J Appl Physiol 128: 785–794, 2020.
First published March 12, 2020; doi:10.1152/japplphysiol.00651.2019.

RESEARCH ARTICLE | *Translational Control of Muscle Mass*

Mechanisms of neuromuscular fatigue and recovery in unilateral versus bilateral maximal voluntary contractions

Jérôme Koral,[1,2] Dustin J. Oranchuk,[1,3] James G. Wrightson,[1] Rosie Twomey,[1] and Guillaume Y. Millet[1,2]

[1]*Human Performance Laboratory, Faculty of Kinesiology, University of Calgary, Calgary, Alberta, Canada;* [2]*Université de Lyon, Université Jean Monnet-Saint-Etienne, Laboratoire Interuniversitaire de Biologie de la Motricité, EA 7424, Saint-Etienne, France; and* [3]*Sports Performance Research Institute New Zealand, Auckland University of Technology, Auckland, New Zealand*

Submitted 16 September 2019; accepted in final form 9 March 2020

Koral J, Oranchuk DJ, Wrightson JG, Twomey R, Millet GY. Mechanisms of neuromuscular fatigue and recovery in unilateral versus bilateral maximal voluntary contractions. *J Appl Physiol* 128: 785–794, 2020. First published March 12, 2020; doi:10.1152/japplphysiol.00651.2019.—The aim of this study was to investigate differences in neuromuscular function and corticospinal excitability in response to sustained unilateral (UNIL) and bilateral (BIL) isometric maximal voluntary contraction (IMVC) of the knee extensors. Eleven men performed a 1-min sustained IMVC of the knee extensors with one or both legs. Central and peripheral measures of neuromuscular function and corticospinal excitability were assessed via surface electromyography (EMG), peripheral nerve stimulation, and transcranial magnetic stimulation before, immediately after, and during recovery from IMVC. IMVC force and root-mean-squared EMG decreased during the fatiguing 1-min IMVC, with a larger decrease in EMG during BIL. All neuromuscular function indexes decreased significantly after the IMVC ($P < 0.005$), but the magnitude of these decreases did not differ between conditions. Changes in corticospinal excitability (motor evoked potential) and inhibition (silent period) did not differ between conditions. In contrast to previous studies utilizing submaximal exercise, no more peripheral fatigue was found after UNIL vs. BIL conditions, even though central drive was lower after BIL 1-min IMVC. Corticospinal excitability and inhibition were not found to be different between UNIL and BIL conditions, in line with maximal voluntary activation.

NEW & NOTEWORTHY The present experiment used peripheral nerve stimulation and transcranial magnetic stimulations during a sustained isometric maximal voluntary contraction to investigate the influence of muscle mass on neuromuscular fatigue. Contrary to previous studies that used submaximal exercise, peripheral fatigue was not found to be greater in unilateral vs. bilateral knee extensions even though central drive was lower during bilateral contractions. Corticospinal excitability and inhibition were not found to be different between unilateral and bilateral conditions.

isometric maximal voluntary contraction; maximal voluntary activation; muscle mass; nerve stimulation; transcranial magnetic stimulation

INTRODUCTION

Neuromuscular fatigue is a multifactorial phenomenon that can occur in various sites along the pathway of force production, typically ascribed to "central" and "peripheral" factors. Central fatigue is characterized as a reduction of voluntary activation due to suboptimal output from the motor cortex (17) and/or a failure originating at the lower motor neurons (15). Peripheral fatigue, defined as a reduction in muscle force output in response to a given neural input, is due to changes occurring at or distal to the neuromuscular junction. To examine neuromuscular fatigue etiology, short-duration sustained isometric maximal voluntary contractions (IMVCs) can be utilized (e.g., Refs. 8, 9, 14, 18, 52). The magnitude and etiology of neuromuscular fatigue depend on many factors such as exercise intensity and duration, environmental conditions, fitness level, and mode of contraction.

Another factor known to influence neuromuscular fatigue is the amount of muscle mass involved. Rossman et al. (41) measured time to task failure in exercises involving a large (cycling) versus small (limited to the knee extensors, KE) amount of exercising muscle mass. They reported longer exercise time and greater peripheral fatigue in KE versus typical cycling exercise (41). Similarly, previous studies have compared performance and fatigue induced by unilateral versus bilateral maximal isometric contractions of the KE. Matkowski et al. (32) measured time to task failure during submaximal isometric contractions with one versus two legs and reported a longer time to task failure with the one-leg condition. Because the results of their first study could have been due to task specificity, Rossman et al. (42) compared time to exhaustion at 85% of peak workload obtained in a previous incremental test performed on a modified cycle ergometer in single- versus double-leg KE and found greater peripheral fatigue at exhaustion with one- versus two-leg KE. The finding that exercise time is reduced and the magnitude of peripheral fatigue is also lower when the amount of active muscle mass is increased suggests that afferent feedback from the periphery (mainly via group III and IV muscle afferents) to the central nervous system is also increased. That is, increased afferent feedback may decrease central drive, thereby limiting the development of peripheral fatigue under these circumstances (4, 17, 36, 37, 42). Behm et al. (6) reported that a bilateral knee extension may require greater trunk stabilization and thus increase the

Address for reprint requests and other correspondence: J. Koral, Laboratoire Interuniversitaire de Biologie de la Motricité, Campus Santé Innovations–IRMIS, 10 Rue de la Marandière, 42270 Saint Priest en Jarez, France (e-mail: jerome.koral@univ-st-etienne.fr).

muscle mass involved, contributing to a more rapid onset of fatigue than during a unilateral contraction.

The factors that lead to voluntary cessation of exercise are unclear and controversial. The psychobiological model (27, 28, 30, 46) states that sense of effort results from the corollary discharge associated with the central drive, explaining the alteration of ratings of perceived exertion (RPE) and performance when central drive is increased to compensate muscle fatigue (39). According to this model, the willingness to continue exercising versus the decision to stop is not influenced by afferent signals (10, 13, 28, 29, 45). Millet (33) proposed a different model (the flush model) that considers the perception of effort, environmental conditions, nociceptive information (feedback mechanisms), as well as feedforward mechanisms due to peripheral fatigue and spinal and/or supraspinal inhibition, since these alterations imply a higher central drive for a given power output. Other models, such as the central governor (35) and sensory tolerance limit (24), also emphasize a role for both afferent and efferent processes. This balance between the effects of descending central processes and afferent signals resulting from peripheral disturbances on exercise tolerance may explain why, compared with unilateral contractions, exercise time is reduced during bilateral contractions without similar concomitant alterations to peripheral function or with actually a lower level of peripheral fatigue reached at exhaustion (42). The (presumably) higher demands on central processes required to perform bilateral maximal contractions may result in a reduction in performance (i.e., a reduction in exercise time), which is primarily caused by a reduction in the systems involved in efferent central drive rather than by peripheral alterations to neuromuscular function. It could also help explain why a higher central drive in bilateral tasks may also partly explain the lower level of peripheral fatigue reached at exhaustion.

Although these previous studies help us to better understand how neuromuscular fatigue is influenced by active muscle mass, further work on the unilateral versus bilateral comparison in a well-controlled experimental design is needed. In particular, the use of transcranial magnetic stimulation (TMS) has been proven to be useful in understanding corticospinal excitability (motor evoked potential, MEP) and inhibition (silent period, SP) but to our knowledge has not been used in this context. Therefore, the aim of this study was to investigate differences in the magnitude and etiology of neuromuscular fatigue and the corticospinal responses to a sustained isometric maximal voluntary contraction (IMVC) of the knee extensors performed bilaterally (BIL) versus unilaterally (UNIL). On the basis of previous studies of exercising muscle mass, we hypothesized that the UNIL IMVC would result in a higher magnitude of peripheral fatigue whereas exercise in the BIL condition would induce a greater reduction in central drive. Because of the difference in central drive during the fatiguing exercise, we also hypothesized that MEP and SP changes would be different between the conditions.

METHODS

Participants

Eleven healthy men (28.8 ± 8.4 yr, 176.4 ± 7.8 cm, 75.5 ± 9.1 kg) voluntarily participated. Written and verbal explanation of the experimental protocol and associated risks was provided to all participants before written informed consent was obtained. The study was approved by the University of Calgary ethics board (REB 15-2432) according to the standards of the Declaration of Helsinki, with the exception of registration in a database. Participants were instructed to avoid the consumption of caffeine on the testing days and to refrain from performing any exhaustive exercise for 48 h before each testing session.

The sample size calculation was based on the results of Rossman et al. (42). The authors found a mean difference of 44% with standard deviation of 6% for the reduction of potentiated peak twitch (as an index of peripheral fatigue) between pre- and postexercise in the single-leg KE condition. The decrease in potentiated peak twitch was 33% with standard deviations of 7% for the double-leg KE condition. This gives a standardized effect size (dz) of 2. In case this effect size was inflated, we powered the study for an effect size 50% of this magnitude, i.e., $dz = 1$. For an α of 0.05 and a power of 80%, we calculated that 10 participants should be included. Taking into account an abandon rate of 10%, 11 participants needed to be included.

Experimental Design

Each participant completed one familiarization and two experimental trials. During the familiarization trial, participants were taught to perform maximal and submaximal voluntary isometric contractions of the KE with and without TMS and peripheral nerve stimulation (PNS). Participants were also instructed to recontract to the prestimulus force level as quickly as possible after TMS delivery (Fig. 1). The order of the experimental trials, *1*) UNIL 1-min IMVC and *2*) BIL 1-min IMVC, was counterbalanced. Experimental trials were separated by 3–5 days and took place at the same time of day (±1 h).

Neuromuscular Testing Protocol

Before each condition, participants performed two neuromuscular function evaluations, PRE1 and PRE2, separated by 3 min, with TMS and peripheral stimulation. At the end of each 1-min IMVC, participants were allowed to relax for 1 s before the neuromuscular function evaluation was performed (POST). Additional neuromuscular function evaluations were performed 1 min (R1), 2 min (R2), 4 min (R4), and 8 min (R8) after the 1-min IMVC (Fig. 1).

Force and Electromyography

Voluntary and evoked isometric contraction forces were recorded from the right limb of each participant with the knee and hip flexed at 90° from full extension. Each participant was secured to a custom-built isometric chair with noncompliant nylon straps across the hips and chest to minimize body movement. KE force was measured by a calibrated strain gauge (LC101-2K; Omegadyne, Sunbury, OH). A PowerLab (16/30-ML880/P; ADInstruments, Bella Vista, Australia) data acquisition system was set at a sampling rate of 2,000 Hz. The strain gauge was attached to the right leg immediately superior to the medial malleolus of the ankle joint. The investigators provided strong verbal encouragement, and custom software and macros (LabChart 8; ADInstruments Ltd., Oxford UK) provided live visual feedback. Force data were processed and analyzed off-line with LabChart 8 software (ADInstruments).

The electrical activity of right vastus lateralis was recorded with pairs of self-adhesive silver chloride surface electrodes (10-mm recording diameter, Meditrace 100; Covidien, Mansfield, MA) in bipolar configuration with a 20-mm interelectrode distance and the reference on the patella. To reach low impedance controlled by a digital multimeter (<5 kΩ), participants' skin was previously shaved, softly abraded, and cleaned with alcohol swabs. Force and EMG signals were analog-to-digitally converted at a sampling rate of 2,000 Hz by a PowerLab system (16/35; ADInstruments, Bella Vista, Australia) and octal bio-amplifier (ML138; ADInstruments; common-mode rejection ratio = 85 dB, gain = 500) with a band-pass filter (5–500 Hz) and analyzed off-line with LabChart 8 software (ADInstruments).

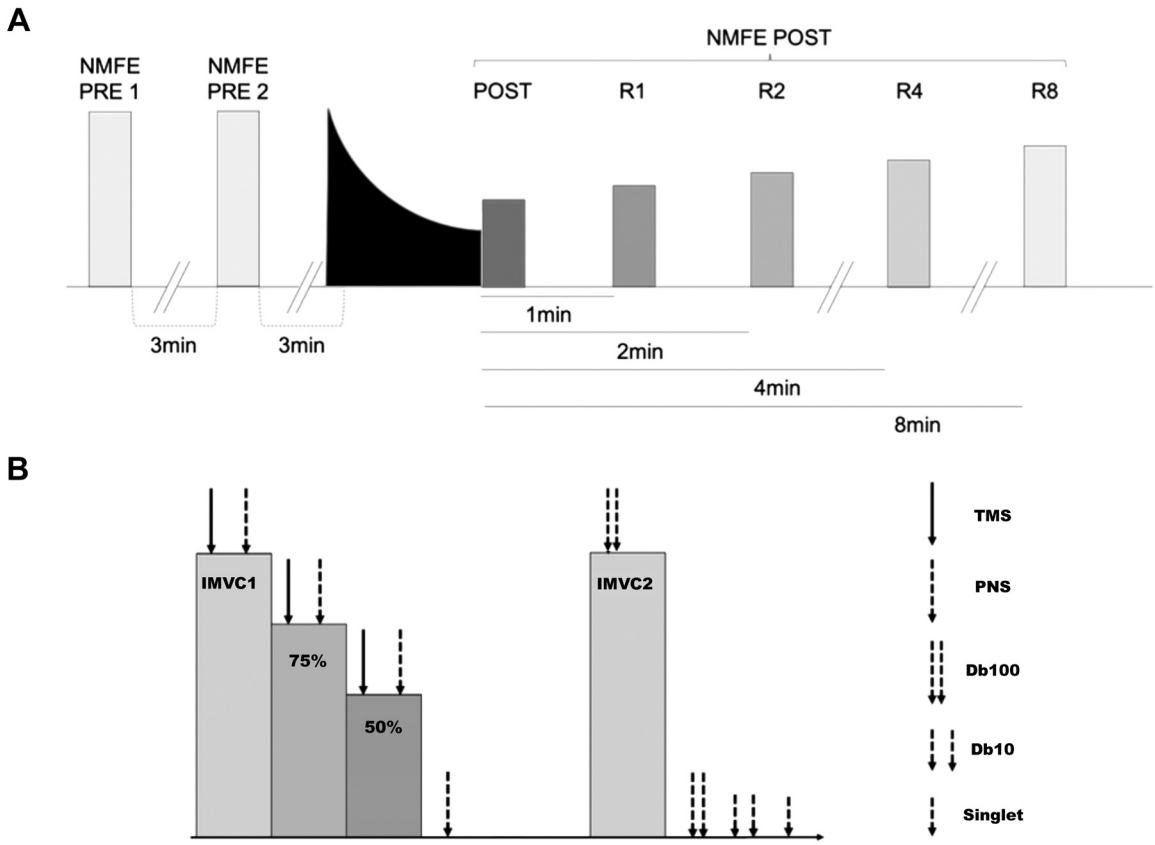

Fig. 1. *A*: knee extension neuromuscular function assessment protocol. NMFE, neuromuscular function evaluation. *B*: NMFE was utilized to assess central and peripheral fatigue. Solid arrow indicates transcranial magnetic stimulation (TMS), whereas dashed arrow indicates peripheral nerve stimulation (PNS) with doublets at 100 Hz (Db100) and at 10 Hz (Db10). Two NMFEs were performed before the 1-min isometric maximal voluntary contraction (IMVC) (PRE1 and PRE2), and additional NMFEs were used 1 min (R1), 2 min (R2), 4 min (R4), and 8 min (R8) after the end of the 1-min IMVC.

Peripheral Stimulation

The femoral nerve was percutaneously stimulated via a constant-current stimulator (DS7A; Digitimer, Welwyn Garden City, UK). The cathode electrode (10-mm, Meditrace 100; Covidien, Watford, UK) was carefully taped over the femoral nerve in the inguinal triangle, and the anode (50 × 90 mm, Durastick Plus; DJO Global, Vista, CA) was directly positioned above the gluteal fold. Participants sat and were asked to relax while single electrical stimuli were manually delivered, increasing in intensity until the M wave, and twitch amplitudes reached a stable state. To ensure maximal M-wave (M_{max}) area and maximal twitch responses throughout the session, 130% of this intensity was applied. The entire protocol was repeated at the beginning of each visit.

Transcranial Magnetic Stimulation

To elicit MEP and superimposed twitch (SIT) during voluntary contractions of KE, the participants' skull was covered by a Lycra swim cap on which the vertex was identified by drawing two lines: first, between nasion and inion and second, between preauricular points. Subsequently, a total of six points were materialized on the cap: three along the nasion-inion line, vertex and 1 cm and 2 cm posterior to the vertex, and three others parallel to the first three but over the left motor cortex separated by 1 cm from the nasion-inion line. To determine the optimal coil position, each of the aforementioned sites was stimulated by a magnetic stimulator (Magstim 2002; The Magstim Company Ltd, Whitland, UK) with a 110-mm concave double-cone coil (maximum output of 1.4 T) to induce a posteroanterior current. Participants were asked to perform a KE contraction at 20% IMVC, and when plateaued a single TMS pulse was manually delivered at 50% maximal stimulator output. The optimal coil position was considered the site where the largest vastus lateralis MEP was elicited. The optimal site was drawn on the swim cap and used throughout the session. The TMS intensity was determined from a stimulus-response curve constituted of four brief consecutive contractions at 50%, 60%, 70%, and 80% maximal stimulator output, in randomized order. The selected stimulus intensity was the lowest intensity eliciting maximal MEP amplitudes in vastus lateralis (with minimal antagonist responses) during brief voluntary contractions at 20% IMVC. TMS was always delivered once the participant had contracted to the appropriate force level and the force had stabilized during voluntary contractions. Participants wore a cervical collar during all TMS measures to stabilize the head and neck.

Neuromuscular Function Evaluation

Participants performed an IMVC, and once maximal force was attained TMS was delivered. Participants were instructed to recontract to the prestimulus force level as quickly as possible, and once they returned to maximal force peripheral stimulation was delivered. Guidelines at 75% IMVC and 50% IMVC were instantaneously displayed on the screen via custom-made macroinstruction to provide real-time visual feedback. Participants were asked to produce 75% and then 50% of maximal force; TMS and PNS were delivered at each force level. Immediately after complete relaxation, a single stimulus was applied to the femoral nerve. Participants rested for 5 s before performing another IMVC, during which a doublet at 100 Hz was delivered. Afterward, the relaxed muscle was stimulated by a high-

frequency doublet (100 Hz), a low-frequency doublet (10 Hz), and a single twitch.

The reliability was calculated as the coefficient of variation (CV) among the four PRE values (i.e., 2 in each condition). The average [±standard deviation (SD)] values of the CV were found to be 4 ± 3% for % MVC at 75% and 4 ± 2% for % MVC at 50%.

Data Analysis

Forces and electromyography. Peak forces measured during IMVC were calculated as the maximal values obtained before the stimuli. At PRE, the higher of the two IMVCs was chosen for further analysis. Throughout the 1-min IMVC, force-time integral (N·s) was calculated over twelve 5-s periods. Vastus lateralis electromyographic (EMG) activity was calculated as root mean square (RMS) over the same 5-s periods during the 1-min IMVC.

Transcranial magnetic stimulation. Modified twitch interpolation with TMS was used to assess voluntary activation during maximal effort (VA_{TMS}). Because the corticospinal excitability increases during voluntary contractions, the resting twitch was not measured directly but estimated as proposed by Todd et al. (50) and validated by Goodall et al. (21) for the KE muscles. Based on our previous work (34), we used a continuous method to assess VA_{TMS}. To allow VA_{TMS} calculation, linear regression between voluntary forces and SIT amplitude elicited by TMS at respectively 100%, 75%, and 50% IMVC was performed. Estimated resting twitch was extrapolated as the *y*-intercept of the regression and corresponds to the value at which voluntary force would be zero (19, 21, 43, 50). VA_{TMS} was then assessed with the conventional equation integrating superimposed twitch (SIT) and evoked resting twitch (ERT): VA_{TMS} (%) = (1 − SIT/ERT) × 100. MEP area was measured and normalized to M_{max} area during voluntary contractions at 100% (MEP_{100}), 75% (MEP_{75}), and 50% (MEP_{50}) IMVC. SPs were measured during voluntary contractions at 100% (SP_{100}), 75% (SP_{75}), and 50% (SP_{50}) IMVC. The duration of the SP was determined visually and defined as the duration from the stimulus to the return of continuous voluntary EMG.

Statistical analysis. All data are reported as means ± standard deviation (SD). All statistical tests were performed with SPSS version 25 (IBM Corporation, Chicago, IL). Data were checked for normality by visual inspection of Q-Q normality plots and descriptive statistics for skewness and kurtosis. Non-Gaussian data were log-transformed.

The effect of condition on force and EMG RMS during the 60-s IMVC was analyzed with a two-way repeated-measures analysis of variance (ANOVA) [condition (UNIL, BIL) × time (5-s intervals)]. Data were checked for assumptions of sphericity with Mauchly's test. PRE to POST 60-s IMVC changes in IMVC peak force, peripheral measures of muscle function [force produced by low-frequency (Db_{10}) and high-frequency (Db_{100}) doublets, peak twitch], and TMS measures (VA_{TMS}, MEP_{100}, MEP_{75}, MEP_{50}, SP_{100}, SP_{75}, or SP_{50}) were compared between trials with a two-way repeated-measures ANOVA [condition (UNIL, BIL) × time (PRE, POST)]. Main and interaction effects were followed up with Bonferroni corrected post hoc pairwise comparisons. Subsequently, the relative (%) change from PRE for each of the post 60-s IMVC time points (POST, R1, R2, R4, R8) was calculated as (POST − PRE)/POST × 100. The effect of condition on the recovery was analyzed with a two-way repeated-measures ANOVA [condition (UNIL, BIL) × time (% change for POST, R1, R2, R4, R8)] followed up with Bonferroni corrected post hoc pairwise comparisons. The threshold to reject the null hypothesis was set at $P < 0.05$.

RESULTS

During Exercise

A significant time effect was observed for both force (UNIL −52 ± 3% vs. BIL −52 ± 15%; $F_{11,110} = 64.9$, $P < 0.001$, $\eta_p^2 = 0.866$) and RMS EMG (UNIL −55 ± 6% vs. BIL −37 ± 60%; $F_{12,120} = 12.8$, $P < 0.001$, $\eta_p^2 = 0.572$) during the 1-min IMVC (Fig. 2). No condition effect or condition × time interaction ($F_{11,110} = 1.0$, $P = 0.434$, $\eta_p^2 = 0.093$) was found for force (Fig. 2*A*). No condition effect ($F_{11,110} = 0.2$, $P = 0.707$, $\eta_p^2 = 0.015$) was observed for the RMS EMG, but a significant condition × time interaction ($F_{12,120} = 2.3$, $P = 0.010$, $\eta_p^2 = 0.190$) was found (Fig. 2*B*). Pairwise comparisons showed that RMS amplitude was higher in BIL compared with UNIL at 10 s (+24%, $P < 0.05$) and 15 s (+35%, $P < 0.01$).

Pre- vs. Postexercise

Force responses. All force parameters showed a significant decrease with time ($P < 0.005$), and none showed a condition effect. A significant condition × time interaction ($F_{1,10} = 8.8$, $P = 0.014$, $\eta_p^2 = 0.468$) was observed for IMVC force. As illustrated in Fig. 3*A*, pairwise comparisons showed no statistical difference between UNIL and BIL at PRE ($P = 0.988$) but UNIL was significantly lower than BIL at POST (−6%, $P = 0.030$). VA_{TMS} (Fig. 3*B*) showed a significant time effect ($P < 0.001$, $\eta_p^2 = 0.855$) where both UNIL and BIL were lower at POST (−23 ± 13% vs. −23 ± 17%, respectively); however, no condition effect ($P = 0.998$, $\eta_p^2 < 0.001$) and no condition × time interaction effect ($P = 0.836$, $\eta_p^2 = 0.006$) were detected.

Corticospinal excitability and inhibition. No significant time effect and condition × time interaction were found for MEP_{100} and M_{max} (M_{max} and peak twitch values are presented in Table 1). MEP_{75} showed a significant condition effect ($F_{1,10} = 5.1$, $P = 0.048$, $\eta_p^2 = 0.337$) where BIL was higher than UNIL, but no time effect ($F_{1,10} = 0.3$, $P = 0.599$, $\eta_p^2 = 0.029$) or condition × time interaction ($F_{1,10} < 0.1$, $P = 0.886$, $\eta_p^2 = 0.002$, Fig. 4*B*) was present. On the contrary, MEP_{50} showed a significant time effect ($F_{1,10} = 14.7$, $P = 0.003$, $\eta_p^2 = 0.595$) with a decrease of both UNIL (−5 ± 26%) and BIL (−13 ± 30%) at POST but no condition × time interaction ($F_{1,10} < 0.1$, $P = 0.312$, $\eta_p^2 = 0.102$, Fig. 4*C*).

SP_{100}, SP_{75}, and SP_{50} showed a time effect where SPs were significantly longer (SP_{100} and SP_{50}, $P < 0.001$, $\eta_p^2 = 0.698$; SP_{75}, $P < 0.01$, $\eta_p^2 = 0.622$) at POST compared with PRE (UNIL SP_{100} +47 ± 38%, SP_{75} +28 ± 30%, SP_{50} +29 ± 26% and BIL SP_{100} +56 ± 38%, SP_{75} +31 ± 24%, SP_{50} +33 ± 26%). There was no effect of condition and no condition × time interaction (Fig. 5).

Recovery

All force and EMG parameters, except for MEP_{100} and MEP_{75}, showed a significant time effect ($P < 0.005$), increasing during recovery compared with POST, but neither condition nor condition × time interaction was significant. MEP_{100} and MEP_{75} did not show any significant effects (Fig. 4).

DISCUSSION

The aim of the present study was to elucidate the influence of muscle mass on central and peripheral fatigue and corticospinal excitability and inhibition with sustained unilateral and bilateral IMVC of the KE. In contrast to our hypothesis, and

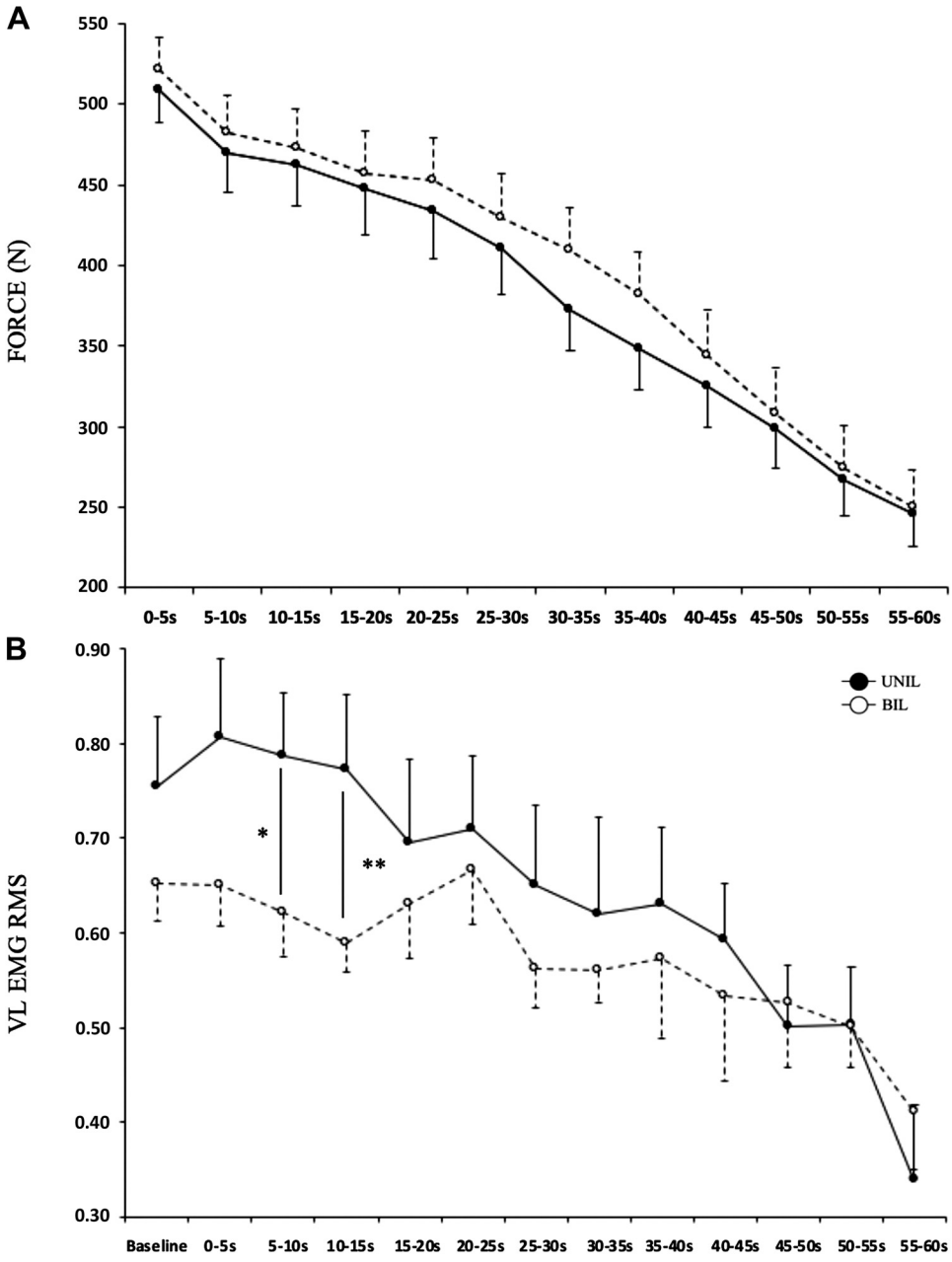

Fig. 2. Isometric force (*A*) and vastus lateralis EMG root mean square (VL EMG RMS; *B*) during the 60-s isometric maximal voluntary contraction. Data are expressed as percentages of 0–5 s values. Each point represents a 5-s window. Values are means ± SE. UNIL and BIL, interventions with unilateral and bilateral protocols, respectively. Significant difference: $*P < 0.05$, $**P < 0.01$.

previous studies utilizing submaximal exercises (32, 41, 42), we did not observe greater peripheral fatigue after an IMVC in UNIL (i.e., lower muscle mass) despite the fact that central drive was lower after the BIL (i.e., higher muscle mass) condition. This result was found despite slight (~6%) but significantly ($P = 0.030$) greater total fatigue (ΔIMVC; Fig. 3*A*) in UNIL and no difference in VA_{TMS} between UNIL and BIL conditions. Additionally, for the first time, corticospinal excitability (MEP) and inhibition (SP) were not found to be different between UNIL and BIL conditions.

Our result (i.e., no difference in VA_{TMS} between UNIL and BIL) is similar to that of Herbert and Gandevia (23) and Jakobi and Cafarelli (25) but differs from other studies. For instance, Behm et al. (6) showed a higher activation during 3-s MVC in BIL versus UNIL but no difference in EMG and force, and Girompaire et al. (18) recently found a significantly lower maximal voluntary activation in BIL with no difference in EMG and force. It is challenging to explain discrepancies between studies for VA_{TMS}. It can be argued that the difference between Girompaire et al. (18) and the present study is that we measured voluntary activation with PNS just after TMS. In Girompaire et al. (18), the participants were first stimulated with peripheral nerve stimulation, followed by TMS after a 10-min break. This was not possible in the present experiment because of the fast recovery following the fatiguing task. Moreover, several studies have highlighted that even if testing is conducted on an appropriately designed dynamometer, factors associated with subjects' body adjustments or mechanical configuration of the dynamometer itself would contribute to the differences observed between studies not only on bilateral deficit but also voluntary activation and EMG activity (6, 18, 44). Simoneau-Buessinger et al. (44) reported that body ad-

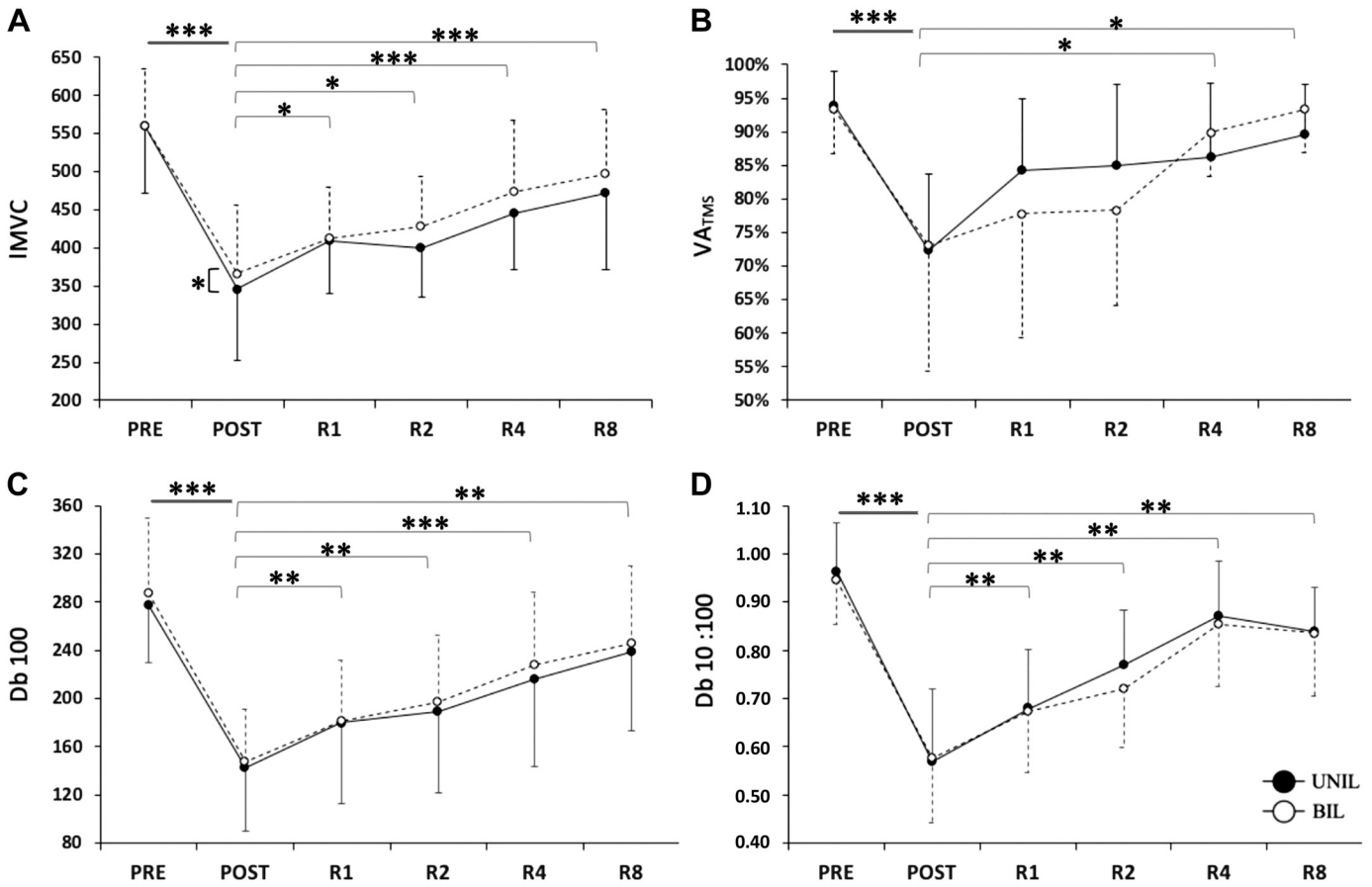

Fig. 3. Isometric maximum force [isometric maximal voluntary contraction (IMVC); *A*], voluntary activation determined by transcranial magnetic stimulation (VA_{TMS}; *B*), force produced by a high-frequency doublet (Db_{100}; *C*), and ratio of force produced by low- to high-frequency doublets ($Db_{10:100}$; *D*) measured before (PRE) and after (POST) a 60-s sustained IMVC and 1, 2, 4, and 8 min after exercise (R1, R2, R4, and R8). Values are means ± SD. UNIL and BIL, interventions with unilateral and bilateral protocols, respectively. Significant difference: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

justments are load dependent, contribute substantially to torque, and contribute more in bilateral conditions. Behm et al. (6) have proposed that stabilizer muscles contribute more to bilateral contractions because of a greater stability challenge. Therefore, the discrepancy between the present and previous findings may be due to the contributions of assistive muscles in which the effects of IMVC were not recorded.

A Lack of Greater Peripheral Fatigue in UNIL

Previous studies investigating the effect of muscle mass on fatigue employed submaximal exercises and found greater peripheral fatigue following UNIL exercise (32, 41, 42), contrary to the present study. We suggest that this may be related to the fact that the exercise utilized in the present investigation was maximal, as existing literature demonstrates that the effects of an intervention on performance differ between maximal and submaximal exercises because maximal exercise may not be as reliant on the contribution of top-down psychobiological processes. For instance, Temesi et al. (48) reported that sleep deprivation affected cycling exercise performance at submaximal intensity; however, no between-condition differences were found for IMVC or VA_{TMS}. These findings are in line with the general observation that sleep deprivation has less effect on maximal performance and maximal strength than on submaximal exercise (7, 40, 51). The effects of mental fatigue on physical performance is another area of research where the

Table 1. *Raw measures of peak twitch and M_{max} area at PRE, immediately POST, and during recovery (+1 min, +2 min, +4 min, and +8 min)*

Measure	Condition	PRE	POST	R1	R2	R4	R8
Peak twitch, N	Unilateral	190 ± 30	68 ± 31***	90 ± 33**	110 ± 37***	137 ± 37***	153 ± 38***
	Bilateral	190 ± 32	73 ± 31***	93 ± 31**	107 ± 34***	140 ± 38***	150 ± 43***
M_{max} area, %	Unilateral	93.7 ± 19.4	113.3 ± 19.0	114.7 ± 25.7	111.9 ± 20.3*	108.8 ± 20.0	102.5 ± 22.5
	Bilateral	94.0 ± 12.0	110.0 ± 21.8	113.5 ± 17.6	113.3 ± 16.7*	108.3 ± 11.3	92.6 ± 8.9

Values are raw (mean ± standard deviation) measures of peak twitch and maximal M-wave (M_{max}) area at PRE, immediately POST, and during recovery [+1 min (R1), +2 min (R2), +4 min (R4), and +8 min R8)]. For peak twitch, differences were tested between *1*) PRE and POST and *2*) POST and R1, R2, R4, and R8. For M_{max}, differences were tested between PRE and POST, R1, R2, R4, and R8. Significant difference: $*P < 0.05$, $**P < 0.01$, $***P \leq 0.001$.

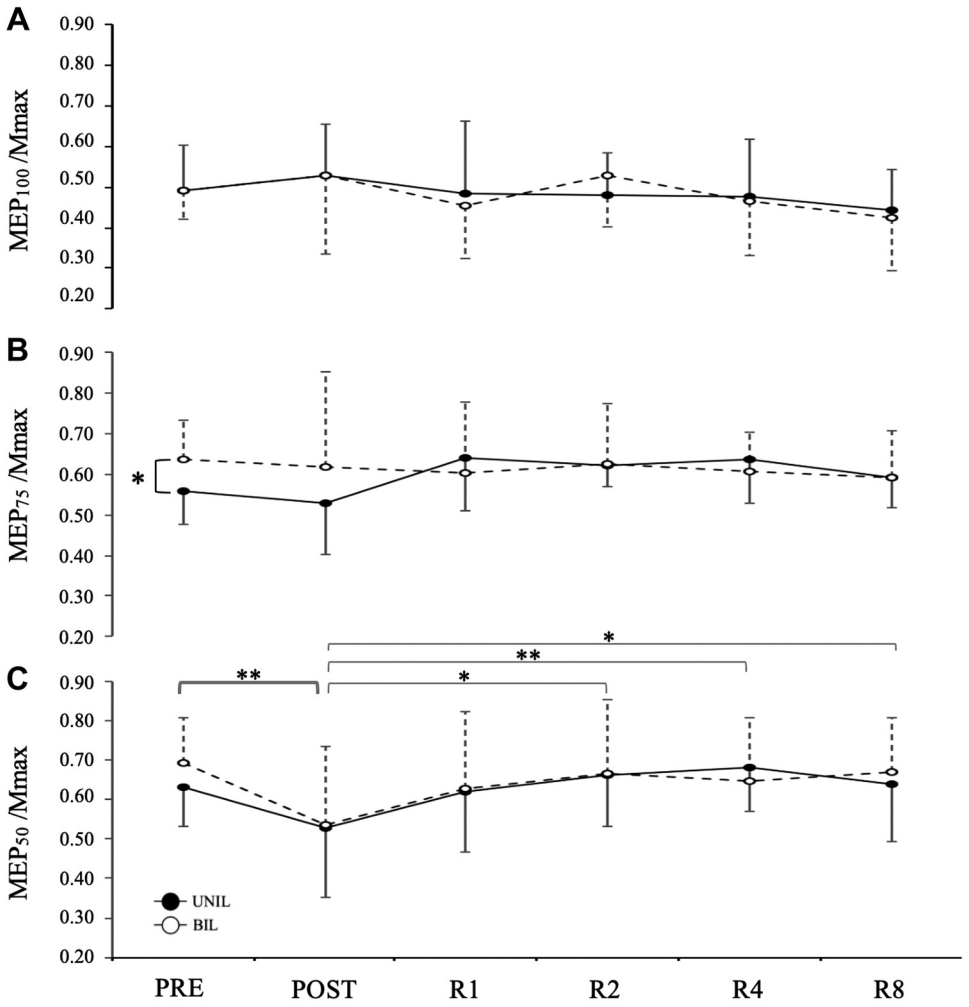

Fig. 4. Motor evoked potential (MEP) area normalized to M-wave (M_{max}) area at 100% (MEP_{100}; *A*), 75% (MEP_{75}; *B*), and 50% (MEP_{50}; *C*) isometric maximal voluntary contraction (IMVC) measured before (PRE) and after (POST) a 60-s sustained IMVC and 1, 2, 4, and 8 min after exercise (R1, R2, R4, and R8). Values are means ± SD. All data are expressed as percentages. UNIL and BIL, interventions with unilateral and bilateral protocols, respectively. Significant difference: $*P < 0.05$), $**P < 0.01$.

results differ between maximal and submaximal exercise. Marcora et al. (28) showed that submaximal cycling time to exhaustion was ~15% shorter with mental fatigue compared with control conditions and was associated with higher feelings of fatigue, whereas Martin et al. (31) reported no difference in performance between mental fatigue and control conditions for maximal anaerobic exercises. Pageaux and Lepers (38) also showed that mental fatigue impairs sport-related performance during exercises performed at submaximal, but not maximal and supramaximal, intensities. The idea that RPE plays a less important role in maximal than in submaximal exercise in both sleep deprivation and mental fatigue studies may explain the differences between the present study and the existing literature (32, 41, 42). This could also explain why EMG RMS was lower in BIL at the end but not at the beginning of the fatiguing task.

A Lack of Differences in Corticospinal Excitability and Inhibition Between Conditions

To the best of our knowledge, this is the first study that compared the effects of UNIL and BIL fatiguing exercises on corticospinal excitability and inhibition. Interestingly, no significant condition × time interaction was found, during either fatigue or recovery, for both MEP and SP at any force level. As explained above, significant discrepancies have been reported in the literature about the effects of muscle mass on VA_{TMS} at rest. Although it is tempting to link functional outcomes such as VA_{TMS} to corticospinal excitability and inhibition, this direct relationship does not exist, to our knowledge. In the present study, no significant VA_{TMS} difference was reported at rest between UNIL and BIL, whereas MEP_{75} showed a condition effect, i.e., this parameter was significantly higher in BIL than UNIL. The reasons are unclear. The direct link between changes in corticospinal excitability and inhibition and changes in VA_{TMS} has also been challenged. Indeed, several studies have shown that MEPs can increase while VA_{TMS} is decreasing (e.g., Ref. 49). Similarly, it has been shown that MEP but not VA_{TMS} recovered after a fatiguing task when a muscle group was put to ischemia (16).

Why MEP_{75} was higher in BIL while MEP_{100} and MEP_{50} did not differ between conditions is also unclear. As MEPs were measured in a consistent order during 100%, 75%, and 50% MVC, the effect of contraction order may have played a role in our results. This issue has not been examined in the literature so far, yet the fact that contraction intensity during TMS delivery influences the direction of changes in MEP amplitude has been reported several times (e.g., Refs. 12, 22).

Although SP usually increases with fatigue for sustained contractions at high force levels (e.g., Refs. 26, 47, 52), the outcomes are more diverse for MEP changes. Indeed, as for the

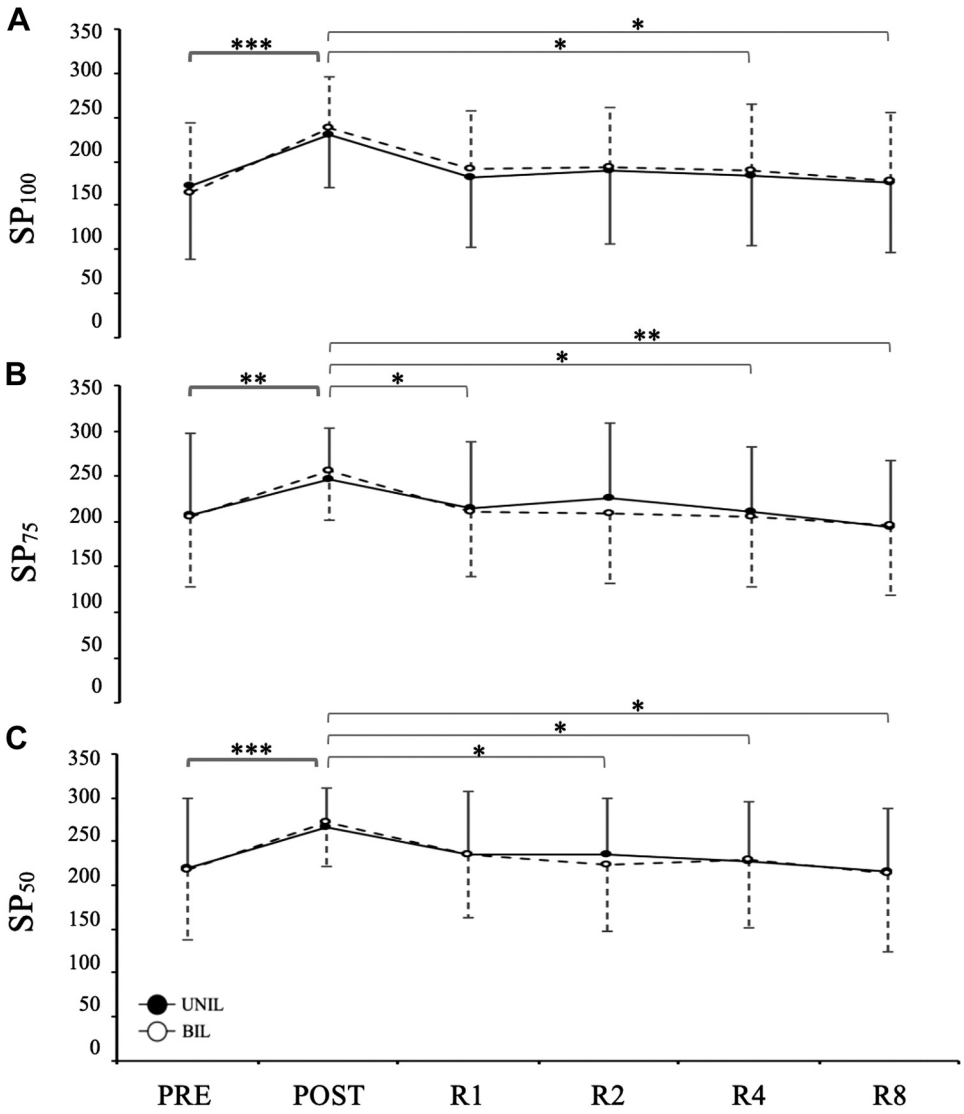

Fig. 5. Silent period (SP) at 100% (SP_{100}; *A*), 75% (SP_{75}; *B*), and 50% (SP_{50}; *C*) isometric maximal voluntary contraction (IMVC) measured before (PRE) and after (POST) a 60-s sustained IMVC and 1, 2, 4, and 8 min after exercise (R1, R2, R4, and R8). Values are means ± SD. All data are expressed in milliseconds. UNIL and BIL, interventions with unilateral and bilateral protocols, respectively. Significant difference: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

condition effects, the time effects depend on the force level at which corticospinal excitability is measured (1–3, 5, 20). MEP_{100} and MEP_{75} did not change between PRE and POST, whereas MEP_{50} significantly decreased. These results were, however, not completely unexpected. The reduction of MEP_{50} may correspond to a reduction of central drive, i.e., a reduction of postsynaptic excitatory potentials. On the contrary, at higher force levels the motoneuron discharge rate is higher (11). A greater probability of TMS-induced volley to reach the motoneuron pool during the refractory period may explain why MEP is usually lower at rest. However, as discharge rates decrease with fatigue this probability is reduced, and this may explain why, contrary to what is found for MEP_{50}, MEP_{75} and MEP_{100} did not decrease. As the effects of muscle mass seem more pronounced during submaximal than maximal contraction, comparing the evolution of corticospinal excitability and inhibition between UNIL and BIL submaximal fatiguing conditions would be an interesting area for future research.

Limitations

The main limitation of the present study is the small sample size, which is limiting the statistical power of our results. One may also consider that discussing RPE without measuring it is a limitation. However, as sustained MVC was used in the present study, it is likely that the participants would always have reached the maximal RPE after each experimental condition. In other words, measuring RPE would have only been relevant if submaximal versus maximal conditions had been compared. Finally, the lack of applicability in clinical populations could also be pointed out as a limitation, since our participants were untrained but healthy.

Conclusions

This study was the first to examine the effect of muscle mass on corticospinal excitability and inhibition using a maximal exercise. Corticospinal excitability and inhibition were not found to be different between UNIL and BIL. Contrary to previous studies that used submaximal exercises, we did not show greater peripheral fatigue in UNIL even though central drive was lower in BIL. It is speculated that a less important role of RPE in maximal versus submaximal exercise as a "saturation" of the RPE during maximal exercise may have occurred.

ACKNOWLEDGMENTS

The authors acknowledge Dr. Gustavo Mota's assistance in the data collection process.

GRANTS

We acknowledge the support of the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grants Program.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

G.Y.M. conceived and designed research; J.K. and D.J.O. performed experiments; J.K., D.J.O., J.G.W., and G.Y.M. analyzed data; J.K. and G.Y.M. interpreted results of experiments; J.K. prepared figures; J.K., D.J.O., J.G.W., R.T., and G.Y.M. drafted manuscript; J.K., D.J.O., J.G.W., R.T., and G.Y.M. edited and revised manuscript; J.K., D.J.O., J.G.W., R.T., and G.Y.M. approved final version of manuscript.

REFERENCES

1. **Aboodarda SJ, Mira J, Floreani M, Jaswal R, Moon SJ, Amery K, Rupp T, Millet GY.** Effects of endurance cycling training on neuromuscular fatigue in healthy active men. Part II: Corticospinal excitability and voluntary activation. *Eur J Appl Physiol* 118: 2295–2305, 2018. doi:10.1007/s00421-018-3951-7.
2. **Aboodarda SJ, Šambaher N, Millet GY, Behm DG.** Knee extensors neuromuscular fatigue changes the corticospinal pathway excitability in biceps brachii muscle. *Neuroscience* 340: 477–486, 2017. doi:10.1016/j.neuroscience.2016.10.065.
3. **Aboodarda SJ, Šambaher N, Behm DG.** Unilateral elbow flexion fatigue modulates corticospinal responsiveness in non-fatigued contralateral biceps brachii. *Scand J Med Sci Sports* 26: 1301–1312, 2016. doi:10.1111/sms.12596.
4. **Amann M.** Central and peripheral fatigue: interaction during cycling exercise in humans. *Med Sci Sports Exerc* 43: 2039–2045, 2011. doi:10.1249/MSS.0b013e31821f59ab.
5. **Bachasson D, Temesi J, Gruet M, Yokoyama K, Rupp T, Millet GY, Verges S.** Transcranial magnetic stimulation intensity affects exercise-induced changes in corticomotoneuronal excitability and inhibition and voluntary activation. *Neuroscience* 314: 125–133, 2016. doi:10.1016/j.neuroscience.2015.11.056.
6. **Behm DG, Power KE, Drinkwater EJ.** Muscle activation is enhanced with multi- and uni-articular bilateral versus unilateral contractions. *Can J Appl Physiol* 28: 38–52, 2003. doi:10.1139/h03-004.
7. **Blumert PA, Crum AJ, Ernsting M, Volek JS, Hollander DB, Haff EE, Haff GG.** The acute effects of twenty-four hours of sleep loss on the performance of national-caliber male collegiate weightlifters. *J Strength Cond Res* 21: 1146–1154, 2007. doi:10.1519/R-21606.1.
8. **Brownstein CG, Dent JP, Parker P, Hicks KM, Howatson G, Goodall S, Thomas K.** Etiology and recovery of neuromuscular fatigue following competitive soccer match-play. *Front Physiol* 8: 831, 2017. doi:10.3389/fphys.2017.00831.
9. **Brownstein CG, Ansdell P, Škarabot J, Frazer A, Kidgell D, Howatson G, Goodall S, Thomas K.** Motor cortical and corticospinal function differ during an isometric squat compared with isometric knee extension. *Exp Physiol* 103: 1251–1263, 2018. doi:10.1113/EP086982.
10. **Christensen MS, Lundbye-Jensen J, Geertsen SS, Petersen TH, Paulson OB, Nielsen JB.** Premotor cortex modulates somatosensory cortex during voluntary movements without proprioceptive feedback. *Nat Neurosci* 10: 417–419, 2007. doi:10.1038/nn1873.
11. **Enoka RM, Duchateau J.** Rate coding and the control of muscle force. *Cold Spring Harb Perspect Med* 7: a029702, 2017. doi:10.1101/cshperspect.a029702.
12. **Fernandez-del-Olmo M, Rodriguez FA, Marquez G, Iglesias X, Marina M, Benitez A, Vallejo L, Acero RM.** Isometric knee extensor fatigue following a Wingate test: peripheral and central mechanisms. *Scand J Med Sci Sports* 23: 57–65, 2013. doi:10.1111/j.1600-0838.2011.01355.x.
13. **Fontes EB, Smirmaul BP, Nakamura FY, Pereira G, Okano AH, Altimari LR, Dantas JL, de Moraes AC.** The relationship between rating of perceived exertion and muscle activity during exhaustive constant-load cycling. *Int J Sports Med* 31: 683–688, 2010. doi:10.1055/s-0030-1255108.
14. **Froyd C, Beltrami FG, Noakes TD.** Neuromuscular fatigue at task failure and during immediate recovery after isometric knee extension trials. *Sports (Basel)* 6: 156, 2018. doi:10.3390/sports6040156.
15. **Gandevia SC, Enoka RM, McComas AJ, Stuart DG, Thomas CK.** Neurobiology of muscle fatigue. Advances and issues. *Adv Exp Med Biol* 384: 515–525, 1995. doi:10.1007/978-1-4899-1016-5_39.
16. **Gandevia SC, Allen GM, Butler JE, Taylor JL.** Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. *J Physiol* 490: 529–536, 1996. doi:10.1113/jphysiol.1996.sp021164.
17. **Gandevia SC.** Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* 81: 1725–1789, 2001. doi:10.1152/physrev.2001.81.4.1725.
18. **Girompaire L, Morel B, Lapole T.** Reduced cortical voluntary activation during bilateral knee extension. *Hum Mov Sci* 52: 17–23, 2017. doi:10.1016/j.humov.2017.01.005.
19. **Goodall S, Howatson G, Romer L, Ross E.** Transcranial magnetic stimulation in sport science: a commentary. *Eur J Sport Sci* 14, *Suppl* 1: S332–S340, 2014. doi:10.1080/17461391.2012.704079.
20. **Goodall S, Ross EZ, Romer LM.** Effect of graded hypoxia on supraspinal contributions to fatigue with unilateral knee-extensor contractions. *J Appl Physiol (1985)* 109: 1842–1851, 2010. doi:10.1152/japplphysiol.00458.2010.
21. **Goodall S, Romer LM, Ross EZ.** Voluntary activation of human knee extensors measured using transcranial magnetic stimulation. *Exp Physiol* 94: 995–1004, 2009. doi:10.1113/expphysiol.2009.047902.
22. **Gruet M, Temesi J, Rupp T, Levy P, Verges S, Millet GY.** Dynamics of corticospinal changes during and after high-intensity quadriceps exercise. *Exp Physiol* 99: 1053–1064, 2014. doi:10.1113/expphysiol.2014.078840.
23. **Herbert RD, Gandevia SC.** Muscle activation in unilateral and bilateral efforts assessed by motor nerve and cortical stimulation. *J Appl Physiol (1985)* 80: 1351–1356, 1996. doi:10.1152/jappl.1996.80.4.1351.
24. **Hureau TJ, Romer LM, Amann M.** The "sensory tolerance limit": a hypothetical construct determining exercise performance? *Eur J Sport Sci* 18: 13–24, 2018. doi:10.1080/17461391.2016.1252428.
25. **Jakobi JM, Cafarelli E.** Neuromuscular drive and force production are not altered during bilateral contractions. *J Appl Physiol (1985)* 84: 200–206, 1998. doi:10.1152/jappl.1998.84.1.200.
26. **Kennedy DS, McNeil CJ, Gandevia SC, Taylor JL.** Effects of fatigue on corticospinal excitability of the human knee extensors. *Exp Physiol* 101: 1552–1564, 2016. doi:10.1113/EP085753.
27. **Marcora SM, Staiano W.** The limit to exercise tolerance in humans: mind over muscle? *Eur J Appl Physiol* 109: 763–770, 2010. doi:10.1007/s00421-010-1418-6.
28. **Marcora SM, Staiano W, Manning V.** Mental fatigue impairs physical performance in humans. *J Appl Physiol (1985)* 106: 857–864, 2009. doi:10.1152/japplphysiol.91324.2008.
29. **Marcora S.** Perception of effort during exercise is independent of afferent feedback from skeletal muscles, heart, and lungs. *J Appl Physiol (1985)* 106: 2060–2062, 2009. doi:10.1152/japplphysiol.90378.2008.
30. **Marcora SM.** Do we really need a central governor to explain brain regulation of exercise performance? *Eur J Appl Physiol* 104: 929–931, 2008. doi:10.1007/s00421-008-0818-3.
31. **Martin K, Thompson KG, Keegan R, Ball N, Rattray B.** Mental fatigue does not affect maximal anaerobic exercise performance. *Eur J Appl Physiol* 115: 715–725, 2015. doi:10.1007/s00421-014-3052-1.
32. **Matkowski B, Place N, Martin A, Lepers R.** Neuromuscular fatigue differs following unilateral vs bilateral sustained submaximal contractions. *Scand J Med Sci Sports* 21: 268–276, 2011. doi:10.1111/j.1600-0838.2009.01040.x.
33. **Millet GY.** Can neuromuscular fatigue explain running strategies and performance in ultra-marathons?: the flush model. *Sports Med* 41: 489–506, 2011. doi:10.2165/11588760-000000000-00000.
34. **Mira J, Lapole T, Souron R, Messonnier L, Millet GY, Rupp T.** Cortical voluntary activation testing methodology impacts central fatigue. *Eur J Appl Physiol* 117: 1845–1857, 2017. doi:10.1007/s00421-017-3678-x.
35. **Noakes TD.** Fatigue is a brain-derived emotion that regulates the exercise behavior to ensure the protection of whole body homeostasis. *Front Physiol* 3: 82, 2012. doi:10.3389/fphys.2012.00082.

36. **Nybo L, Nielsen B.** Hyperthermia and central fatigue during prolonged exercise in humans. *J Appl Physiol (1985)* 91: 1055–1060, 2001. doi:10.1152/jappl.2001.91.3.1055.
37. **Nybo L, Rasmussen P.** Inadequate cerebral oxygen delivery and central fatigue during strenuous exercise. *Exerc Sport Sci Rev* 35: 110–118, 2007. doi:10.1097/jes.0b013e3180a031ec.
38. **Pageaux B, Lepers R.** The effects of mental fatigue on sport-related performance. *Prog Brain Res* 240: 291–315, 2018. doi:10.1016/bs.pbr.2018.10.004.
39. **Pageaux B.** The psychobiological model of endurance performance: an effort-based decision-making theory to explain self-paced endurance performance. *Sports Med* 44: 1319–1320, 2014. doi:10.1007/s40279-014-0198-2.
40. **Reilly T, Piercy M.** The effect of partial sleep deprivation on weight-lifting performance. *Ergonomics* 37: 107–115, 1994. doi:10.1080/00140139408963628.
41. **Rossman MJ, Venturelli M, McDaniel J, Amann M, Richardson RS.** Muscle mass and peripheral fatigue: a potential role for afferent feedback? *Acta Physiol (Oxf)* 206: 242–250, 2012. doi:10.1111/j.1748-1716.2012.02471.x.
42. **Rossman MJ, Garten RS, Venturelli M, Amann M, Richardson RS.** The role of active muscle mass in determining the magnitude of peripheral fatigue during dynamic exercise. *Am J Physiol Regul Integr Comp Physiol* 306: R934–R940, 2014. doi:10.1152/ajpregu.00043.2014.
43. **Sidhu SK, Bentley DJ, Carroll TJ.** Cortical voluntary activation of the human knee extensors can be reliably estimated using transcranial magnetic stimulation. *Muscle Nerve* 39: 186–196, 2009. doi:10.1002/mus.21064.
44. **Simoneau-Buessinger E, Leteneur S, Toumi A, Dessurne A, Gabrielli F, Barbier F, Jakobi JM.** Bilateral strength deficit is not neural in origin; rather due to dynamometer mechanical configuration. *PLoS One* 10: e0145077, 2015. doi:10.1371/journal.pone.0145077.
45. **Smirmaul BP.** Sense of effort and other unpleasant sensations during exercise: clarifying concepts and mechanisms. *Br J Sports Med* 46: 308–311, 2012. doi:10.1136/bjsm.2010.071407.
46. **Smirmaul BP, Dantas JL, Nakamura FY, Pereira G.** The psychobiological model: a new explanation to intensity regulation and (in)tolerance in endurance exercise. *Rev Bras Educ Fís Esporte* 27: 333–340, 2013. doi:10.1590/S1807-55092013005000008.
47. **Taylor JL, Butler JE, Allen GM, Gandevia SC.** Changes in motor cortical excitability during human muscle fatigue. *J Physiol* 490: 519–528, 1996. doi:10.1113/jphysiol.1996.sp021163.
48. **Temesi J, Arnal PJ, Davranche K, Bonnefoy R, Levy P, Verges S, Millet GY.** Does central fatigue explain reduced cycling after complete sleep deprivation? *Med Sci Sports Exerc* 45: 2243–2253, 2013. doi:10.1249/MSS.0b013e31829ce379.
49. **Temesi J, Rupp T, Martin V, Arnal PJ, Féasson L, Verges S, Millet GY.** Central fatigue assessed by transcranial magnetic stimulation in ultratrail running. *Med Sci Sports Exerc* 46: 1166–1175, 2014. doi:10.1249/MSS.0000000000000207.
50. **Todd G, Taylor JL, Gandevia SC.** Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. *J Physiol* 551: 661–671, 2003. doi:10.1113/jphysiol.2003.044099.
51. **Vaara JP, Oksanen H, Kyröläinen H, Virmavirta M, Koski H, Finni T.** 60-Hour sleep deprivation affects submaximal but not maximal physical performance. *Front Physiol* 9: 1437, 2018. doi:10.3389/fphys.2018.01437.
52. **Vernillo G, Temesi J, Martin M, Millet GY.** Mechanisms of fatigue and recovery in upper versus lower limbs in men. *Med Sci Sports Exerc* 50: 334–343, 2018. doi:10.1249/MSS.0000000000001445.