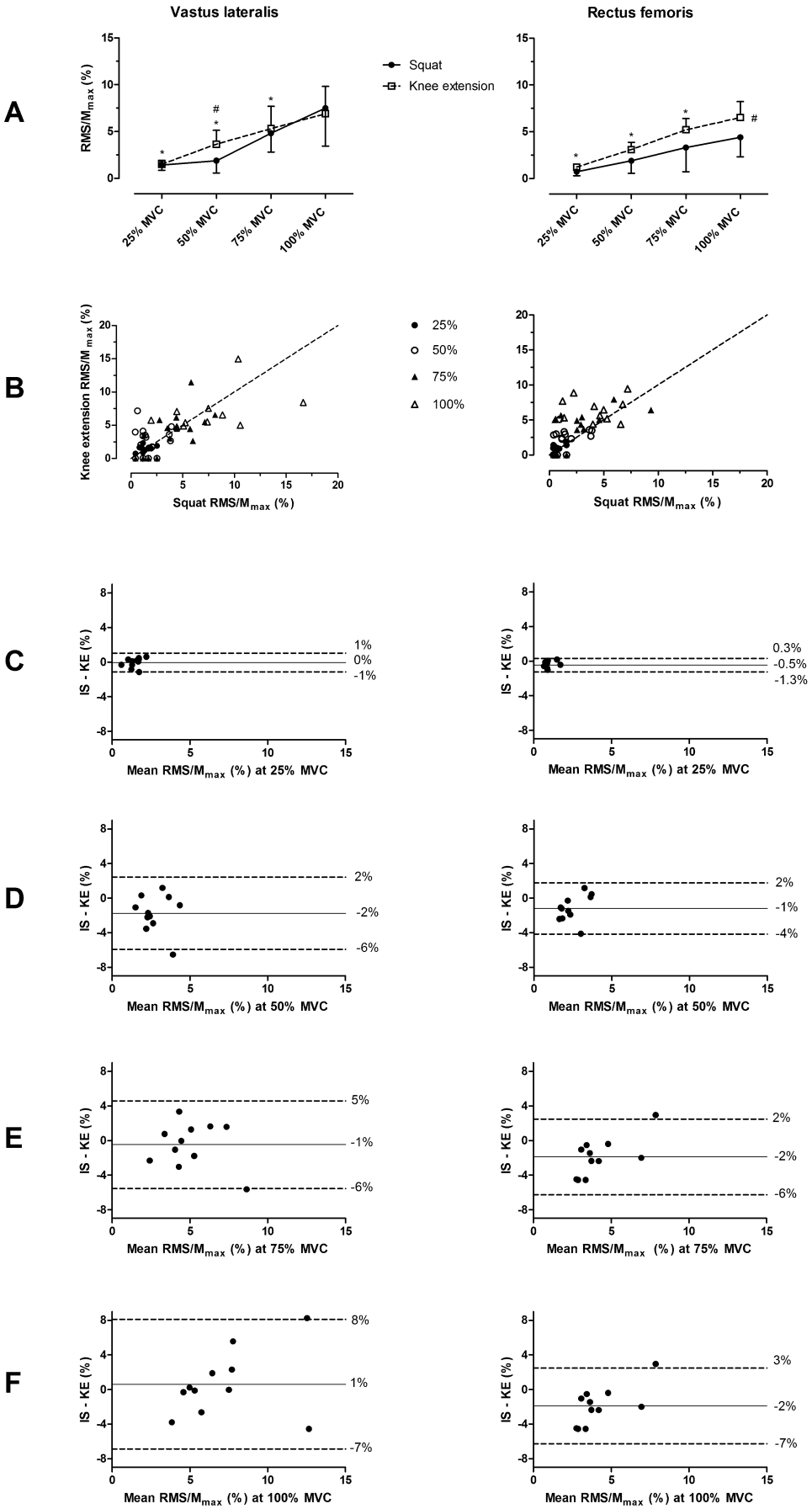
A
Vastus lateralis
Rectus femoris
Squat
Knee extension
RMS/Mmax (%)
25% MVC
50% MVC
75% MVC
100% MVC
B
Knee extension RMS/Mmax (%)
Squat RMS/Mmax (%)
25%
50%
75%
100%
C
D
E
F
IS - KE (%)
Mean RMS/Mmax (%) at 25% MVC
Mean RMS/Mmax (%) at 50% MVC
Mean RMS/Mmax (%) at 75% MVC
Mean RMS/Mmax (%) at 100% MVC
1%
0%
-1%
0.3%
-0.5%
-1.3%
2%
-2%
-6%
-1%
-4%
5%
2%
8%
1%
-7%
3%

Figure 5. The EMG-force relationship during the isometric squat and knee extension in *vastus lateralis* and *rectus femoris* with determination coefficients and associated p-values (n = 11).