TITLE PAGE

Title: Do changes in neuromuscular activation contribute to the knee extensor angle-torque relationship?

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Running Title: Neuromuscular activation at different knee joint angles

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New Findings (limited 100 words)

What is the central question of the study?

- Do changes in neuromuscular activation contribute to knee extensor angle-torque relationship?

What is the main finding and its importance?

- Both agonist (quadriceps) and antagonist (hamstrings) co-activation differed with knee joint angle during maximal isometric knee extensions and thus both likely contribute to the angle-torque relationship. Specifically, two independent measurement techniques showed quadriceps activation to be lower at more extended positions. These effects may influence the capacity for neural changes in response to training and rehabilitation at different knee joint angles.

Abstract

The influence of joint angle on knee extensor neuromuscular activation is unclear, owing in part to the diversity of surface electromyography (sEMG) and/or interpolated twitch technique (ITT) methods used. The aim of the study was to compare neuromuscular activation, using rigorous contemporary sEMG and ITT procedures, during isometric maximal voluntary contractions (iMVCs) of the quadriceps femoris at different knee joint angles and examine whether activation contributes to the angle–torque relationship. Sixteen healthy active men completed two familiarization sessions and two experimental sessions of isometric knee extension and knee flexion contractions. The experimental sessions included the following at each of four joint angles (25, 50, 80 and 106 deg): iMVCs (with and without superimposed evoked doublets); submaximal contractions with superimposed doublets; and evoked twitch and doublet contractions whilst voluntarily passive, and knee flexion iMVC at the same knee joint positions. The absolute quadriceps femoris EMG was normalized to the peak-to-peak amplitude of an evoked maximal M-wave, and the doublet–voluntary torque relationship was used to calculate activation with the ITT. Agonist activation, assessed with both normalized EMG and the ITT, was reduced at the more extended compared with the more flexed positions (25 and 50 *versus* 80 and 106 deg; $P \geq 0.016$), whereas antagonist coactivation was greatest in the most flexed compared with the extended positions (106 *versus* 25 and 50 deg; $P \geq 0.02$). In conclusion, both agonist and antagonist activation differed with knee joint angle during knee extension iMVCs, and thus both are likely to contribute to the knee extensor angle–torque relationship.

INTRODUCTION

The torque a muscle group can generate across a joint's range of motion impacts mobility (Penninx et al. 2001; Hunter et al. 2004), locomotion (Morrison, 1970; Engin & Korde, 1974) and athletic performance (Paasuke et al. 2001; Janura et al. 2016). Indeed, the relationship between joint angle and torque has been carefully described for many joints and muscle groups, including the quadriceps femoris (Q; Leedham & Dowling, 1995; Kubo et al. 2004; Arampatzis et al. 2006; Kooistra et al. 2007). Although changes in sarcomere length are considered the primary factor underpinning the angle–torque relationship (Rassier et al. 1999), it has been suggested that both excitatory and inhibitory feed- back to the motoneuron pool may vary with joint angle (Johansson et al. 1991), and thus changes in neuromuscular activation could also contribute to the angle–torque relationship (Gandevia & McKenzie, 1988). However, whether maximal neuromuscular activation changes with joint angle and contributes to the angle–torque relationship remains unclear (Babault et al. 2001; Newman et al. 2003; Kooistra et al. 2007).

Previous studies using surface electromyography (sEMG) and the interpolated twitch technique (ITT) to investigate neuromuscular activation of the Q during isometric maximal voluntary contractions (iMVCs) at distinct knee joint angles have reported conflicting findings. Specifically, higher Q activation has been demonstrated at both more flexed positions (Becker & Awiszus, 2001; Kubo et al. 2004; Pincivero et al. 2004) and more extended positions (Hasler et al. 1994; Babault et al. 2003), whereas others have documented no differences in Q activation across knee joint positions (Zabik & Dawson, 1996; Newman et al. 2003; Kooistra et al. 2007). In

addition, one study even reported conflicting results between EMG and ITT measures within the same study, with Q EMG found to be highest in a flexed position (90 deg) but activation measured via ITT to be greater in an extended position (Suter & Herzog, 1997). Likewise, differences in antagonist activation have been documented across knee joint angles in some studies (Kubo et al. 2004) but not others (Babault et al. 2003; de Ruiter et al. 2004). These inconsistent findings might be attributable to methodological deficiencies or discrepancies between studies.

Comparison of sEMG measurements at different joint angles may be confounded by any changes in the electrical propagation and recording conditions as the joint angle changes, including any shift in the tissues beneath the recording electrode, especially the muscle fibres as they lengthen, shorten and change orientation (Schulte et al. 2004). Normalizing EMG measured during voluntary contractions to the amplitude of an evoked maximal M-wave (MMAX) at the same joint angle may account for many of these changes in the recording conditions (Gandevia et al. 2001) and facilitate a more valid comparison between joint angles. However, none of the previous studies investigating neural drive at different joint angles with EMG has used this normalization approach. In addition, many of these studies measured EMG from only a limited number of agonist (Q; Suter & Herzog, 1997; Newman et al. 2003; de Ruiter et al. 2004) and antagonist (hamstrings; H) muscles (Babault et al. 2003; de Ruiter et al. 2004; Kubo et al. 2004), which might not provide a very thorough representation of the whole muscle group (Aagaard et al. 2000).

Studies using the ITT to assess voluntary activation at different joint angles have invariably used the following formula: voluntary activation 1 twitch amplitude during an iMVC/control twitch amplitude (e.g. Suter & Herzog, 1997; Becker & Awiszus,

2001; Babault et al. 2003; Newman et al. 2003; Kubo et al. 2004; Kooistra et al. 2007). However, this formula assumes a linear reciprocal twitch–voluntary force relationship, to calculate activation (ACT), despite the fact that the twitch–voluntary force relationship has been widely demonstrated not to be a linear reciprocal (Behm et al. 1996; Scaglioni et al. 2002; Kooistra et al. 2007; Folland & Williams, 2007a). This discrepancy tends to lead to overestimated activation values (Kooistra et al. 2007; Folland & Williams 2007a) and could also be affected by joint angle. Therefore, defining the twitch–voluntary force relationship at each joint angle for each participant in order to extrapolate up to the theoretical maximal torque (TMT) and calculate activation may be a more rigorous approach (Folland & Williams, 2007a).

Consequently, a study using rigorous EMG and ITT measures is warranted to help determine whether differences in neuromuscular activation contribute to the knee extension angle–torque relationship. Therefore, the aim of this study was to compare neuromuscular activation, using contemporary EMG and ITT procedures, during maximal voluntary isometric contractions of the Q at different knee joint angles.

METHODS

Participants

Sixteen healthy, recreationally active men (21 2 years old; 1.78 0.07 m; 73 5 kg) who had no previous lower-body injuries and had not taken part in systematic lower body training for at least 12 months participated in the study and they also provided written informed consent before their participation. The study was approved by the Loughborough University ethical advisory committee (R15-P040) and was conducted in accordance with the Declaration of Helsinki.

Overview

Participants attended four laboratory sessions at a consistent time of day, each separated by 3–6 days, and were instructed to avoid strenuous exercise in the 48 h before each session. The first two laboratory visits were familiarization sessions with identical procedures to the main experimental sessions (visits 3 and 4) but no analysis. All sessions involved unilateral isometric knee extension and flexion contractions of the right leg at four different knee joint angles (25, 50, 80 and 106 deg, where 0 deg is full knee extension), with a constant hip joint angle of 65 deg (0 deg is full hip extension). The different knee joint angles were tested in a counterbalanced order during the two main experimental sessions. After a brief warm-up, the following tasks were performed at each angle before moving to the next task: (i) knee extension iMVCs (x2; ascending/descending angle order); (ii) evoked knee extensor twitch and doublet contractions (via femoral nerve stimulation) whilst voluntarily passive and knee extensor submaximal voluntary contractions (at

60, 75 and 90%) and iMVCs (x2), all with superimposed doublets, to define the twitch–voluntary force relationship and calculate maximal activation with a variant of the ITT (ACTITT; opposite order); and (iii) knee flexion iMVCs, for normalization of antagonist EMG (original angle order; Table 1). The knee extension/flexion torque and EMG of the superficial quadriceps and hamstrings were recorded during all contractions. The EMG amplitude at knee extension maximal voluntary torque (MVT) was expressed as absolute values (root mean square; RMS) and also normalized (agonist was normalized to M_{MAX} and antagonist to EMG at maximal knee flexion; $HEMG_{MAX}$).

Recording Procedures

Torque, sEMG and video recordings. All contractions were performed with participants seated on a custom-made isometric testing chair, adjustable for knee joint angle, and securely strapped at the waist and across the chest to minimize extraneous bodily movement. The different knee joint positions were established with a hand-held goniometer during the first familiarization session, and the chair configuration was replicated thereafter. The knee joint angles were measured by digitizing video images (Kinovea 0.8.15 software, https://www.kinovea.org/) recorded with a video camera (Panasonic HC-V110; Secaucus, NJ, USA), specifically the angle between the greater trochanter, knee joint space and lateral ankle malleolus during the iMVCs of the first laboratory visit.

Force production was measured with a calibrated S-beam strain gauge (linear range 0–1500 N; Force Logic, Swallowfield, UK). The strain gauge was attached perpendicular to the participant's tibia with a custom reinforced non-extendable

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webbing strap (35 mm width) fastened ~3 cm superior to the lateral malleolus. Force was sampled and recorded at 2000 Hz using an A/D converter (A/D Micro 1401; CED, Cambridge, UK) and PC using Spike2 software (CED). The force signal was low-pass filtered at 500 Hz with a fourth-order zero-lag Butterworth digital filter and notch filtered at 50 Hz with an infinite impulse response digital filter (q-factor of 10) to remove mains frequency noise. Torque during each task was calculated as the product of force and lever arm length (the distance between the knee joint centre and the middle of the strap).

Surface EMG was recorded during the main experimental sessions using a wireless EMG system (Trigno; Delsys, Inc., Boston, MA, USA). After preparation of the skin (shaving, abrading, and cleansing with 70% ethanol), single differential Trigno Standard EMG sensors (Delsys Inc.), each with a fixed 1 cm interelectrode distance, were attached over the quadriceps and hamstrings using adhesive interfaces. A total of six separate EMG sensors were located over the superficial quadriceps muscles, two per muscle, at the following percentages of thigh length (the distance from the knee joint centre to the greater trochanter) above the superior border of the patellar: vastus medialis (VM; 25 and 35%), vastus lateralis (VL; 50 and 60%) and rectus femoris (RF; 55 and 65%). In addition, single EMG sensors were also placed at 45% of thigh length above the popliteal fossa on the biceps femoris long head (BF) and semitendinosis (ST). The EMG signals were amplified and filtered at source (×300; 20–450 Hz bandwidth) before further amplification (overall effective gain, ×909) and were sampled at 2000 Hz using the same external A/D converter and computer software as the force recordings. During the offline analysis, the EMG data were time

aligned with the force signal (inherent 48 ms delay of EMG signal). Whole muscle group (Q and H) EMG amplitude values were the average of six and two sites, respectively.

Femoral nerve stimulation. Tanscutaneous femoral nerve stimulation was delivered by a constant current, variable voltage stimulator (DS7AH; Digitimer Ltd, Welwyn Garden City, UK) via a cathode probe (10 mm diameter, protruding 20 mm from a 35 mm 55 mm plastic base; Electro-Medical Supplies, Greenham, UK) positioned over the femoral nerve in the femoral triangle and an anode electrode (70 mm 100 mm carbon rubber electrode; Electro-Medical Supplies) over the greater trochanter. The anode and cathode were both coated in conductive gel, and a low-level current (30–50 mA) was delivered. Once an appropriate twitch response was elicited, according to the optimal twitch response from a small stimulus current, the cathode was secured with transpore tape. The M_{MAX} and doublet were configured as 200 µs square-wave pulses with a10 ms interval between each stimulus.

Protocol

Knee extensor MVCs. A warm-up of submaximal contractions was performed at the first angle only, at percentages of perceived maximal effort [50% (3), 75% (3) and 90% (1)]. Participants then completed an initial sequence of two iMVCs at each knee joint angle (in an ascending or descending order). Later in the protocol, participants performed two further iMVCs at each angle, in the opposite order, with superimposed doublets to facilitate measurement of activation with the ITT. During the iMVCs, participants were instructed to extend their knee and 'push as hard as possible' for 3–5 s with a 2':30 s recovery period between each iMVC. Biofeedback

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was provided after the first iMVC with a horizontal cursor on the torque–time curve, displayed on a computer monitor in front of the participant, to indicate the greatest force produced and encourage participants to produce greater force with subsequent attempts. Additionally, verbal encouragement was given during all iMVCs. Knee extension isometric maximal voluntary torque (iMVT) was identified as the highest voluntary torque produced from all four iMVCs at each angle (torque increments attributable to superimposed doublet stimulation were excluded). The absolute EMG amplitude from each recording site was measured as the RMS, over a 500 ms epoch at iMVT (250 ms either side, unless this coincided with a superimposed doublet, in which case the epoch was time shifted, forwards or backwards, to avoid the doublet artefact in the EMG signal, but still incorporated iMVT). For individual agonist muscles, the EMG amplitude during maximal voluntary torque from the two recording sites over each muscle was averaged (VM, VL and RF EMGMVT), and the values from all six sites were averaged to calculate a whole quadriceps value (QEMG$_{MVT}$). The EMG amplitude from the two antagonist sites (BF and ST) at knee extension iMVT was also averaged to calculate an overall hamstrings coactivation (i.e. simultaneous activation of the antagonists; HEMG$_{CO-ACT}$). Agonist EMG amplitude for each recording site was also normalized to site- and angle-specific maximal M-wave peak to peak (M$_{MAX}$ P-P; see below) before averaging across sites and muscles as above. Antagonist EMG amplitude for the BF and ST was normalized to HEMGMAX during knee flexion iMVCs (see below). After the initial sequence of two iMVCs at each angle and 5 min of rest, all of the following were done at each angle in the opposite order to the initial iMVCs: current calibration and MMAX responses, as well as doublets at rest and superimposed on submaximal and maximal voluntary

contractions (Table 1). This meant there was a minimum of 3 min between the voluntary contractions at each subsequent angle.

Stimulation calibration and M_{MAX} responses. The stimulation current was calibrated with single stimuli or evoked twitch contractions at each angle before the measurement of supramaximal MMAX responses and the delivery of evoked doublets. Therefore, at each angle, twitch responses were evoked every ~10 s, first at a low current, and increased until twitch force and the peak-to-peak amplitude of the M-wave plateaued. Thereafter, three further single stimuli were delivered at a current of 150% of the plateau level to ensure supramaximal stimulation and M-wave responses. The MMAX P-P was calculated as the mean of these three MMAX responses at each joint angle. Evoked doublets at rest, superimposed on submaximal and maximal voluntary contractions. After the MMAX recordings, the stimulator current was set to 100% of the plateau level (to minimize participant discomfort), and doublet amplitude (>25% MVT) was checked by delivering two doublet contractions, each involving two stimuli 10 ms apart, while the participant was voluntarily passive, with 10 s between each. Doublets were used for the ITT because they provide a larger signal-to-noise ratio, which facilitates identification of the superimposed torque increment, and are less sensitive to confounding potentiation (Oskouei et al. 2003; Folland & Williams, 2007a). Participants then performed three submaximal contractions (60, 75 and 90% MVT) and two iMVCs, with two doublet contractions (separated by ~1 s) superimposed during the plateau phase of each voluntary contraction, and two further doublet contractions delivered after each voluntary contraction (~5 s afterwards, and separated by ~1 s), while

participants were voluntarily passive. An interval of >30 s was given between voluntary contractions. Before each submaximal contraction, participants were instructed to achieve the required force (indicated by a horizontal target line on the screen) and hold the desired force as steadily as possible for ~5 s.

Calculation of activation using the interpolated twitch. For each resting or superimposed doublet torque response, values immediately before and at the peak of the doublet were recorded, and the difference was calculated as the doublet magnitude. For the superimposed doublet, torque immediately before the doublet also provided a measure of voluntary torque at the instant the doublet was delivered. The magnitude of the two superimposed doublets during each voluntary contraction was averaged and expressed as a percentage of the doublet contraction evoked at rest immediately after the contraction. The voluntary torque at the instant the superimposed doublets were delivered was also averaged. Thereafter, doublet torque was plotted against voluntary torque for the three submaximal and two maximal contractions of each participant at each angle. A linear function was found to provide a good fit to these relationships (Fig. 1) and was used to extrapolate up to the TMT (i.e. x-axis intercept and the point where the superimposed doublet declines to zero because of 'full activation'; Folland & Williams, 2007b). The MVT (i.e. the highest voluntary torque during any iMVCs at that angle) was compared with the TMT in order to calculate the individual's maximal activation at each angle using the following equation: ACT_{ITT} MVT/TMT ×100 (Folland & Williams, 2007a).

Knee flexion maximal voluntary contractions. Knee flexion iMVCs were performed in an identical manner to the knee extensor iMVCs. The EMG RMS amplitudes from

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the two agonist sites, BF and ST, were calculated over a 500 ms epoch at iMVT (250 ms either side) and averaged to calculate an overall HEMGMAX.

Statistical analysis

Statistical analysis Between-sessions reliability of MVT and EMG from the main trial sessions was assessed by calculating the within-participant coefficient of variation [CV_W (SD/mean) × 100]. All the CV_W values presented are averaged across the four joint angles and all participants. Torque, EMG, TMT and ACTITT measurements at each knee joint angle during both the main experimental sessions were averaged to provide criterion values for statistical analysis. Repeated-measures one-way ANOVA was used to analyse the differences between angles for MVT, absolute and normalized EMG, ACTITT and TMT. Repeated-measures two-way ANOVA (muscle versus angle) was used to compare the changes in absolute and normalized EMG across angles for the different muscles (i.e. the interaction effect). Post hoc comparisons were conducted using Bonferroni post hoc tests. Statistical analysis was performed using SPSS version 22 (IBM Corporation, Armonk, NY, USA), and the significance level was set at $P < 0.05$. The standardized effect size (ES; Cohen's *d*) are included, and an ES of <0.2 was considered trivial, ≥ 0.2 small, ≥ 0.5 moderate and ≥ 0.8 large (Cohen, 1988). Data are reported as means ± SD.

RESULTS

Reliability

Knee extension MVT had an average CV_W of 4.3% (across angles and participants), and absolute $QEMG_{MVT}$ and $HEMG_{CO\text{-}ACT}$ had an average CV_W of 12.6 and 20.2%, respectively. Normalized $QEMG_{MVT}$ and $HEMG_{MAX}$ showed an average CV_W of 15.4 and 24.3%, respectively; and ACT_{ITT} had a CV_W of 5.2%.

Maximal Isometric Voluntary Torque

The MVT showed a distinct inverted 'U' relationship, as expected (Fig. 2). MVT at 25 deg was lower than all the other positions (one-way ANOVA, $P < 0.001$), and MVT at 106 deg was also lower than at 50 and 80 deg ($P \leq 0.001$, $ES \geq 2.97$ 'large'; Fig. 2).

Neuromuscular and Voluntary Activation

Absolute agonist EMG. Although absolute QEMGMVT indicated a difference according to knee joint angle (one-way ANOVA, $P = 0.005$), post hoc analysis did not show any specific differences between angles, only a tendency for 25 deg to be lower than 80 deg ($P = 0.075$; $ES = 0.05$ 'trivial'; Fig. 3A). Within each individual agonist muscle, $VMEMG_{MVT}$ (one-way ANOVA, $P = 0.010$) and $VLEMG_{MVT}$ (one-way ANOVA, $P = 0.031$) varied with knee angle, whereas $RFEMG_{MVT}$ was indifferent for angle (one-way ANOVA, P 0.259). Specifically, $VMEMG_{MVT}$ 25 deg was lower than 80 deg (P 0.038, ES 0.49 'small'), and $VLEMG_{MVT}$ showed a tendency for 50 deg to be lower than that at 80 deg ($P = 0.092$, ES 0.2 'small'). Consequently, there was a

muscle angle interaction (P = 0.020), with greater changes for the $VMEMG_{MVT}$ across joint angles than for the $VLEMG_{MVT}$ or $RFEMG_{MVT}$ (P ≤ 0.026).

Normalized agonist EMG. The $QEMG_{MVT}$ normalized to M_{MAX} P-P showed a main effect of angle (one-way ANOVA, P < 0.001), with progressively higher values at more flexed positions such that 106 deg was 29% higher than at 25 deg [25 and 50 deg < 80 and 106 deg, P ≤ 0.016; ES ≤ 0.8 'large' (25 vs 80 and 50 vs 106 deg) or ES = 0.4 'small' (50 vs 80 deg); Fig. 4A], and normalized $QEMG_{MVT}$ at 106 deg was 29% higher than at 25 deg. Within each individual agonist muscle, both $VMEMG_{MVT}$ and $VLEMG_{MVT}$ changed with knee joint angle (P < 0.001), whereas $RFEMG_{MVT}$ was indifferent to angle (one-way ANOVA, P = 0.120). Specifically, normalized $VMEMG_{MVT}$ at 25 deg was lower than the other angles (P ≤ 0.016; ES ≤ 0.94 'large') and 50 deg was lower than 80 deg (P < 0.001, ES = 0.6 'moderate'); normalized $VLEMG_{MVT}$ showed lower values at 25 and 50 deg than at 80 and 106 deg [P ≤ 0.002; ES ≤ 1.05 'large' (25 with 80 and 106 deg, and 50 with 106 deg) or ES = 0.65 'moderate' (50 with 80 deg)]. Subsequently, there was a muscle × angle interaction (P = 0.028), with greater changes in the $VLEMG_{MVT}$ than in the $RFEMG_{MVT}$ and $VMEMG_{MVT}$ [P ≤ 0.039; ES = 0.65 'moderate' (VL versus VM) and ES = 1.22 (VL versus RF)].

Voluntary Activation. A main effect of angle was found for ACTITT (ANOVA, P< 0.001), with ACTITT at 25 and 50 deg being ~16% lower than at 80 and 106 deg (P ≤ 0.001; ES ≤ 2.08 'large'; Fig. 5).

Antagonist Coactivation. Antagonist coactivation. Absolute $HEMG_{CO-ACT}$ during knee extension showed no significant difference between angles (P =0.459; Fig. 3C), but

normalized coactivation (to $HEMG_{MAX}$) revealed differences for angle (P 0.002), with 106 deg greater than 50 and 80 deg (P ≤ 0.02; ES ≤ 0.81 'large'), and also a tendency to be greater than 25 deg (P = 0.084; ES = 0.81 'moderate'; Fig. 4C).

Evoked M_{Max} Responses

There were clear differences in Q M_{MAX} P-P between angles (ANOVA, P < 0.001), with post hoc tests showing differences between all the angles [P ≤ 0.022; ES = 0.7 'moderate' (25 vs 106 deg) or ES ≤ 0.21 'small' (all other combinations); Fig. 6A] and values at 25 deg being 17% higher than at 106 deg. Within the individual agonist muscles, VL and VM M_{MAX} P-P was different across angles (ANOVA, P < 0.001; Fig. 6B; 17% range for the VM and 26% range for the VL), whereas the RF did not show differences (ANOVA, P < 0.378). Specifically, the VL was higher at 25 deg than at the other angles (P ≤ 0.015), and at 106 deg it was lower than at the other angles (P ≤ 0.002). For VM, 25 deg was higher than the other angles [P ≤ 0.001; ES = 0.58 'moderate' (25 with 106 deg), ES ≤ 0.25 'small' (25 with 50 and 80 deg)] and at 50 deg was higher than at 80 and 106 deg (P ≤ 0.046; or ES = 0.13 'trivial').

DISCUSSION

In the present study, we assessed neuromuscular activation of the agonist and antagonist muscles during maximal isometric knee extension contractions at four angles, and found activation of both muscle groups to change with joint angle and thus likely contribute to the angle-torque relationship. Both normalised $QEMG_{MVT}$ and ACT_{ITT} indicated that quadriceps neuromuscular activation was lower at more extended (25 and 50 deg), compared to more flexed, knee-joint angles (80 and 106 deg). Normalised EMG from the individual agonist muscles showed this effect to be specific to the vasti muscles (VL and VM) as only these muscles, and not the RF, showed lower neural drive at the most extended positions. Finally, normalised hamstrings co-activation, also showed higher co-activation simultaneous to knee extension MVT at the most knee flexed position (106 deg).

As expected we found a marked knee extensor angle-torque relationship with higher values at the middle angles compared to more extreme positions (Babault *et al.*, 2003; Newman *et al.*, 2003; de Ruiter *et al.*, 2004; Pincivero *et al.*, 2004). The differences in torque production (Fig. 2) are thought to be primarily due to the muscle force-length relationship (Herzog *et al.*, 1990; Rassier *et al.*, 1999) whereby changes in joint angle and muscle length affect the extent of cross-bridge overlap and thus sarcomere force generation (Lieber et al. 1994; Rassier et al. 1999). However, the present study provides convincing evidence that agonist and antagonist activation also contribute to the shape of the angle-torque relationship. These findings were based on duplicate measurement sessions, following two familiarisation sessions, and involved more rigorous EMG and ITT measurements than previous studies, specifically agonist EMG normalised to M_{MAX} and ACT_{ITT} calculated with appropriate extrapolation for each individual and joint angle, which provides some confidence in the findings.

Quadriceps activation

We found no differences in absolute EMG amplitude at different joint angles. Previous studies reported contrary results for maximal EMG at different knee joint positions, with reports of higher EMG at more flexed positions (Becker & Awiszus, 2001; Kubo et al. 2004; Pincivero et al. 2004) or more extended positions (Hasler et al. 1994; Babault et al. 2003) or no differences between joint angles (Zabik & Dawson, 1996; Newman et al. 2003; Kooistra et al. 2007). However, in the present study the electrical response to the same stimulus, amplitude of a compound muscle action potential (MMAX P-P), was markedly different according to the knee angle for both vastii muscles and, consequently, the whole quadriceps (25 deg was 17% higher than 106 deg). This provides strong evidence that the electrical signal propagation and recording conditions are influenced by knee joint angle in a manner entirely independent of voluntary neural drive, and probably invalidates the comparison of absolute EMG amplitude values between angles. This finding of higher MMAX P-P values at more extended positions is in accordance with previous studies with other muscle groups (e.g. tibialis anterior, Marsh et al. 1981; tibialis anterior and soleous, Frigon et al. 2007). Differences in MMAX P-P might be expected when the muscle changes length because of the shift of the electrode position relative to the underlying fibres, which may lead to recording of different motor units (Rainoldi et al. 2000), and a change in the conduction velocity of fibre electrical activity (initiation, propagation, termination and slow repolarization) through to the skin. These effects are likely to explain the joint angle dependence of MMAX P-P (Lateva et al. 1996).

In contrast, the normalized EMG (absolute EMG amplitude/MMAX P-P), which might be expected to remove the differences in signal propagation and recording conditions across angles, revealed differences in maximal voluntary neural drive between joint angles. We are not aware of any previous studies that have examined normalized quadriceps EMG across knee joint angles during iMVCs. However, studies of other muscle groups have used normalized EMG with mixed findings, such as higher activation of elbow flexors at shortened positions (Kasprisin & Grabiner, 2000; Linnamo et al. 2006) or no differences for joint angle (Leedham & Dowling, 1995; Doheny et al. 2008), as well as higher plantar flexor activation at lengthening positions (Kluka et al. 2016) or no differences for joint angles (Papaiordanidou et al. 2016).

The normalized EMG findings of our study are reinforced by the ACTITT results, which also showed higher activation at the most flexed knee joint positions. Some previous ITT studies have made similar findings (Suter & Herzog, 1997; de Ruiter et al. 2004; Kubo et al. 2004), but there are also contrary reports of higher activation at the most extended knee joint positions (Newman et al. 2003; Kooistra et al. 2007) and no differences across the angles (Babault et al. 2003). In the present study, we deliberately used a doublet stimulus to generate a high signal-to-noise ratio and carefully defined the twitch–voluntary force relationship at each joint angle for each participant in order to extrapolate up to the TMT and calculate activation (Folland & Williams, 2007a). The fact that both independent measures of activation in this study (normalized QEMG and ITT) found lower activation at the more extended knee joint angles provides some confidence that these effects are genuine and that quadriceps

activation is inhibited at more extended positions. These findings might have implications for the adaptation to resistance training; specifically, neural adaptations, and thus also functional changes, could be angle specific, with greater scope for neural changes at extended knee joint positions because of the initial failure in activation at these positions. Reduced activation at some positions (e.g. extended knee joint angles) could also impair knee joint stabilization and might contribute to the observation that knee injuries typically occur at relatively extended positions (Krosshaug et al. 2007).

The anterior displacement of the tibia during forceful knee extension contractions at extended knee joint angles (Hirokawa et al. 1992) has been suggested to explain lower agonist activation at these positions (Suter & Herzog, 1997). During knee extension contractions between 0 and ~60 deg, anterior tibial displacement (and rotation) occurs and applies stress to the anterior cruciate ligament (ACL) as the primary passive restraint to this movement (Howell, 1990; Hirokawa et al. 1992). The sensory mechanoreceptors in the ACL could activate reflex pathways, such as Ia and Ib interneurons (Johansson et al. 1991), and so alter the activation of the muscles arround the knee. This suggestion is supported by Solomonow et al. (1987), who showed that ACL stress generates a moderate inhibition of the Q, whereas during knee extension contractions at more flexed positions there is a posterior displacement of the tibia, resulting in unloading of the ACL (Hirokawa et al. 1992) and, consequently, no inhibitory feedback would be expected.

The capability for neuromuscular activation at different joint angles could also be influenced by changes in the motoneuron pool of the Q. It has been hypothesized

that longer muscle spindle lengths lead to higher input from Ia afferents providing excitation of the Q motoneuron pool, resulting in an increase in neuromuscular activation (Becker & Awiszus, 2001; Kubo et al. 2004). However, evidence also suggests that differences in neuromuscular activation and/or voluntary activation across joint angles could suffer influence from the spinal and supraspinal level. A plantar flexor study (Papaiordanidou et al. 2016) found differences in voluntary activation (ITT) during iMVC across different ankle joints but with no differences in spinal excitability measure by H reflex; however, when the H reflex was normalized by MMAX, the H reflex presented a lower activation during most lengthening positions. The same study also investigated the effect of different knee joint angles on the plantar flexor neural mechanisms (V-wave and H reflex), and a knee joint angle effect in the plantar flexors was found for neuromuscular activation (ITT) and V-wave normalized by MMAX. Therefore, it was suggested by the authors that interferences from the spinal and supraspinal level might be responsible for those changes.

Co-activation of the hamstrings

In the present study, we measured coactivation from both hamstring muscles (BF and ST) and found greater normalized coactivation at the most knee flexed position. The majority of previous studies did not find coactivation during knee extensor iMVCs to vary with joint angle (Babault et al. 2003; Newman et al. 2003; de Ruiter et al. 2004), but two studies corroborate our findings (Baratta et al. 1988; Kubo et al. 2004). The contrasting evidence may be in part because previous findings were

based on purely BF EMG measurements (Babault et al. 2003; Newman et al. 2003; de Ruiter et al. 2004; Kubo et al. 2004), which may underestimate activation of the whole hamstrings (Aagaard et al. 2000), or were not normalized to the maximal EMG during knee flexion (Newman et al. 2003). The increased coactivation we have observed at the most knee flexed position is not readily explained by knee joint mechanics, because tibial displacement during knee extension at the most knee flexed position is posterior (Hirokawa et al. 1992) and there is no obvious need for coactivation of the hamstrings to stabilize the knee. The normalization procedure used for the antagonist coactivation in the present study (to HEMG$_{MAX}$ during knee flexion MVCs) was not as rigorous as the agonist normalization (to MMAX, i.e. a maximal involuntary response) because we are not aware of a method for evoking hamstrings MMAX. Consequently, given the differences in quadriceps maximal activation with knee joint angle we have demonstrated, it is conceivable that hamstring maximal activation also varies with knee joint angle, in which case HEMGMAX might not represent a consistent level of neural drive at different joint angles and thus might not be an ideal reference for normalization across joint angles.

CONCLUSION

In conclusion, both agonist and antagonist activation differed with knee joint angle during maximal isometric knee extension contractions and thus both are likely to contribute to the angle–torque relationship. Agonist activation, assessed with both normalized EMG and ACT_{ITT}, was reduced at the more extended positions, whereas antagonist coactivation was greatest at the most flexed position. These findings may have implications for the scope of training-induced adaptations and the risk of knee injury at extended knee angles.

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COMPETING INTEREST

None declared.

AUHTOR CONTRIBUTIONS

All experiments for this study was conducted in the muscle function laboratory of the School of Sport, Exercise and Health Sciences at Loughborough University, UK.

Conception and design of the experiments: MBL, T.G.B, J.P.F.

Collection, analysis and interpretation of data: MBL, T.G.B, J.P.F.

Drafting the article or revising it critically for important intellectual content: MBL, T.G.B, J.P.F.

All authors approved the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed

TABLES

Table 1. Order of the isometric contractions performed during the main experimental sessions. All orders were counterbalanced across the two main experimental sessions.

	Task performed at each angle	Angle Order
1	Knee extensions iMVCs (×2)	Ascending/Descending **
2	Knee extension evoked Twitch & Doublet (voluntarily passive), and submaximal (60, 70 & 90%) and iMVCs (×2) with superimposed doublets.	Opposite order to 1
3	Knee flexion iMVCs	Same order as 1

** Ascending (25°, 50°, 80°, 106°) /Descending (106°, 80°, 50°,25°).

Figures

Superimposed doublet torque relationship.

Figure 1. The voluntary torque – superimposed doublet torque relationship for one participant at 80° knee-joint angle from the first experimental session. The solid line represents the linear regression fit for the data points and the dashed line represents the extrapolation to the x-axis in order to find the true maximum torque (TMT).
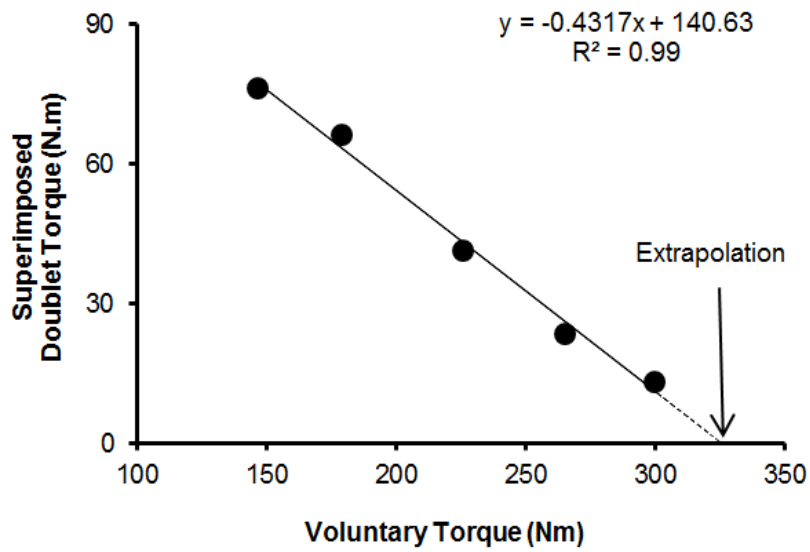
Knee Extensor angle-torque relationship

Figure 2. The knee extensor angle-torque relationship for isometric maximal voluntary torque at knee-joint angles of 25°, 50°, 80° and 106°. One way ANOVA showed differences between angles (P<0.001). # indicate lower than 50°, 80° and 106° (P<0.001), * indicate lower than 50° and 80° (P ≤ 0.001). Data are mean ± SD (n=16).
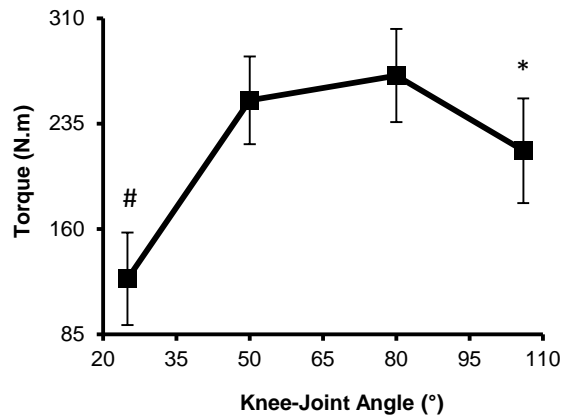
Absolute sEMG across angle positions

Figure 3. EMG at knee extensor maximal voluntary torque (MVT) for knee joint angles of 25°, 50°, 80° and 106°. (A) Absolute EMG of the quadriceps femoris at MVT ($QEMG_{MVT}$). Post hoc analysis found no differences between angles. (B) Absolute EMG of vastus medialis (open diamond), rectus femoris (open triangle) and vastus lateralis (open circle), VM and VL EMG changed with knee joint angle ($P \leq 0.031$). Average standard deviation across angle positions are represented with the symbols at the right side of the figure. (C) Absolute EMG of the hamstrings at knee extension MVT ($HEMG_{CO\text{-}ACT}$). $HEMG_{CO\text{-}ACT}$ was unaffected by angle ($P = 0.459$). Data are mean ± SD (n=16).
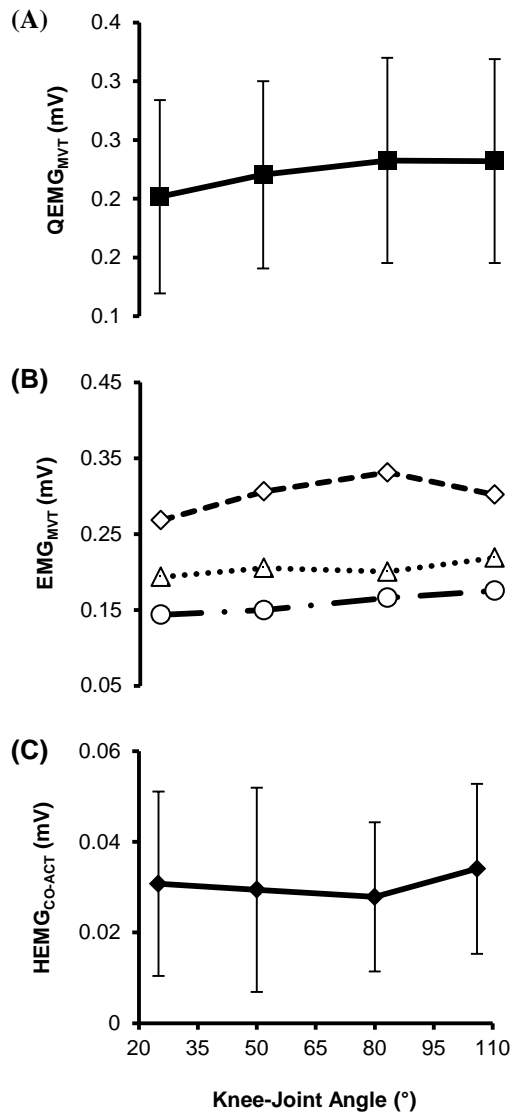
Normalised sEMG across angle positions

Figure 4. Normalized agonist and antagonist EMG at knee extension MVT at knee joint angles of 25, 50, 80 and 106 deg. A, normalized QEMG$_{MVT}$ [to maximal M-wave peak-to-peak (M$_{MAX}$ P-P)] differed according to joint angle ($P < 0.001$). B, normalized EMG (to M$_{MAX}$ P-P) for rectus femoris (open triangles), vastus medialis (open diamonds) and vastus lateralis (open circles). The VM and VL changed with knee joint angle ($P < 0.001$). Average standard deviations across angle positions are represented with the symbols at the right side of the figure. C, normalized hamstring EMG (to HEMG$_{MAX}$) during knee extension MVT differed between angles ($P = 0.002$). Symbols indicate: * lower than 80 and 106 deg ($P \_ 0.011$); and †higher than 50 and 80 deg ($P \_ 0.020$). Data are means ± SD (n = 16).
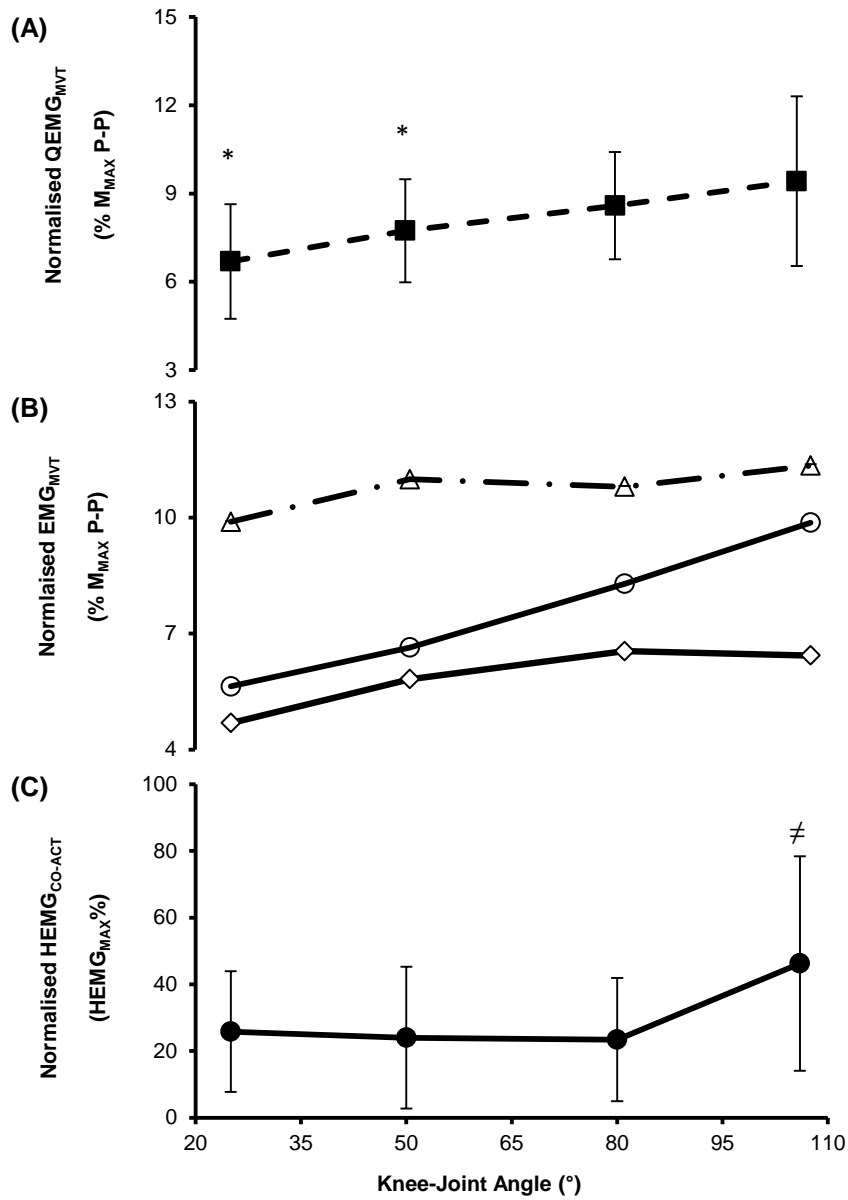
Voluntary activation (ITT)

Figure 5. Neuromuscular activation measured with the interpolated twitch technique (ACT_{ITT}) during isometric maximal voluntary contractions at knee joint angles of 25°, 50°, 80° and 106°. ACT_{ITT} showed differences between angles ($P<0.001$). Symbols indicate: * higher than 25° and 50° ($P≤0.001$). Data are mean ± SD (n=16).
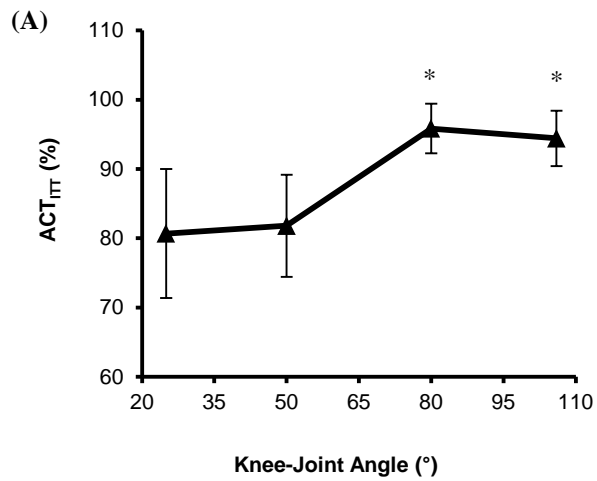
M_{MAX} P-P across angle positions

Figure 6. The supramaximal compound muscle action potential peak-to-peak amplitude (M_{Max} P-P) at different knee joint angles of 25°, 50°, 80° and 106°for: (A) Overall quadriceps (Q), which showed presented differences between angles (P<0.001); and (B) individual quadriceps muscles (VM, open diamond; RF open triangle; VL open circle). VM and VL showed differences between angle positions (P<0.01). Average standard deviation across angle positions are represented with the symbols at the right side of the figure. Symbols indicate: * higher than the other angles (P≤0.015), ≠ higher than 80° and 106° (P≤0.046), § higher than 106° (P≤0.002). Data are mean ± SD (n=16).