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Spinal contribution to neuromuscular recovery differs between elbow-flexor and knee-extensor muscles after a maximal sustained fatiguing task

Running title: Muscle-related differences in spinal excitability recovery

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ABSTRACT

Data from studies of elbow-flexor (EF) or knee-extensor (KE) muscles suggest that a fatigue-related decrease in motoneuron excitability only occurs in EF. It is unknown how motoneuron excitability changes after sustained fatiguing maximal voluntary isometric contractions (MVICs) in EF and KE in the same participants. In two sessions, eight healthy men performed a 2-min MVIC of EF or KE to induce fatigue with brief MVICs before and six times after the 2-min MVIC. Electromyographic responses elicited by corticospinal tract stimulation at the transmastoid [cervicomedullary motor-evoked potential (CMEP)] or thoracic [thoracic motor-evoked potential (TMEP)] level were recorded from EF and KE, respectively. To account for muscle excitability, CMEPs and TMEPs were normalized to maximal M-wave (M_{\max}) elicited by peripheral nerve stimulation during each brief MVIC. Immediately after the 2-min MVIC, *biceps brachii* and *brachioradialis* CMEP/ M_{\max} were 88% (SD 11%) ($P = 0.026$) and 87% (SD 12%) ($P = 0.029$) of pre-MVIC values, respectively, and remained lower than PRE after 5 s of recovery [91% (SD 8%), $P = 0.036$ and 87% (SD 13%), $P = 0.046$, respectively]. No subsequent time points differed from PRE (all $P \geq 0.253$). TMEP/ M_{\max} for *rectus femoris* and *vastus lateralis* were not different from PRE at any time during the recovery period (all $P > 0.050$). A different recovery pattern in motoneuron excitability occurred in EF as it recovered by 60 s whereas KE motoneurons were unaffected by the fatiguing task. The present findings may contribute to better understand muscle-specific neurophysiological differences in spinal excitability.

Key words: fatigue; inhibition; maximal voluntary contraction; motoneuron; spinal excitability

24 **NEW & NOTEWORTHY**

25 By comparing the changes in motoneuron excitability in elbow-flexor and knee-extensor
26 muscles after sustained fatiguing maximal voluntary contractions, this study shows that
27 motoneuron recovery behavior depends on the muscle performing the exercise. A different
28 recovery pattern in motoneuron excitability occurs in elbow flexors as it recovered by 60 s
29 whereas knee extensors were unaffected by fatigue. This finding can help to increase
30 understanding of the effect of a fatigue and subsequent recovery on neural processes.

INTRODUCTION

After a 2-min sustained maximal voluntary isometric contraction (MVIC), a classical model to study fatigue, approximately one-quarter of the reduction in maximal force can be attributed to processes within the central nervous system (Taylor et al. 2016). The central-mediated force loss is due to some combination of spinal and supraspinal mechanisms (Gandevia 2001), resulting in a suboptimal activation of muscle fibers and reduced discharge rate of the motoneuron pool (Bigland-Ritchie et al. 1983a, 1983b). This reduction depends on fatigue-induced changes in intrinsic properties of the motoneurons (Butler et al. 2003) and the sum of the multiple inputs received by the motoneurons (Hounsgaard 2017). When testing the excitability of motoneurons in humans, corticospinal tract stimulation provides the best available method to assess motoneuron excitability (Martin et al. 2008) because the corticospinal/motoneuron synapse is not modified by Ia presynaptic inhibition, unlike both the H-reflex and F-wave (McNeil et al. 2013). During 2-min MVICs, this technique has been conducted by means of non-invasive electrical stimulation of the descending spinal tracts either between the mastoid processes [for elbow-flexor muscles (EF)] (Butler et al. 2003; Martin et al. 2006b; McNeil et al. 2011; McNeil et al. 2009) or over the upper thoracic spine [for knee-extensor muscles (KE)] (Kennedy et al. 2016). Indeed, both activate corticospinal axons in the spinal cord (Ugawa et al. 1991) and evidence shows that descending spinal tracts are not subject to presynaptic inhibition (Nielsen and Petersen 1994). Corticospinal tract stimulation can evoke large, short-latency and predominantly monosynaptic responses in arm and leg muscles [cervicomedullary motor-evoked potentials (CMEPs) and thoracic motor-evoked potentials (TMEPs), respectively] (Taylor and Gandevia 2004). The consequent sizes of CMEPs and TMEPs reflect motoneuron excitability when normalized to the size of the maximal M-wave (Martin et al. 2008) and their reductions indicate decreased responsiveness of the motoneuron pool to descending input.

After a 2-min MVIC, the excitability of the motoneuron pool declined in EF [e.g. (Butler et al. 2003; Gandevia et al. 1999; McNeil et al. 2011; McNeil et al. 2009)] but not in KE (Kennedy et al. 2016). These results may occur due to different fatigue-related changes in intrinsic motoneuron properties (e.g. reduced efficacy of the motoneuronal synapse; or activity-dependent changes in corticospinal axons) and descending input (Temesi et al. 2019), all of which can affect motoneuron excitability (Taylor and Gandevia 2004). However, motoneuron excitability after sustained MVICs has been investigated while fatiguing only EF or KE, focusing on neurophysiological responses to the exercise model itself, rather than on muscle-specific physiological differences. Therefore, it cannot be assumed these studies' conclusions also apply when the same participants perform the same fatiguing model using both EF and KE. When the corticospinal responsiveness in EF and KE muscles of the same participants was tested during a 2-min MVIC, motoneuron excitability was reduced in EF, but not KE (Temesi et al. 2019). Therefore, sustained fatiguing isometric MVICs elicit different responses in motoneuron excitability in elbow-flexor and knee-extensor muscles of the same participants. However, the time course of recovery of motoneuron excitability was unreported.

After a 2-min MVIC, voluntary force declined to 42% and 30% of baseline for EF and KE, with a partial recovery over the first few minutes after exercise cessation (Vernillo et al. 2018). Furthermore, both the excitatory (motor-evoked potential) and inhibitory (silent period) responses elicited by transcranial magnetic stimulation of the corresponding motor cortical area returned to baseline values within 5 s for both EF and KE (Vernillo et al. 2018). This suggests that the full capacity of corticospinal outputs to appropriately drive motoneurons at maximal voluntary force-generating capacity may have recovered to control levels within a few seconds after contraction cessation. This does not preclude continued impairment of the excitability of the motoneuron pool from being a possible reason for prolonged impairment in the maximal voluntary force-generating capacity. However, whether time courses of recovery in the

motoneuron excitability in EF and KE of the same participants reflect that of the functional recovery in voluntary force has not yet been elucidated. A better understanding of the time course of recovery in motoneuron excitability in EF and KE muscles after fatiguing exercises may be relevant to better understand muscle-specific neurophysiological differences in spinal excitability and inhibition.

Therefore, this study investigated the effects of a 2-min MVIC on the time course of recovery in the excitability of the motoneuron pool of EF and KE in the same participants.

MATERIAL AND METHODS

Participants

Based on CMEP changes observed after a 2-min MVIC with or without ischemia in 8 participants (Butler et al. 2003), the mean effect size of the change in the main outcome (CMEP for BB) was 1.3. Using this value, an α [threshold probability for rejecting the null hypothesis (type I error)] at 0.05 and a β [probability of failing to reject the null hypothesis under the alternative hypothesis (type II error) at 0.2], a sample size of 7 participants was considered sufficient to detect meaningful changes. From 12 healthy males who participated in a series of investigations [see (Temesi et al. 2019; Vernillo et al. 2019; Vernillo et al. 2018) for further details], 4 of them chose not to participate in this study because they found spinal stimulations prohibitively painful during the familiarization sessions. Therefore, 8 participated in the sessions comprising this study. Of those tested, 1 participant was excluded from the analysis of the EF motoneuron pool due to difficulties in consistently eliciting CMEP responses. Therefore, results are reported for 8 participants (age: 32 ± 10 years; height: 180 ± 7 cm; body mass: 75 ± 9 kg) for KE and 7 participants (age: 33 ± 11 years; height: 179 ± 7 cm; body mass: 74 ± 9 kg) for EF. Participants were instructed to avoid the consumption of caffeine on the day of the experiment and avoid performing any strenuous exercise during the 48 h prior to testing.

The experimental protocol was approved by the University of Calgary Conjoint Health Research Ethics Board (#REB14-1625). All participants gave written informed consent.

Experimental protocol

Each participant completed one familiarization session and two experimental sessions. During the familiarization session, participants performed maximal and submaximal voluntary isometric contractions of EF and KE with and without electrical spinal and peripheral nerve stimulation. The two experimental sessions were performed in a pseudo-randomized and counter-balanced order and consisted of either a 2-min EF or KE MVIC with spinal and peripheral stimulation. Sessions were separated by between 3 and 7 days and each participant performed all tests at the same time of day to control for within-participant diurnal variation. Participants were highly motivated and instructed to perform at maximal effort until asked to relax. During the 2-min MVICs, participants received continuous visual feedback and were strongly encouraged throughout the experiments by the investigators.

Neuromuscular testing protocol

Two to 3 min before each 2-min MVIC (PRE), the neuromuscular testing protocol consisted of two brief 2-3 s MVICs (separated by 60 s) with spinal and peripheral stimulation (see Neuromuscular function evaluation section). As an estimate of true MVIC force, we compared the peak forces of the two MVICs before exercise by means of a real-time display of MVIC values on a computer screen. Peak force from the second brief MVIC was always within 5% of peak force from the first brief MVIC for all participants. The neuromuscular function evaluation consisted of a brief 2-3 s MVIC with visual feedback of the force produced provided to the participants. At the end of the 2-min MVIC, participants were not permitted to relax and they were required to continue their maximal effort for the first assessment post 2-min MVIC.

(POSTimm). Additional brief MVICs were performed 5 s after relaxation (POSTrelax) and 1 (POST 1), 2 (POST 2), 4 (POST 4), and 8 (POST 8) min after the end of the 2-min MVIC (Fig. 1).

****Figure 1 near here****

Force and electromyography recordings

EF force was assessed by a calibrated force transducer (2712-200 daN, Sensy, Jumet, Belgium). Participants sat upright in a chair with their right arm in a custom-built dynamometer. Both shoulder and elbow joints were at 90°, with the forearm in a supinated position. A noncompliant strap secured the wrist to the dynamometer.

KE force was measured by a calibrated force transducer (LC101-2K; Omegadyne, Sunbury, OH). Participants sat upright in a custom-built chair with the hips and right knee at 90° of flexion. A noncompliant strap secured the leg immediately proximal to the malleoli to the dynamometer.

EMG of EF [*biceps brachii* (BB) and *brachioradialis* (BR)] and KE [*rectus femoris* (RF) and *vastus lateralis* (VL)] was recorded with pairs of self-adhesive surface electrodes (10-mm recording diameter, Meditrace 100; Covidien, Mansfield, MA) in bipolar configuration with a 30-mm interelectrode distance and the reference on the medial epicondyle of the *humerus* (for EF) or the patella (for KE). Placement of EMG electrodes for BB was on the line between the medial acromion and the cubital fossa at 1/3 the distance from the cubital fossa (Hermens et al. 2000) and placement for BR was over the muscle midbelly (Martin et al. 2006a). Placement of EMG electrodes for RF was between the anterior superior iliac spine and the superior border of the patella, on the distal portion of the muscle belly (Botter et al. 2011) while for VL, electrodes were placed between the apex of the greater trochanter and the

superolateral border of the patella, on the distal portion of the muscle belly (Botter et al. 2011). A low impedance ($<5\text{ k}\Omega$) between electrodes was obtained by shaving and gently abrading the skin and then cleaning it with isopropyl alcohol. Force and EMG signals were converted from analog-to-digital at a sampling rate of 2000 Hz by PowerLab system (16/35, ADInstruments, Bella Vista, Australia) and octal bioamplifier (ML138; ADInstruments; common mode rejection ratio = 85 dB, gain = 500) with band pass filter (5-500 Hz) and analyzed offline using Labchart 8 software (ADInstruments).

Spinal stimulation

The corticospinal tract was stimulated with single electrical stimuli of 500- μ s duration via a constant-current stimulator (DS7A; Digitimer, Welwyn Garden City, Hertfordshire, UK). For BB and BR, CMEP responses were evoked by electrical stimulation at the transmatoid level during voluntary contractions of EF. The electrical stimulus passed between two electrodes of 10-mm diameter (Meditrace 100) fixed to the skin over the left (cathode) and right (anode) mastoid processes (Ugawa et al. 1991). For RF and VL, TMEP responses were evoked by electrical stimulation of the descending corticospinal tract at the upper-thoracic level during voluntary contractions of KE. The electrical stimulus passed between two electrodes of 10-mm diameter (Meditrace 100) fixed over the thoracic spine. The cathode was placed between the spinal processes of T3-T4 vertebrae and the anode ~5-10 cm above, but below the C7 vertebra (Kennedy et al. 2016). BB and RF were the main muscles of interest and stimulation intensity was determined for these muscles. The stimulus intensity was determined during brief voluntary isometric contractions at 50% MVIC and increased until the amplitude of BB CMEPs and RF TMEPs (normalized to the corresponding M_{\max}) matched approximately 50% of M_{\max} amplitude, since this was conducted as part of previously reported sessions (Temesi et al. 2019).

The stimulus intensity was verified from the mean amplitude of 4 CMEPs or TMEPs. Mean stimulus intensities were 151 ± 44 mA and 578 ± 125 mA in EF and KE, respectively. Raw traces showing CMEPs and TMEPs before and after the 2-min MVIC and during the recovery period are displayed for a single participant in Fig. 2.

****Figure 2 near here****

Peripheral stimulation

To evoke maximal M-wave (M_{\max}) in BB, BR, RF and VL, single electrical stimuli of 200- μ s duration were delivered via a constant-current stimulator (DS7AH, Digitimer). For BB and BR, stimuli were delivered to the brachial plexus trunk at Erb's point with a cathode (Meditrace 100) in the supraclavicular fossa and a 50×90 mm rectangular anode (Durastick Plus; DJO Global, Vista, CA) on the acromion. For RF and VL, stimuli were delivered to the femoral nerve trunk via a cathode taped into the femoral triangle (Meditrace 100) and a 50×90 mm rectangular anode (Durastick Plus) in the gluteal fold. During peripheral nerve stimulation of both the brachial plexus and the femoral nerve trunk, a small gauze ball was placed over the cathodes before securing it with tape in order to apply pressure over the stimulation site. Single stimuli were delivered incrementally in the relaxed muscle state until M_{\max} and twitch amplitudes plateaued. A stimulus intensity of 130% of the intensity to elicit M_{\max} and maximal twitch responses was used throughout the rest of the experiment. The supramaximal stimulus intensity was 153 ± 95 mA for EF and 158 ± 50 mA for KE. Raw traces showing M_{\max} before and after the 2-min MVIC and during the recovery period are displayed for a single participant in Fig. 2.

Neuromuscular function evaluation

The neuromuscular function evaluation consisted of a brief 2-3 s MVIC with visual feedback of the force produced provided to the participants by means of a real-time display on a computer screen. The participants contracted to maximal force and once maximal force was attained, stimulation of the spinal tract was delivered. Once the participant returned to maximal force after the induced silent period, peripheral stimulation was delivered. To avoid possible contamination of the EMG signal by stimulation of either the spinal tract or peripheral nerves, participants were instructed to avoid inadvertent contractions in anticipation of the stimulus. They were also instructed to avoid inadvertent changes in head position that may have changed the CMEP responses since changes in CMEP size may occur due to movement of the electrodes relative to the point of stimulation (Taylor and Gandevia 2004).

Data Analysis

Force values were measured for the duration of the 2-min MVIC and for the brief 2-3 s MVICs constituting the neuromuscular testing protocol. During the 2-min MVIC, force was measured for each successive 5-s window for the entire duration of the fatiguing contraction. During the brief 2-3 s MVICs, mean force was measured over the 500 ms before spinal electrical stimulation.

Area values for M_{\max} , CMEPs and TMEPs were measured between cursors marking the initial deflection from the baseline to the second crossing of the horizontal axis (Martin et al. 2006a). The durations of the silent period after spinal electrical stimulation (SP_{CMEP} and SP_{TMEP}) were measured by visually inspecting the interval from the stimulus to the return of continuous voluntary EMG (Taylor et al. 1996). To account for any changes in the compound muscle action potential, CMEPs and TMEPs were normalized to M_{\max} values (CMEP/M_{\max} or TMEP/M_{\max} , respectively) recorded during the same contraction. All data during the post 2-min MVIC contractions were normalized as a percentage of the PRE evaluation except for

force values during the 2-min MVIC for which force data were normalized as a percentage of the PRE evaluation and averaged in 5-s time windows.

Statistical analysis

Results are given as means (SD). To test differences between PRE and POSTimm, as well as during the recovery time, the longitudinal analysis (muscle group \times time for force and muscle \times time for EMG parameters) was performed using generalized estimating equations (GEE; i.e. GEE under ‘Generalized Linear Model’ procedure in SPSS v. 26) to take into account the unbalanced nature of the measurements ($n = 7$ for EF session and $n = 8$ for KE session) (Liang and Zeger 1986). Furthermore, GEE was used to take into account the correlated nature of observations within each participant (i.e. within-participant measurements) (Twisk 2013). GEE is considered to be robust against the choice of an incorrect correlation structure (Liang and Zeger 1986). When significant main effects or interactions were observed, Bonferroni’s test was used for *post-hoc* analysis. As a measure of effect size, Cohen’s d (d) was calculated with 95% confidence intervals (CI). Values of 0.2, 0.5, and above 0.8 were considered *small*, *medium*, and *large*, respectively (Cohen 1988). Statistical analysis was conducted using IBMTM SPSSTM Statistics (version 26, IBM Corp., Somers, New York, NY). Statistical significance was set at $\alpha < 0.050$.

RESULTS

Table 1 presents values before the 2-min MVIC for maximal voluntary force, M_{\max} area, CMEP/ M_{\max} for both BB and BR, TMEP/ M_{\max} for both RF and VL, SP_{CMEP} for both BB and BR, SP_{TMEP} for both RF and VL,

****Table 1 near here****

Force

Mean force profiles for each 5-s window during the 2-min MVICs for both EF and KE are presented in Figure 3 (Panel A). Force profiles during the 2-min MVICs showed a time effect [$\chi^2(8) = 2.941\text{E}+14$, $P < 0.001$], a muscle group effect [$\chi^2(1) = 8.978$, $P = 0.003$], and muscle group \times time interaction [$\chi^2(8) = 1.403\text{E}+14$, $P < 0.001$]. The force decreased in a comparable manner until 30 s. Then the difference in force between EF and KE became visually appreciable from 35 s and this difference reached significance at 65 s when EF was 68% (SD 12%) of PRE and KE force was 59% (SD 11%) of PRE [$P = 0.011$, $d = 0.8$ (95% CI -0.3-1.8)]. KE force remained significantly lower than EF until the end of the sustained MVICs [mean normalized difference of PRE MVIC of 12% (SD 3%) from 65 s to 120 s]. Force values at the end of the 2-min MVICs were 32% (SD 7%) [$P < 0.001$, $d = 13.7$ (95% CI 7.9-17.6)] and 23% (SD 5%) [$P < 0.001$, $d = 21.8$ (95% CI 13.4-27.8)] of PRE for EF and KE, respectively, being also lower than those observed at POSTimm (both $P < 0.001$, see below).

Figure 3 (Panel B) shows the MVIC force immediately after the 2-min contractions and during recovery. MVIC force showed a time effect [$\chi^2(6) = 222157.0$, $P < 0.001$] and muscle group \times time interaction [$\chi^2(6) = 420.3$, $P < 0.001$], but not a muscle group effect [$\chi^2(1) = 0.416$, $P = 0.519$]. MVIC force at POSTimm was 48% (SD 5%) [$P < 0.001$, $d = 14.7$ (95% CI 8.5-18.8)] and 31% (SD 3%) [$P < 0.001$, $d = 32.5$ (95% CI 20.0-41.4)] of PRE values for EF and KE, respectively. Then MVIC force remained lower than PRE values through POST 2 for both EF [81% (SD 9%) of PRE values, $P = 0.042$, $d = 3.0$ (95% CI 1.3-4.2)] and KE [76% (SD 17%) of PRE values, $P = 0.030$, $d = 2.0$ (95% CI 0.7-3.1)], but had recovered by POST 4 [89% (SD 9%), $P = 0.405$, and 84% (SD 15%), $P = 0.917$, of PRE values for EF and KE, respectively]. The decrease in MVIC force was greater in KE than EF only at POSTimm [by 17%, $P < 0.001$, $d = 4.2$ (95% CI 2.2-5.7)].

****Figure 3 near here****

Peripheral stimulation

M_{\max} results are presented in Figure 4. A time effect [χ^2 (6) = 841.7, $P < 0.001$], muscle effect [χ^2 (3) = 14.9, $P = 0.002$], and muscle \times time interaction [χ^2 (7) = 60.9, $P < 0.001$] were observed.

At POSTimm, M_{\max} for BB increased to 150% (SD 46%) [$P = 0.035$, $d = 1.5$ (95% CI 0.3-2.7)] of PRE values, while no subsequent time points were different from PRE (all $P \geq 0.129$).

M_{\max} for BR increased to 189% (SD 41%) [$P < 0.001$, $d = 3.1$ (95% CI 1.4-4.4)] of PRE values at POSTimm. Then M_{\max} for BR remained greater than PRE values through POST 2 [134% (SD 21%) of PRE values, $P < 0.001$, $d = 2.3$ (95% CI 0.8-3.4)], while no subsequent time points were different from PRE ($P = 0.390$).

At POSTimm, M_{\max} for RF increased to 126% (SD 14%) [$P < 0.001$, $d = 2.6$ (95% CI 1.2-3.8)] of PRE values. Then M_{\max} remained greater than PRE values through POST 1 [129% (SD 21%) of PRE values, $P = 0.002$, $d = 1.9$ (95% CI 0.7-3.0 while no subsequent time points were different from PRE (all $P = 1.000$).

M_{\max} for VL increased to 143% (SD 40%) [$P = 0.022$, $d = 1.5$ (95% CI 0.3-2.5)] of PRE values at POSTimm. Then M_{\max} remained greater than PRE values through POST 1 [118% (SD 19%) of PRE values, $P = 0.001$, $d = 1.3$ (95% CI 0.2-2.3)], while no subsequent time points were different from PRE ($P \geq 0.119$).

At POSTimm, the increase in M_{\max} as a percentage change of PRE values was similar between BB, BR, RF and VL (all $P \geq 0.184$).

Spinal stimulation

CMEP/M_{max} for both BB and BR, as well as TMEP/M_{max} for both RF and VL are presented in Figure 4. A time effect [$\chi^2(6) = 24.5, P < 0.001$] and muscle \times time interaction [$\chi^2(7) = 105.5, P < 0.001$], but not a muscle effect [$\chi^2(3) = 2.1, P = 0.543$], were observed.

At POSTimm, CMEP/M_{max} for BB decreased to 88% (SD 11%) of PRE values [$P = 0.026, d = 1.5$ (95% CI 0.3-2.6)]. Then CMEP/M_{max} remained lower than PRE at POSTrelax [91% (SD 8%) of PRE values, $P = 0.036, d = 1.6$ (95% CI 0.3-2.7)] while no subsequent time points were significantly different from PRE (all $P \geq 0.253$).

CMEP/M_{max} for BR decreased to 87% (SD 12%) [$P = 0.029, d = 1.5$ (95% CI 0.3-2.6)] of PRE values at POSTimm. Then CMEP/M_{max} remained lower than PRE at POSTrelax [87% (SD 13%) of PRE values, $P = 0.046, d = 1.4$ (95% CI 0.2-2.5)] while no subsequent time points were different from PRE (all $P = 1.000$).

TMEP/M_{max} for RF was not different from PRE at POSTimm [104% (SD 9%) of PRE values, $P = 1.000, d = 0.6$ (95% CI -0.4-1.6)] or at any time during the recovery period (all $P = 1.000$).

TMEP/M_{max} for VL was not different from PRE at POSTimm [105% (SD 10%) of PRE values, $P = 1.000, d = 0.7$ (95% CI -0.3-1.7)] or at any time during the recovery period (all $P = 1.000$).

At POSTimm, the decrease in CMEP/M_{max} for BB as a percentage of PRE values was 16% and 17% greater than that in TMEP/M_{max} for RF [$P = 0.046, d = 1.6$ (95% CI 0.4-2.7)] and VL [$P < 0.001, d = 1.6$ (95% CI 0.4-2.7)], respectively. Similarly, the decrease in CMEP/M_{max} for BR was 17% and 18% greater than that in TMEP/M_{max} for RF [$P = 0.032, d = 1.5$ (95% CI 0.4-2.7)] and VL [$P = 0.008, d = 1.6$ (95% CI 0.4-2.7)], respectively.

****Figure 4 near here****

SP_{CMEP} for both BB and BR, as well as SP_{TMEP} for both RF and VL are presented in Figure 5. SP showed a time effect [χ^2 (6) = 479.4, $P < 0.001$] and muscle \times time interaction [χ^2 (7) = 105.0, $P < 0.001$], but not a muscle effect [χ^2 (3) = 2.3, $P = 0.513$].

At POSTimm, SP_{CMEP} for BB increased to 144% (SD 20%) [$P < 0.001$, $d = 3.1$ (95% CI 1.4-4.4)] while no other time points were different from PRE (all $P \geq 0.249$).

SP_{CMEP} for BR increased to 148% (SD 12%) [$P < 0.001$, $d = 5.7$ (95% CI 3.1-7.5)] of PRE values at POSTimm. Then SP_{CMEP} for BR remained greater than PRE at POSTrelax [125% (SD 13%) of PRE values, $P < 0.001$, $d = 2.7$ (95% CI 1.1-3.9)] while no subsequent time points were different from PRE (all $P = 1.000$).

At POSTimm, SP_{TMEP} for RF increased to 153% (SD 28%) [$P < 0.001$, $d = 2.7$ (95% CI 1.2-3.8)] of PRE values. SP_{TMEP} for RF remained greater than PRE through POST 1 [116% (SD 13%) of PRE values, $P = 0.008$, $d = 1.7$ (95% CI 0.5-2.8)] while no subsequent time points were different from PRE (all $P = 1.000$).

SP_{TMEP} for VL increased to 148% (SD 17%) [$P < 0.001$, $d = 4.0$ (95% CI 2.1-5.4)] of PRE values at POSTimm. Then SP_{TMEP} for VL remained greater than PRE through POST 1 [113% (SD 11%) of PRE values, $P = 0.018$, $d = 1.7$ (95% CI 0.5-2.7)] while no subsequent time points were different from PRE (all $P \geq 0.447$).

At POSTimm, the increase in SP as a percentage change of PRE values was similar between BB, BR, RF and VL (all $P = 1.000$).

****Figure 5 near here****

DISCUSSION

Despite a similar and gradual recovery of voluntary force for both elbow-flexor and knee-extensor muscles after a sustained maximal isometric voluntary contraction, the present study showed that time courses of recovery in the motoneuron excitability of the two muscle groups in the same participants differs, i.e. it did not reflect the functional recovery in maximal voluntary force. Therefore, this study is the first to describe that responses at the motoneuron level recovered differently in elbow-flexor and knee-extensor muscles after an intense fatiguing task in the same participants. Specifically, only the excitability of the motoneuron pool of *biceps brachii* and *brachioradialis* was reduced and responses to corticospinal tract stimulation for *biceps brachii* and *brachioradialis* required 5 to 60 s to return to pre-exercise levels.

Motoneuron excitability and fatigue

Compared with baseline, maximal force decreased by 69% in KE and by 52% in EF when assessed immediately after the 2-min MVIC (i.e. POSTimm). This observation is in line with previous studies (Goodall et al. 2009; Kennedy et al. 2016; McNeil et al. 2009; Vernillo et al. 2018) and confirms the fatiguing nature of the 2-min MVIC. Furthermore, although MVIC force declined at the end of the 2-min MVIC for both EF and KE, M_{\max} of BB, BR, RF and VL increased in size as previously observed after a 2-min EF (Butler et al. 2003; Gandevia et al. 1999; Vernillo et al. 2018) or KE (Vernillo et al. 2018) MVICs. Although the neurophysiological mechanisms of the increased M_{\max} following a sustained maximal isometric contraction remain unclear, our result suggests that excitation had not failed, at least not at the sarcolemmal level.

During the brief MVIC performed as an extension of the 2-min MVIC, CMEP/ M_{\max} for BB was smaller compared to the PRE values. This decrease is consistent with previous studies examining responses of motoneuron pools of BB to corticospinal stimulation at the end of 2-

380 min MVICs either by means of conditioned [i.e. the corticospinal stimulation was delivered in
381 the silent period following a conditioning transcranial magnetic stimulation pulse (McNeil et
382 al. 2011; McNeil et al. 2009)] or unconditioned [i.e. when the corticospinal stimulation was
383 delivered in isolation (Butler et al. 2003; McNeil et al. 2009; Temesi et al. 2019)] CMEPs.
384 Evidence suggests the depression of the responses to the corticospinal tract stimulation may
385 reflect changes in the motoneurons, consequently becoming less excitable to a given input
386 (Butler et al. 2003; McNeil et al. 2009) as our group recently observed during a 2-min EF
387 MVIC (Temesi et al. 2019). The concomitant fatigue-induced lengthening of SP_{CMEP} may also
388 suggest a decrease in excitability of the motoneuron pool. However, we cannot completely rule
389 out the lengthening of SP_{CMEP} to a slowing of the conduction velocity of the repeatedly-
390 activated muscle fibers (see below) (Bigland-Ritchie et al. 1979; Mortimer et al. 1970). Several
391 possible mechanisms may have contributed to the decreased excitability of the motoneuron
392 pool. For instance, repetitive activation of motoneurons can lead to an insufficient release of
393 neurotransmitters, in particular monoamine neurotransmitters such as serotonin and
394 norepinephrine, from the synaptic vesicles, thus compromising synaptic efficacy (Heckman et
395 al. 2009). This level of neuromodulatory input to motoneurons has been suggested to account
396 for some of the decrease in motoneuronal excitability immediately after exercise (Gandevia et
397 al. 1999; Petersen et al. 2003). Besides intrinsic changes of the motoneuron properties with
398 repetitive activity and through neurotransmitters, the excitability of the motoneuron pool could
399 have also been modulated by afferent feedback. Synaptic input received by the motoneuron
400 during fatiguing contractions comprises concurrent increases in excitatory (i.e. descending
401 drive and muscle spindle) and inhibitory (i.e. group Ib, group III and IV and Renshaw cell)
402 afferent feedback (Taylor et al. 2016). The inhibitory influence of group Ib afferents (Golgi
403 tendon organs) and Renshaw cells should not have played a substantial role since a diminished
404 activity is generally observed with fatigue (Gandevia 2001). Furthermore, the excitatory

influence of muscle spindles is unlikely to have played a major role in reducing the excitability of the motoneuron pool with muscle fatigue since tendon vibration during a prolonged fatiguing muscle contraction showed no effects on conditioned CMEP size (McNeil et al. 2011). Conversely, an increased firing of group III and IV muscle afferents is a well-accepted explanation for the observed reduction in the excitability of the motoneuron pool (Taylor et al. 2016). Indeed, during a prolonged fatiguing muscle contraction, group III-IV afferents become increasingly excited (Butler et al. 2003), presumably mediating an increase in the motoneuronal afterhyperpolarization period which reduces the likelihood for neuronal discharge (Matthews 1999).

The size of TMEP/ M_{\max} for both RF and VL responses did not change at POSTimm, in agreement with Temesi et al. (Temesi et al. 2019) who showed that TMEP/ M_{\max} responses did not change from 5 to 115 s of a 2-min MVIC. Furthermore, as previously shown for VL (Kennedy et al. 2016), the present study observed that although TMEP/ M_{\max} responses did not change after the 2-min MVIC, SP_{TMEP} for both RF and VL increased in duration. While this may be seen as a potential indicator of decreasing motoneuron excitability, Kennedy et al. (Kennedy et al. 2016) argued that it may also owe to a slowing of the conduction velocity of the repetitively-activated muscle fibers, ultimately manifesting as increased TMEP duration (Bigland-Ritchie et al. 1979; Mortimer et al. 1970). Moreover, changes in voluntary descending drive can affect motoneuron excitability, likely creating a confounding interpretation of the results because measuring motoneuron excitability during changing levels of descending drive would result in the evoked response reflecting changes both in motoneuron excitability and level of the voluntary descending drive. Therefore, by only analyzing TMEP/ M_{\max} responses it can be hard to isolate the true contribution of spinal mechanisms (Finn et al. 2018). To control the ongoing descending drive on measures of motoneuron excitability, the technique elicits CMEPs or TMEPs during the silent period that follows a transcranial magnetic stimulation

pulse upon the motor cortex during a voluntary contraction (McNeil et al. 2009). The resultant CMEP or TMEP responses may better reflect the excitability of motoneurons when they are not being acted upon by descending drive and not actively firing. When this technique was used during a submaximal 10-min KE contraction at a constant level of integrated EMG (Finn et al. 2018), TMEP/ M_{\max} responses in RF were reduced. Future studies should employ the above mentioned technique to study changes at the level of the motoneurons for a KE sustained MVIC, as previously shown in EF (McNeil et al. 2009).

Motoneuron excitability during recovery

After a 2-min MVIC, the excitatory (motor-evoked potential) or inhibitory (silent period) responses elicited by transcranial magnetic stimulation of the corresponding motor cortical area quickly returned to baseline values for both EF and KE (Vernillo et al. 2018). Findings from the present study showed that only CMEP/ M_{\max} decreased at the end of the 2-min MVIC and remained lower than PRE 5 s after contraction cessation. Thus, only spinal motoneurons innervating EF became less responsive with fatigue. Moreover, CMEP/ M_{\max} returned to pre-exercise values by 1 min after contraction cessation (at POST 1), in line with a previous study that found that CMEP/ M_{\max} depression was evident when tested 2-5 s after a 2-min MVIC (Gandevia et al. 1999). Other studies performed the first post-exercise contractions either 15 s (Butler et al. 2003) or 30 s (McNeil et al. 2009) after the end of a 2-min MVIC, failing to observe a reduction from control values. Thus, motoneuron excitability in EF recovers rapidly after a 2-min MVIC, suggesting that the fatigue-related decrease in the motoneuron excitability could be underestimated if measured with any delay. This consideration is further reinforced by a recent study showing how post-fatigue assessments should be initiated immediately following task cessation because spinal mechanisms substantially recover within 30 s of recovery (Aboodarda et al. 2019).

Differences between extensor and flexor motoneuron pool

Rather than limb-specific differences in the behavior of motoneuron pool excitability, the observed results could reflect differences between flexor (i.e. BB and BR) and extensor (i.e. RF and VL) muscles. With activation of group III and IV afferents during 2-min MVICs of both the elbow extensors (Martin et al. 2006b) and EF (Butler et al. 2003), inhibition of the motoneuron pool has been observed (although smaller CMEP/ M_{\max} reflecting reduced intrinsic excitability due to repetitive activation cannot be ruled out). However, CMEP/ M_{\max} responses during the subsequent recovery period differed between elbow extensors and flexors under ischemic conditions. Indeed, CMEP/ M_{\max} elicited in the elbow extensors did not recover during the first 2 min of recovery (Martin et al. 2006b); whereas in EF, CMEP/ M_{\max} recovered within 15 s of the end of the sustained contraction (Butler et al. 2003). These observations suggest that the effects of group III and IV afferents differ among motoneuron pools. In the lower limbs, TMEP/ M_{\max} responses evoked in VL did not change after a 2-min MVIC (Kennedy et al. 2016). Similarly, in the present study, the excitability of the motoneuron pool of the extensor muscle (i.e. RF and VL) was maintained after the 2-min MVIC. Conversely, excitability of the motoneuron pool for the flexor muscle (i.e. BB and BR) decreased by ~12%. Given that inhibition of the motoneuron pool has been demonstrated in the proximal muscles of the upper limb [i.e. both elbow flexors (Butler et al. 2003) and extensors (Martin et al. 2006b)] but not in KE [i.e. VL (Kennedy et al. 2016) or RF and VL in the present study], there is insufficient evidence to suggest that the changes reported in the study may be due to functional (i.e. flexor *versus* extensor) muscle differences. Instead, the above-mentioned results could suggest that upper- *versus* lower-limb differences determined the behavior of motoneuron pool excitability and, therefore, a different balance of fatigue-related changes in the intrinsic motoneuron properties (as well as in sensory and descending input) of different limbs.

Limitations

Although our study provides evidence that fatigue and recovery of motoneuron excitability depends on the muscle performing the exercise in young men, women exhibit different fatigue characteristics than men (Hunter 2009) and are generally less fatigable than men for sustained isometric contractions (Hunter 2014). Nevertheless, recent evidence shows no effect of sex on motoneuron excitability after an isometric sustained contractions (Yacyshyn et al. 2018). Furthermore, healthy aging causes changes in the intrinsic properties of the motoneurons such that there is a decrease in both the number of motoneurons (Tomlinson and Irving 1977), the excitability of motoneurons (Kido et al. 2004), and the maximal firing rate of motor units (Kamen et al. 1995). Nevertheless, fatiguing intermittent maximal isometric KE contractions showed no effect on motoneuron excitability in older males (Weavil et al. 2016). However, whether the same results we observed apply to older males has yet to be determined. Consequently, we can only generalize our findings to young adults.

Conclusion

The present study is the first to show for the same participants that a diminished output from spinal motoneurons after a sustained maximal isometric exercise model occurs for the elbow-flexor but not the knee-extensor muscles. Specifically, while excitability of *rectus femoris* and *vastus lateralis* motoneurons was not altered by a fatiguing 2-min MVIC, reduced excitability of spinal motoneurons was observed in *biceps brachii* and *brachioradialis* with rapid recovery (within 60 s). Therefore, spinal contribution to neuromuscular fatigue and subsequent recovery may differ for elbow-flexor and knee-extensor muscles. The present findings may contribute to better understand muscle-specific neurophysiological differences in spinal excitability and inhibition. Indeed, elucidating the neurophysiological mechanisms underlying muscle-specific

adaptations in spinal excitability and inhibition can be important for interpreting alterations in the properties of the nervous system associated with aging and disease.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

JT and GYM conceived of and designed the research. GV, JT and MM performed the experiment. GV, JT, MM and RLK analyzed the data. GV, JT, MM, RLK, and GYM interpreted the data of the experiment. GV prepared the figures. GV and JT drafted the manuscript. GV, JT, MM, RLK, and GYM edited and revised the manuscript. GV, JT, MM, RLK, and GYM approved the final version of the manuscript.

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625

TABLE AND FIGURE CAPTIONS

Table 1. Participants' control values before the fatiguing contraction (i.e. 2-min maximal voluntary contraction). Data are presented as mean (standard deviation) and ranges and were recorded during brief (2-3 s) maximal voluntary contractions.

Figure 1. The fatigue protocol performed in two separate sessions for both elbow-flexor and knee-extensor muscles. Each protocol began with a neuromuscular function evaluation before (PRE) the fatiguing contraction [2-min sustained maximal voluntary isometric contraction (MVIC), represented by the black trapezoid). The neuromuscular function evaluation required participants to perform a brief (~2-3 s) MVIC (white bars). Once maximal force was attained, either transmastoid or thoracic stimulation was delivered. When the participant returned to maximal force after the silent period induced by the spinal stimulus, peripheral stimulation (i.e. femoral nerve or brachial plexus electrical stimulation) was delivered. At the end of the 2-min MVIC, the same neuromuscular function evaluation was performed as an extension of the 2-min MVIC (POSTimm) and additional evaluations were performed after 5 s of relaxation (POSTrelax) and 1 (POST 1), 2 (POST 2), 4 (POST 4) and 8 (POST 8) min after the end of the 2-min MVIC. Time 'zero' corresponds to the beginning of the recovery period.

Figure 2. Single-participant data of raw electromyographic (EMG) responses. Responses were evoked in the *biceps brachii* (Panel A) and *brachioradialis* (Panel B) by transmastoid stimulation (CMEP) and peripheral nerve stimulation to the brachial plexus trunk at Erb's point [M_{max} , (Panels E and F)]. Responses were also evoked in the *rectus femoris* (Panel C) and *vastus lateralis* (Panel D) by thoracic stimulation (TMEP) and peripheral nerve stimulation to the femoral nerve trunk [M_{max} , (Panels G and H)]. CMEP, TMEP and M_{max} are highlighted by the shaded areas. Stimuli were delivered at time 0 ms (represented by the continuous vertical

lines) before the 2-min MVIC (PRE), at the end of the 2-min MVIC (POSTimm), after 5 s of relaxation (POSTrelax), and 1 (POST 1), 2 (POST 2), 4 (POST 4) and 8 (POST 8) min after the end of the 2-min MVIC. Arrows indicate the time at which the silent period after CMEP and TMEP ended.

Figure 3. Panel A: Means and standard deviations of force values (as percentage of the PRE values) of the elbow flexors (EF) and knee extensors (KE) muscles during the 2-min sustained maximal voluntary isometric contraction (MVIC). Each point represents a 5-s window. Significant differences between EF and KE were observed during the second half of the 2-min MVIC (as indicated by the shaded area, $P < 0.05$). For differences within muscle relative to the PRE 2-min MVIC: ‡, $P < 0.001$. At sign (@) denotes within muscle differences between the end of the 2-min MVICs and POSTimm: $P < 0.05$. For differences between muscles within the same time-points: \$, $P < 0.001$. Panel B: Changes in force after the sustained 2-min MVIC for elbow flexors (EF, $n = 7$) and knee extensors (KE, $n = 8$). At the end of the 2-min MVIC a neuromuscular function evaluation was performed as an extension of the 2-min MVIC (POSTimm) and additional evaluations were performed after 5 s of relaxation (POSTrelax) and 1 (POST 1), 2 (POST 2), 4 (POST 4) and 8 (POST 8) min after the end of the 2-min MVIC. The shaded box indicates the sustained 2-min MVIC and time 'zero' corresponds to the beginning of the recovery period. Values are means and standard deviations and expressed as a percentage of the PRE evaluation. For differences between time-points within the same muscle *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$. For differences between muscles within the same time-points \$, $P < 0.001$.

Figure 4. Changes after the 2-min maximal voluntary isometric contraction (MVIC) in the maximal M-wave (M_{max}) and spinal motor-evoked potentials [either as cervicomedullary

motor-evoked potentials (CMEP/ M_{\max}) in *biceps brachii* and *brachioradialis* ($n = 7$), or as thoracic motor-evoked potentials (TMEP/ M_{\max}) in *rectus femoris* and *vastus lateralis* ($n = 8$)] normalized to M_{\max} . At the end of the 2-min MVIC a neuromuscular function evaluation was performed as an extension of the 2-min MVIC (POSTimm) and additional evaluations were performed after 5 s of relaxation (POSTrelax) and 1 (POST 1), 2 (POST 2), 4 (POST 4) and 8 (POST 8) min after the end of the 2-min MVIC. The shaded box indicates the sustained 2-min MVIC and time ‘zero’ corresponds to the beginning of the recovery period. Values are means and standard deviations and expressed as a percentage of the PRE evaluation. For differences between time-points within the same muscle: *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$. For differences between muscles within the same time-points: *biceps brachii* was different than *rectus femoris* and *vastus lateralis* ($\# < 0.05$); *brachioradialis* was different than *rectus femoris* ($\$ < 0.001$) and *vastus lateralis* ($\& < 0.01$).

Figure 5. Changes after the 2-min maximal voluntary isometric contraction (MVIC) in silent period duration after transmastoid stimulation delivered to either the *biceps brachii* or the *brachioradialis* ($n = 7$) and thoracic stimulation delivered to either the *rectus femoris* or the *vastus lateralis* ($n = 8$). At the end of the 2-min MVIC a neuromuscular function evaluation was performed as an extension of the 2-min MVIC (POSTimm) and additional evaluations were performed after 5 s of relaxation (POSTrelax) and 1 (POST 1), 2 (POST 2), 4 (POST 4) and 8 (POST 8) min after the end of the 2-min MVIC. The shaded box indicates the sustained 2-min MVIC and time ‘zero’ corresponds to the beginning of the recovery period. Values are means and standard deviations and expressed as a percentage of the PRE evaluation. For differences between time-points within the same muscle: *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$.

Variable	EF (<i>n</i> = 7)		KE (<i>n</i> = 8)	
MVC (N)	285 (SD 44)		590 (SD 85)	
	Range: 244-377		Range: 481-679	
	BB (<i>n</i> = 7)	BR (<i>n</i> = 7)	RF (<i>n</i> = 8)	VL (<i>n</i> = 8)
M _{max} area (mV·s)	0.095 (SD 0.023)	0.047 (SD 0.018)	0.034 (SD 0.019)	0.081 (SD 0.017)
	Range: 0.062-0.131	Range: 0.025-0.072	Range: 0.007-0.056	Range: 0.058-0.104
CMEP area (mV·s)	0.055 (SD 0.015)	0.032 (SD 0.022)		
	Range: 0.038-0.076	Range: 0.010-0.069		
TMEP area (mV·s)			0.026 (SD 0.014)	0.042 (SD 0.020)
			Range: 0.007-0.047	Range: 0.023-0.078
SP _{CMEP} (ms)	55 (SD 4)	55 (SD 6)		
	Range: 49-60	Range: 49-66		

SP_{TMEP} (ms)

57 (SD 4)

60 (SD 6)

Range: 52-63

Range: 50-68

EF, elbow flexors; KE, knee extensors; BB, *biceps brachii*; BR, *brachioradialis*; RF, *rectus femoris*; VL, *vastus lateralis*; MVC, isometric maximal voluntary contraction; M_{max}, maximal M-wave; CMEP, cervicomedullary motor-evoked potential; TMEP, thoracic motor-evoked potential; SP_{CMEP}, silent period after transmastoid electrical stimulation; SP_{TMEP}, silent period after thoracic electrical stimulation.









