Mechanisms of Fatigue and Recovery in Upper versus Lower Limbs in Men

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ABSTRACT

VERNILLO, G., J. TEMESI, M. MARTIN, and G. Y. MILLET. Mechanisms of Fatigue and Recovery in Upper versus Lower Limbs in Men. *Med. Sci. Sports Exerc.*, Vol. 50, No. 2, pp. 334–343, 2018. **Purpose**: To compare the mechanisms of fatigue and recovery between upper and lower limbs in the same subjects. **Methods**: Twelve healthy young men performed a 2-min sustained maximal voluntary isometric contraction (MVC) of the knee extensors (KE) and on another day a 2-min MVC of the elbow flexors (EF). Neuromuscular function evaluations were performed with both transcranial magnetic and peripheral stimulations before (PRE), at the end of the 2-min MVC, and five more times within 8 min of recovery. **Results**: Decreases in MVC and cortical voluntary activation were approximately 12% ($P < 0.001$) and approximately 25% greater ($P = 0.04$) in KE than EF at end of the 2-min MVC. Conversely, twitch response decreased approximately 29% more ($P = 0.02$) in EF than KE. Changes in motor-evoked potential with fatigue were not different between upper and lower limbs ($P > 0.05$), whereas the increase in silent period duration was approximately 30% greater in EF than KE ($P < 0.05$). **Conclusions**: Upper and lower limbs presented different magnitudes of total, central and peripheral fatigue. Total neuromuscular fatigue and central fatigue were greater in KE than EF. Conversely, peripheral fatigue and corticospinal inhibition were greater in EF than KE. **Key Words:** CORTICOSPINAL EXCITABILITY, INHIBITION, MAXIMAL VOLUNTARY CONTRACTION, TRANSCRANIAL MAGNETIC STIMULATION

Neuromuscular fatigue is defined as a reversible, time-dependent decline in the maximal force-generating capacity of a muscle (1). Various sites along the pathway of force production have been implicated in the development of neuromuscular fatigue. The global fatigue effect, assessed by force loss during a maximal voluntary contraction (MVC), originates at one or both of the central and peripheral levels.

Although not linked to a reduction in voluntary activation (VA) (the level of voluntary drive to the muscle during exercise) (2), the electrical responses evoked by transcranial magnetic stimulation (TMS) can, in parallel with peripheral nerve stimulation, provide information about excitability and inhibition within the motor pathway. Notably, the responses evoked by TMS and recorded at the muscle level (i.e., motor-evoked potentials [MEP]) do not solely reflect changes in the motor cortical excitability because changes in the motoneurons and muscle fibers can also influence MEP responses. When single-pulse TMS is delivered during a voluntary contraction, the MEP is followed by a period of near-silence in the EMG activity signal. This period of EMG suppression (the so-called silent period [SP]) is mediated by the activation of long-lasting $GABA_B$ receptors (1), where the first 150 ms is believed to be predominantly influenced by spinal mechanisms (3) and thereafter by intracortical inhibitory mechanisms (4).

Meanwhile, fatigue occurring at the skeletal muscle level is usually referred to as peripheral fatigue (5) and is traditionally assessed by changes in the EMG and mechanical responses elicited at or distal to the neuromuscular junction in a relaxed muscle (6). Several mechanisms (e.g., failure of excitation-contraction coupling and reduced sarcoplasmic reticulum Ca^{2+} release) have been suggested to contribute to muscle fatigue (5). During short-duration sustained isometric MVC (e.g., 2 min), the contribution of the fatigability of the skeletal muscle seems to play a major role in the force reduction compared with the central, including supraspinal, fatigue component (7,8).

Because the nature of a sustained isometric MVC is that there is a maximal effort for the duration of the task, many studies have used this fatiguing exercise as a pure theoretical model to study the physiological processes underlying fatigue [e.g., (9–16)]. Decreases in the maximal force production after sustained isometric MVC have been observed in various upper- and lower-limb muscle groups including the elbow flexors (EF) [e.g., (10,12–14)] and knee extensors (KE) [e.g., (9,11,15)]. After a 2-min sustained MVC, studies reported similar force losses (~60%–70%) in KE (9,11,15) and EF (10,13,14). Immediately after a 2-min MVC, VA decreased

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by approximately 7% in KE (11), whereas the superimposed twitch (SIT) expressed as a percentage of the ongoing MVC (as a surrogate for VA) approximately doubled (13,14) in EF. The MEP area increased by about 18% (9) for lower limbs, whereas for the upper limbs, studies reported increases of approximately 55% for MEP area (10,12) and approximately 28% for MEP amplitude (13). The potentiated resting twitch was reduced by approximately 25% in KE (11) and approximately 70% in EF (14). However, what is currently known about the mechanisms of fatigue in the upper and lower limbs has been inferred across studies using different subjects. To the best of our knowledge, only two studies compared fatigue for the same task in different muscle groups in the same subjects (17,18). Senefeld et al. (18) showed that when subjects performed 90 submaximal isotonic contractions at maximal voluntary shortening velocity, the loss in isometric MVC force was 16% higher in EF compared with KE. Neyroud et al. (17) showed that the loss of MVC force after a sustained contraction at 50% MVC until task failure was similar between muscle groups, with 30%, 37%, 40%, and 34% declines reported for plantar flexors, thumb adductors, EF, and KE, respectively. Further, no significant changes in EF or KE VA (from 96% to 91% and from 89% to 92%, respectively) were found in that study (17). The potentiated evoked doublet amplitudes in relaxed muscle (peripheral fatigue) decreased more for EF (−59%) than for KE (−28%). However, one of the main limitations of Senefeld et al. (18) is that the etiology of fatigue was not determined, whereas in Neyroud et al. (17), VA was only determined by means of the classic twitch interpolation technique. The conceptual limitation of VA assessed by peripheral nerve stimulation is that it does not reveal whether suboptimal maximal drive occurs at the supraspinal level (1,19). The aforementioned limits thereby preclude a complete understanding of the potential differences in the mechanisms of fatigue between upper and lower limbs. Because the upper and lower limbs are functionally different, understanding fatigue-induced corticospinal and peripheral changes is crucial.

Therefore, by using a 2-min sustained maximal isometric exercise model, the aim of this exploratory study was to investigate if the magnitude and etiology of fatigue and recovery are similar between upper and lower limbs.

METHODS

Subjects

Twelve healthy young men participated in this study (Table 1). Subjects were informed of the experimental protocol and all associated risks before giving written informed consent. All procedures conformed to the Declaration of Helsinki and were approved by the local ethics committee (University of Calgary Conjoint Health Research Ethics Board, REB14-1625). Subjects were instructed to avoid the consumption of caffeine on the day of the experiment and avoid performing any strenuous exercise for 48 h before testing.

Experimental Protocol

Each subject completed one familiarization session and two experimental sessions. During the familiarization session, subjects performed maximal and submaximal voluntary isometric contractions of KE and EF with and without TMS and peripheral stimulation. The two experimental sessions were performed in a pseudo-randomized and counter-balanced order and consisted of (i) a 2-min KE MVC with TMS and peripheral stimulation, and (ii) a 2-min EF MVC with TMS and peripheral stimulation. All tests were separated by between 3 and 7 d and each subject performed all tests at the same time of day.

Neuromuscular testing protocol. Before each 2-min MVC (PRE), the neuromuscular testing protocol consisted of two neuromuscular function evaluations (separated by 60 s) with TMS and peripheral stimulation (see Neuromuscular function evaluation section). Peak forces from the second trial were within 5% of the first trial for all subjects. At the end of the 2-min MVC, a neuromuscular function evaluation was performed as an extension of the 2-min MVC (i.e., the subject was not permitted to relax) (POSTimm). Additional evaluations 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 MVC (Fig. 1A).

Force and EMG recordings. Muscle forces were obtained from voluntary and evoked isometric contractions. All measurements were taken from the subjects' right limbs. KE force was measured by a calibrated force transducer (LC101-2K; Omegadyne, Sunbury, OH) attached by a noncompliant strap to the right leg immediately proximal to the malleoli of the ankle joint. Subjects were seated upright in a custom-built chair with both right knee and hips at 90° of flexion and secured by chest and hip straps. The force transducer was fixed to the chair such that force was measured in direct line to the applied force.

EF force was assessed by calibrated force transducer (2712–200 daN, Sensy, Jumet, Belgium). Subjects were seated upright in a chair with right arm in a custom-built dynamometer. Both

TABLE 1. Subjects' baseline characteristics (n = 12).

	Age (yr)	Body Mass (kg)	Height (cm)	MVC (N)		VA_{TMS} (%)		TW_{POT} (N)		M_{max} ($mV \cdot s^{-1}$)		MEP (%M_{max})		SP (ms)	
				KE	EF	KE	EF	KE	EF	RF	BB	RF	BB	RF	BB
Mean	31	75	179	630	320	97	98	153	51	0.061	0.077	59	70	275	195
SD	9	9	7	113	48	2	2	22	13	0.032	0.210	21	19	58	59

Values are presented as means and SD. For VA_{TMS}, the sample size was n = 6 (see TMS section).
MEP area as percent of M_{max} area.

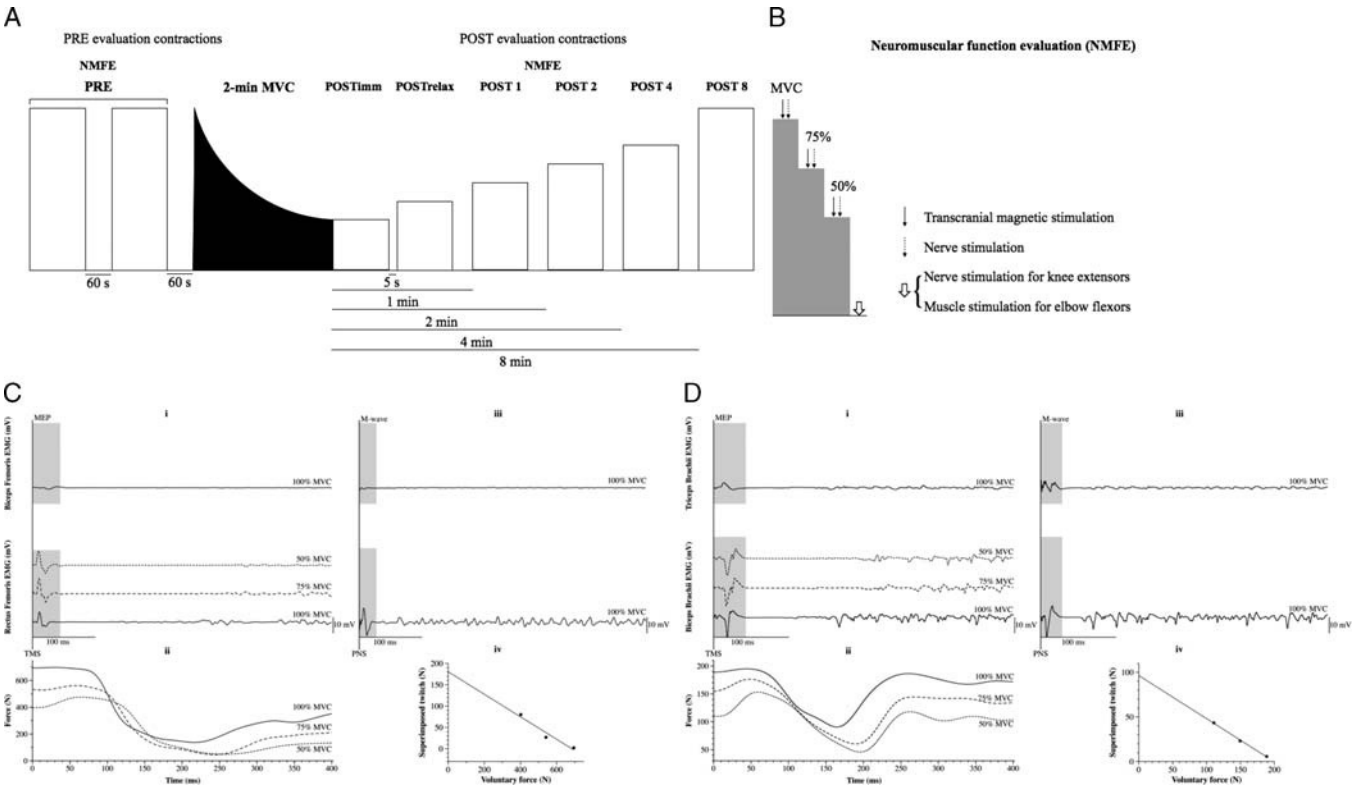

FIGURE 1—Fatigue protocols performed in two separate experiments for both upper and lower limbs. Each protocol was composed of a neuromuscular function evaluation (NMFE) before (PRE) the 2-min MVC. The NMFE required subjects to perform a sustained isometric contraction (A). The subject contracted to maximal force and once maximal force was attained, motor cortex stimulation was delivered. Once the subject returned to maximal force, peripheral stimulation (i.e., femoral nerve or brachial plexus electrical stimulation) was delivered. Guidelines at 75 and 50% of maximal force were instantaneously displayed on the computer screen so that the contraction was sustained at 75% MVC and then 50% MVC. Motor cortex stimulation was delivered at each force level once the subject produced the appropriate amount of force while peripheral stimulation was delivered only at 100% MVC. Each sustained contraction lasted approximately 9 s (~3 s per contraction intensity). Immediately after relaxing completely, a single stimulus was delivered as femoral nerve electrical stimulation in KE or motor point electrical stimulation in the BB (B). At PRE, two NMFE were performed and separated by 60 s. At the end of the 2-min MVC, the same NMFE was performed as an extension of the 2-min MVC (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 MVC (A). Single-subject data showing the force and EMG responses evoked in the KE (C) and EF (D) by the transcranial magnetic stimulation (TMS) over the motor cortex and peripheral nerve stimulation (PNS) of the femoral nerve (A) and brachial plexus (B). Stimuli were delivered at time 0 ms during 50%, 75%, and 100% MVC of KE and EF before the 2-min maximal MVC. (i and iii) Raw EMG traces showing the TMS-elicited MEP and compound muscle action potential (M-wave) evoked by PNS, respectively. (ii) overlaid raw KE and EF force traces showing the size of the SIT that accompanied the EMG data presented in i and iii. (iv) linear regression of the amplitude of the SIT and voluntary force for data shown in panels C ($r = 0.97$) and D ($r = 0.99$). The y-intercept (180.4 N and 96.4 N for KE and EF, respectively) was taken as the estimated amplitude of the resting twitch.

shoulder and elbow joints were at 90°, with the forearm in a supinated position (20). A noncompliant strap secured the wrist to the dynamometer.

EMG of the right KE (rectus femoris [RF]), knee flexors (biceps femoris), EF (biceps brachii [BB]), and elbow extensors (long head of the triceps brachii) was recorded with pairs of self-adhesive surface (10-mm recording diameter) electrodes (Meditrace 100; Covidien, Mansfield, MA) in bipolar configuration with a 30-mm interelectrode distance and the reference on the patella (for KE) or medial epicondyle of the humerus (for EF). A low impedance (<5 kΩ) between electrodes was obtained by shaving and gently abrading the skin and then cleaning it with isopropyl alcohol. Force and EMG signals were analog-to-digitally converted 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).

Peripheral stimulation. During knee extension, single electrical stimuli of 1-ms duration were delivered via constant-current stimulator (DS7A; Digitimer, Welwyn Garden City, Hertfordshire, UK) to the right femoral nerve. Stimuli to the femoral nerve were delivered via a surface cathode securely taped into the femoral triangle (10-mm stimulating diameter, Meditrace 100) and a 50 × 90 mm rectangular anode (Durastick Plus; DJO Global, Vista, CA) in the gluteal fold. During elbow flexion, single electrical stimuli of 200-μs duration were delivered to the BB motor point (for force measurements) and brachial plexus (for M-wave measurements) via constant-current stimulator (DS7AH). For motor point stimulation, the cathode (Meditrace 100) was placed on the motor point (i.e., on the BB muscle belly, midway between the anterior edge of deltoid and the proximal elbow crease with the elbow flexed

at 90°), and the anode (Durastick Plus) over the bicipital tendon. For brachial plexus stimulation, the cathode (Meditrace 100) was securely taped in the supraclavicular fossa and the anode (Durastick Plus) was placed over the acromion.

Single stimuli were delivered incrementally in the relaxed muscle state until M-wave and twitch amplitudes plateaued. A stimulus intensity of 130% of the intensity to elicit maximal M-wave area (M_{max}) and maximal twitch responses was used throughout the rest of the experiment. Stimulus intensity was determined at the start of each session. In KE, the supramaximal stimulus intensity was 84 ± 36 mA. The supramaximal stimulus intensity was 138 ± 65 mA for brachial plexus stimulation and 120 ± 36 mA for EF motor point stimulation.

Transcranial magnetic stimulation. Single TMS pulses were manually delivered to elicit MEP and SIT during voluntary contractions of KE and EF. The left motor cortex was stimulated by a magnetic stimulator (Magstim 200[2]; 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. Subjects wore a Lycra swim cap on which lines were drawn between the preauricular points and from nasion to inion to identify the vertex. During KE, every centimeter was demarcated from the vertex to 2 cm posterior to the vertex along the nasal–inion line and 1 cm laterally over the left motor cortex. At each of these six points, a stimulus was delivered at 50% maximal stimulator output during voluntary contractions at 20% MVC. During EF, every centimeter was demarcated from 3 to 5 cm to the left of the vertex and from the vertex to 2 cm posterior. The optimal coil position was the site where the largest RF and BB MEP were elicited in KE and EF, respectively. These sites were drawn on the swim cap and employed throughout the session. The selected stimulus intensity was the lowest intensity eliciting maximal MEP amplitudes in RF and BB (with minimal antagonist responses) during brief voluntary contractions at 20% MVC (21). The TMS intensity was determined from a stimulus–response curve comprised of four brief consecutive contractions at each of 20%, 30%, 40%, 50%, 60%, 70%, and 80% maximal stimulator output in a randomized order. Mean stimulus intensities were 63% ± 9% and 56% ± 14% in KE and EF, respectively. TMS was always delivered once the subject had contracted to the appropriate force level and the force had stabilized during voluntary contractions. Subjects were also instructed to recontract to the prestimulus force level as quickly as possible after TMS delivery (22).

Neuromuscular function evaluation. The neuromuscular function evaluation consisted of a sustained isometric contraction with visual feedback of the force produced and target force levels provided to the subjects by means of a real-time display on a computer screen. The subject contracted to maximal force, and once maximal force was attained, TMS was delivered. Once the subject returned to maximal force, peripheral stimulation was delivered. Guidelines at 75% and 50% of maximal force were instantaneously displayed on the computer screen so that the contraction could then be sustained at 75% MVC and then 50% MVC (23). Each sustained contraction lasted approximately 9 s (~3 s per contraction intensity). Once the subject produced the appropriate amount of force (24), TMS was delivered at each force level, whereas peripheral stimulation was delivered only at 100% MVC. Immediately after relaxing completely, a single stimulus was delivered as femoral nerve electrical stimulation in KE or motor point electrical stimulation in the EF (Fig. 1B).

Data Analysis

Peripheral stimulation. During the evaluation contractions at 100% MVC, M_{max} area (M_{max100}) was determined by means of femoral nerve or brachial plexus electrical stimulation for KE and EF, respectively. The amplitudes of the potentiated peak twitch (TW_{Pot}) were determined on relaxed muscles by a single stimulus delivered to the femoral nerve for KE and to the muscle motor point for EF (see Neuromuscular function evaluation section). EMG root mean square (RMS) of both RF and BB was calculated for successive 500-ms windows for the first and last 5 s of the 2-min MVC and then averaged. Then RMS was normalized to the RF or BB maximal M_{max} (RMS/M_{max}).

TMS. Areas of MEP (as an index of corticospinal excitability) were measured and normalized to M_{max} area during voluntary contractions at 100 (MEP_{100}) (see Neuromuscular function evaluation section).

Voluntary activation during maximal effort (VA_{TMS}) was assessed with TMS by modified twitch interpolation (23). Because motor cortical and spinal cord excitability increase with activity (25), the amplitude of the resting twitch was estimated rather than measured directly. Specifically, the linear regression between SIT amplitude elicited by TMS at 100%, 75%, and 50% MVC and voluntary force was performed and the estimated twitch amplitude was extrapolated as the y-intercept of the regression, as previously proposed [e.g., (23)]. VA_{TMS} was then assessed with the equation (23):

$$VA_{TMS}(\%) = (1 - SIT/\text{estimated resting twitch}) \times 100$$

VA_{TMS} was deemed appropriate based on the primary criterion that TMS delivered over the motor cortex at 100% MVC produced a large MEP in the agonist muscle (minimum amplitude of 50%–80% of M_{max}), but only a small MEP in the antagonist muscle (amplitude <20% of M_{max}) (19) (Fig. 1C). However, this was true only for the upper limbs because we did not stimulate the sciatic nerve, and thus could not compare M_{max} of the biceps femoris. Accordingly, a second criterion was also used, ensuring that the regression of voluntary force and the SIT force evoked during the contractions was linear ($r > 0.9$) (26). Based on the aforementioned criteria, one and six subjects were excluded from the analysis in KE and EF, respectively. However, to minimize the chances of either type I (false-positive) or type II (false-negative) errors (27), the VA_{TMS} analysis between KE and EF was conducted on the same subjects ($n = 6$). The largest MEP area (85% ± 27% M_{max} for RF and 91% ± 13% M_{max} for BB) occurred at 50% MVC, and the smallest occurred during the MVC (51% ± 19% M_{max} for RF and 70% ± 19% M_{max} for BB).

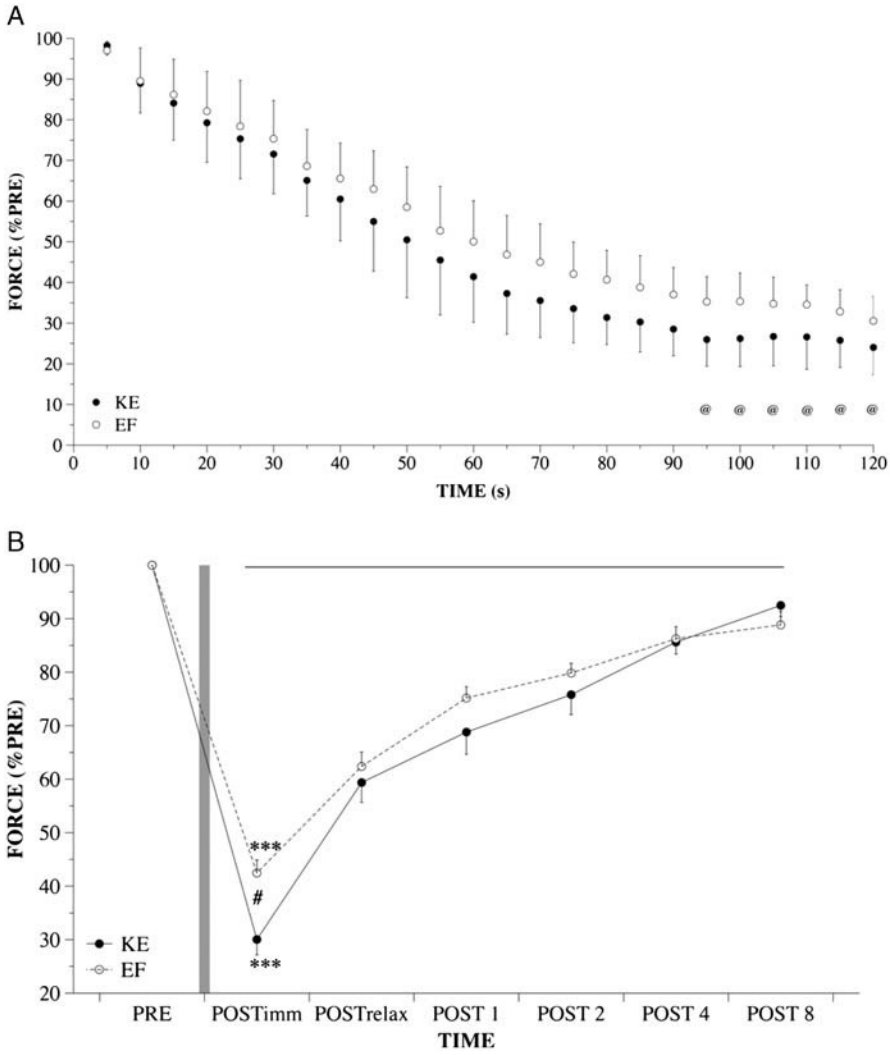

FIGURE 2—(A) Mean force (as percentage of the PRE values) of the KE and EF muscles during the 2-min MVC. Each point represents a 5-s window. Differences between KE and EF force values for each 5-s window were assessed by using the Student's paired *t* test with a Bonferroni-adjusted *P* value for 24 comparisons: $^{@}P < 0.002$. (B) Changes in MVC after the 2-min MVC (*shaded area*) for KE and EF muscles. At the end of the 2-min MVC a neuromuscular function evaluation was performed as an extension of the 2-min MVC (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 MVC. Values are means ± SEM and were normalized as a percentage of the PRE evaluation. Asterisks denote within limb differences between PRE and POSTimm by means of Student's paired *t* test: $***P < 0.001$. Horizontal bar denotes significant limb–time interactions during the recovery period. Number sign (#) denotes differences between KE and EF at POSTimm by means of Student's paired *t* test: $P = 0.003$.

The duration of the SP (as an index of corticospinal inhibition) was determined visually and defined as the duration from the stimulus to the return of continuous voluntary EMG (12).

Statistical Analysis

Results are given in the text as means ± SD and in figures as means ± SEM. Force data during the 2-min MVC contractions were normalized as a percentage of the PRE evaluation and averaged in 5-s time windows for subsequent analysis. All data during the post 2-min MVC contractions were normalized as a percentage of the PRE evaluation except for VA_{TMS}, for which the raw data are presented. The normality of distribution, homogeneity of variances and sphericity were verified using the Shapiro–Wilk normality test, Levene test, and Mauchly test, respectively. If the assumption of sphericity was violated, the Greenhouse–Geisser method to overcome the effects of violation was adopted. Different statistical approaches were performed to analyze the mechanical and EMG responses in our study. (i) First, Student's paired *t* tests were used to determine differences within muscle groups in the percentage changes from PRE to POSTimm and between muscles groups in the percentage changes only at POSTimm. To account for the small sample size ($n = 6$) in VA_{TMS} as described above, Wilcoxon signed-rank test was used to determine differences in VA_{TMS} within muscle groups in percentage change from PRE to POSTimm. Mann–Whitney's *U* test was used to determine differences in VA_{TMS} between muscle groups in percentage change at POSTimm. Student's paired *t* tests were also used to assess within muscle-group differences in

RMS/M_{max} between the first and the last 5-s windows during the 2-min MVC and to determine between muscle-group differences during the last 5-s window of the 2-min MVC. (ii) Then two-way ANOVA with repeated measures (upper/lower limbs × time) were used to test differences during the recovery time for changes in mechanical and EMG parameters. When significant main effects were observed, Bonferroni's test was used for *post hoc* analysis. Comparison of repeated VA_{TMS} during the recovery time was performed using Friedman's test. *Pos hoc* analysis with Mann–Whitney's *U* test was conducted. (iii) Because we were also interested in determining whether there were any differences in force (normalized to PRE values) during the 2-min MVC between KE and EF, multiple pairwise comparisons were performed using the Student's paired *t* test with a Bonferroni-adjusted *P* value for 24 comparisons of < 0.002, comparing KE and EF values for each successive 5-s window for the duration of the 2-min MVC. Statistical analyses were conducted using IBM™ SPSS™ Statistics (version 23.0.0; IBM Corp., Somers, New York, NY). Statistical significance was set at $P < 0.05$.

RESULTS

MVC and central fatigue. Mean force profiles for each 5-s window during the 2-min MVC contractions for both KE and EF are presented in Figure 2A. The force decreased in a comparable manner until 35 s. Then, the difference in force between KE and EF became visually appreciable from 40 s, and this difference reached significance at 95 s when KE force was 26% of baseline and EF 35% of baseline ($P = 0.0007$). KE force remained significantly lower than EF until the end of the sustained contractions (mean normalized difference of PRE MVC of 8% from 95 s to 120 s). After the 2-min contractions, MVC decreased to 30% ± 10% ($P < 0.001$) and 42% ± 8% ($P < 0.001$) of PRE values at POSTimm for KE and EF, respectively. For the recovery period, there was a significant time effect ($P < 0.001$) and limb–time interaction ($P = 0.006$). Student's paired *t* tests show that the decrease in MVC was 12% greater in KE than EF at POSTimm ($P = 0.003$; Fig. 2B).

At the end of the 2-min MVC, RF and BB RMS/M_{max} decreased to 42% ± 17% ($P < 0.001$) and 65% ± 26% ($P = 0.001$) of initial values, respectively. Student's paired *t* tests show that the decrease in RMS/M_{max} was 23% greater in RF than BB ($P = 0.03$) when compared with the values at the beginning of the 2-min MVC.

VA_{TMS} responses are presented in Figure 3. VA_{TMS} significantly decreased from PRE to POSTimm both for KE (from 97% ± 2% to 41% ± 24%, $P = 0.03$) and EF (from 98% ± 2% to 65% ± 21%, $P = 0.03$). There was a significant time effect during the recovery period ($P < 0.001$ for both limbs). Wilcoxon signed-rank test showed that the decrease in VA_{TMS} was 25% greater in KE than EF at POSTimm ($P = 0.04$).

Peripheral function. Twitch responses are presented in Figure 4. Normalized TW_{Pot} decreased to 26% ± 10% ($P < 0.001$) and 14% ± 13% ($P < 0.001$) of PRE values at POSTimm for KE and EF, respectively. For the recovery period, normalized TW_{Pot} showed significant muscle ($P = 0.004$) and time ($P < 0.001$) effects but not a limb–time interaction ($P = 0.26$). Student's paired *t* tests showed that the decrease in the normalized EF TW_{Pot} was 12% greater than that in KE at POSTimm ($P = 0.017$).

M_{max100} results are presented in Figure 5. Between PRE and POSTimm, M_{max100} showed a 28% ± 30% ($P = 0.009$) and a 56% ± 33% ($P = 0.001$) increase for RF and BB, respectively. A significant time effect ($P < 0.001$) and

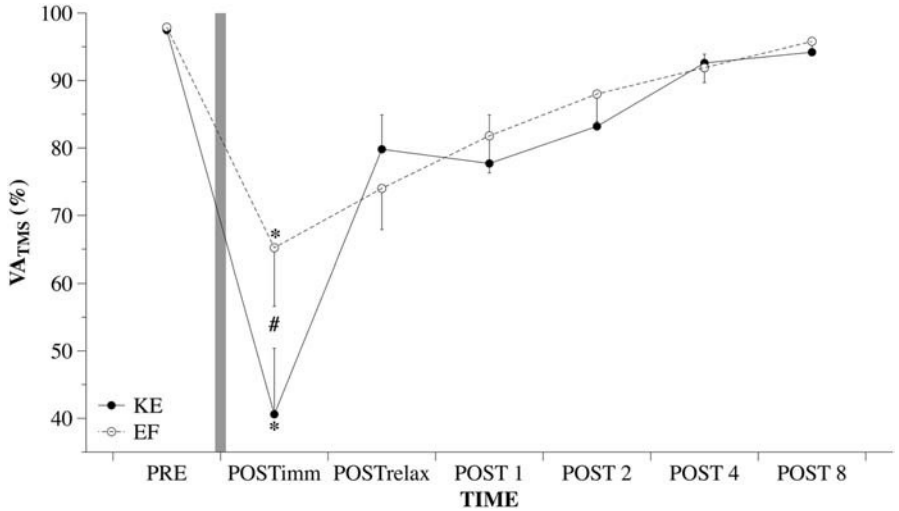

FIGURE 3—Changes in VA determined by VA_{TMS} after the 2-min MVC (*shaded area*) for KE and EF. At the end of the 2-min MVC a neuromuscular function evaluation was performed as an extension of the 2-min MVC (POSTimm) and additional evaluations were performed after 5 s of relaxation (POSTrelax) and 1 (POST 1), 2 (POST 2), 4 (POST 4) and 8 (POST 8) min after the end of the 2-min MVC. VA_{TMS} was calculated using the estimated resting twitch for each subject. Only the values of those subjects with regressions of $r \geq 0.9$ between the voluntary torque and SIT torque are plotted. Values are means ± SEM. Asterisks denote within limb differences between PRE and POSTimm by means of Wilcoxon signed-rank test: *$P < 0.05$ for KE and EF. Number sign (#) denotes differences between KE and EF at POSTimm by means of Mann–Whitney's *U* test: $P = 0.04$.

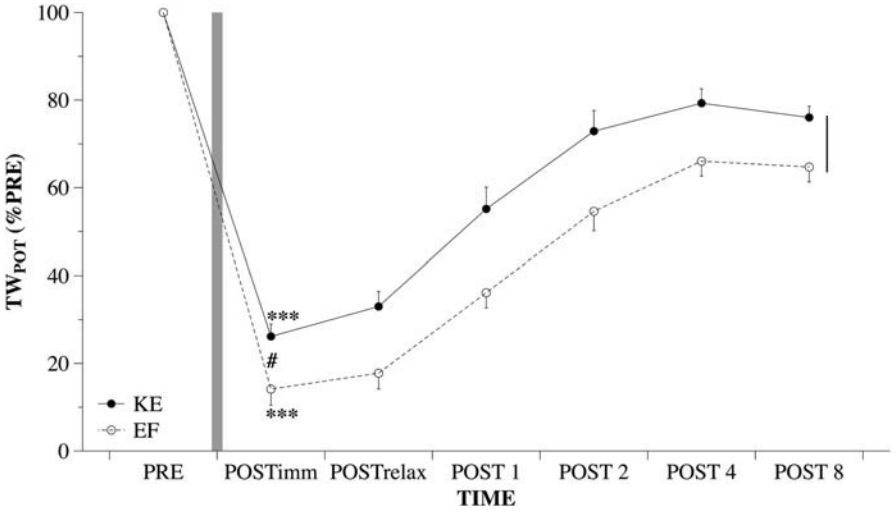

FIGURE 4—Changes in normalized potentiated peak twitch (TW_{Pot}) after the 2-min MVC (*shaded area*) for KE and EF. At the end of the 2-min MVC a neuromuscular function evaluation was performed as an extension of the 2-min MVC (POSTimm) and additional evaluations were performed after 5 s of relaxation (POSTrelax) and 1 (POST 1), 2 (POST 2), 4 (POST 4), and 8 (POST 8) min after the end of the 2-min MVC. Values are means ± SEM and were normalized as a percentage of the PRE evaluation. *Asterisks* denote within limb differences between PRE and POSTimm by means of Student's paired *t* test: ***$P < 0.001$ for KE and EF. *Vertical bar* denotes significant limb effects during the recovery period. Number sign (#) denotes differences between KE and EF at POSTimm by means of Student's paired *t* test: $P = 0.017$.

muscle–time interaction ($P = 0.031$) were observed during the recovery period. Student's paired *t* tests show that the increase in M_{max100} was 28% greater in BB than RF at POSTimm ($P = 0.04$).

Corticospinal excitability and inhibition. Normalized MEP_{100} are presented in Figure 5. MEP_{100} showed a 59% ± 79% ($P = 0.03$) and a 37% ± 35% ($P = 0.003$) increase for RF and BB, respectively, between PRE and POSTimm. During the recovery period, MEP_{100} showed significant muscle ($P = 0.027$) and time ($P = 0.009$) effects but no limb–time interaction ($P = 0.50$). Student's paired *t* tests showed that there were no differences at POSTimm between RF and BB ($P = 0.48$).

SP_{100} are presented in Figure 5. SP_{100} was 16% ± 14% ($P = 0.002$) and 34% ± 40% ($P = 0.01$) longer for RF and BB, respectively, at POSTimm than PRE. SP_{100} showed significant muscle ($P = 0.023$) and time ($P < 0.001$) effects but not a limb–time interaction ($P = 0.22$) during

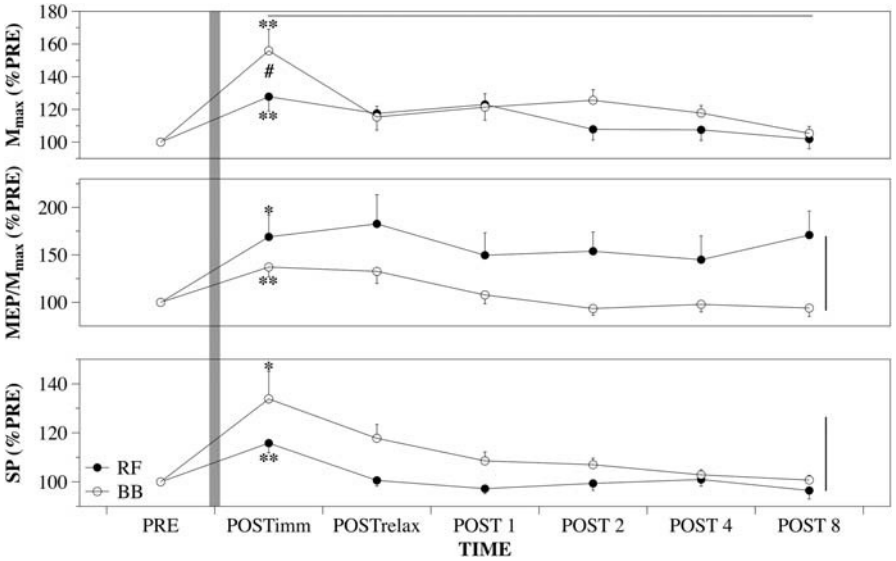

FIGURE 5—Changes in the M_{max}, MEP normalized to M_{max} (MEP/M_{max}) and in the SP, as index of corticospinal inhibition, after the 2-min MVC (shaded area) for KE (RF) and EF (BB). At the end of the 2-min MVC a neuromuscular function evaluation was performed as an extension of the 2-min MVC (POSTimm) 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 MVC. Values are means ± SEM and were normalized as a percentage of the PRE evaluation. Asterisks denote within muscle differences between PRE and POSTimm by means of Student's paired *t* test: *$P < 0.05$; **$P < 0.01$. *Horizontal bar* denotes significant limb–time interactions during the recovery period for M_{max}. *Vertical bars* denote significant muscle effects during the recovery period for MEP/M_{max} and SP. Number sign (#) denotes differences between RF and BB at POSTimm by means of Student's paired *t* test: $P = 0.04$.

the recovery period. Student's paired *t* tests showed that there were no differences at POSTimm between RF and BB ($P = 0.15$).

DISCUSSION

This study examined for the first time whether fatigue magnitude and etiology (including corticospinal characteristics) and recovery postexercise are similar between upper and lower limbs in the same subjects and for the same 2-min sustained maximal isometric-exercise model. By using the same subjects (i.e., within-subjects design), we were able to minimize the influence of subject motivation and fitness. Thus, each subject served as his own control, and this type of study design provides much greater power than a between-subjects design. Our results show that relative maximal strength loss and central fatigue (VA_{TMS} reduction) immediately after the 2-min MVC were higher in KE than EF, whereas TW_{Pot} (an index of peripheral function) showed the opposite result. Finally, the changes in MEP were not significantly different between the muscles and SP was greater in EF.

Magnitude of fatigue and recovery in upper versus lower limbs. MVC force decreased progressively during the 2-min sustained contraction for both KE and EF. The relative force drop was comparable for both KE and EF for the first 35 s, after which KE showed a greater decrease (significantly different force level from 95 s) until the end of the task. This allows direct comparison of the time-course of fatigue in upper vs lower limbs for the first time. Indeed, at the end of the 2-min contractions, MVC had decreased by 70% in KE compared with baseline and by 58% in EF. Previous studies reported similar strength reductions to the present one after sustained isometric MVC protocols. For example, Goodall et al. (15) and Kennedy et al. (9) showed that after a 2-min KE MVC force decreased by a value (~70%) similar to our results. Compared with the EF force reduction in this study (−58%), similar (10) or slightly greater (14) force reductions were reported after 2-min sustained isometric EF MVC. In the present study, we showed for the first time in the same subjects that MVC force loss was significantly greater for KE than EF at POSTimm, with force gradually recovering and approaching baseline values for both muscle groups after 8 min of recovery (Fig. 2). A lack of motivation or a pacing strategy are potential confounding factors, but were considered unlikely in this study. Our subjects were young, motivated, healthy, and physically active adults who were both instructed to perform a real MVC throughout the 2 min and strongly encouraged during the experiments by the investigators. We are confident that the subjects performed a true maximal isometric effort throughout the 2 min, and this is supported by (i) the near maximal VA_{TMS} values at PRE (97% and 98% for KE and EF, respectively) confirming that the subjects voluntarily activated the muscle groups near their maximum and (ii) the lack of significant difference between peak force during MVC PRE and force measured 5 s after the start of the 2-min maximal contraction ($P = 0.45$ and $P = 0.21$ for KE and EF, respectively).

Both muscle groups exhibited a marked reduction in MVC force at POSTimm. This substantial neuromuscular fatigue could be partially attributed to ischemia in the working muscles. Indeed, it has been shown that during sustained muscle contractions, intramuscular pressure at 50% to 60% of the initial MVC is sufficient to occlude blood flow of the contracting muscle (28,29), that is, a force level above which the subjects were for about 1 min. Of note is the fact that KE force was significantly lower compared to EF from 95 s until the end of the 2-min MVC. Also, KE force decreased below 50% MVC earlier compared to EF (Fig. 2A). Thus, KE experienced a greater amount of time compared to EF below this threshold, suggesting that the relative blood flow occlusion was less. This could have induced the EF to develop greater peripheral fatigue compared to KE (in terms of TW_{Pot} decrement) because of the longer time spent under more severe ischemia. However, this part of the discussion is quite speculative, as the present study was not designed to evaluate if muscle-related differences in occlusion contributed to muscle-related differences in fatigability.

Etiology of fatigue and recovery in upper versus lower limbs. As expected, both central (i.e., reduced voluntary drive) and peripheral fatigue that developed during both upper- and lower-limb exercise gradually recovered after the termination of the sustained contraction.

The presence of peripheral fatigue is confirmed by the impairment of TW_{Pot}, reduced by 74% and 86% in KE and EF, respectively. This reduction in twitch force is higher than previously reported for both KE (11) and EF (14). Yet, our results confirm the findings from Neyroud et al. (17), who found that EF contractile responses were more affected by fatigue than KE. Even if not measured in the present study and not unanimously recognized (30), EF seems to present a relatively larger proportion of type II fibers than KE (31,32). If that is the case, a possible explanation for this result is that muscle fiber distribution may influence changes in contractile properties after a fatiguing exercise because a greater decrease in TW_{Pot} after fatiguing exercise has been shown in muscle groups presenting a higher proportion of type II fibers (33). Interestingly, TW_{Pot} did not fully recover for either KE or EF by 8 min after exercise cessation and, the initial intermuscle group difference in peripheral failure at POSTimm remained throughout the recovery period. Further, M_{max100} increased in both muscles during the 2-min MVC and was 28% larger at POSTimm in BB than RF. Despite MVC force and TW_{Pot} declining, M_{max} increased in size, suggesting that excitation had not failed, at least not at the sarcolemmal level. Thus, reduced sarcolemmal excitability was not responsible for the differences in muscle fatigue occurring during the sustained maximal isometric exercise.

VA_{TMS} decreased from 97% at PRE to 41% at POSTimm in KE and from 98% to "only" 65% in EF. This decrease in the ability to drive the KE and EF muscles maximally indicates that central fatigue developed during the 2-min MVC. Normalized MEP changes were not different between the two muscle groups (Fig. 5). Further, although SP

duration increased in both KE and EF as expected (9,10), the SP increased significantly more for EF than KE (Fig. 5).

Because the relationship between force output and VA_{TMS} remains linear ($r > 0.9$) with fatigue in our study, as previously demonstrated for both KE (15) and EF (23), it was possible to estimate the contribution of supraspinal fatigue to the total force loss. For each subject, the linear force–VA_{TMS} relationship was determined immediately after task failure (34,35). Using the regression equation, the force corresponding to preexercise VA_{TMS} was determined and compared with the real postexercise MVC. Any additional force loss was interpreted to be due to supraspinal fatigue, which accounted for approximately 26% and approximately 23% of the total force loss in KE and EF, respectively. These results are in line with previous findings that supraspinal fatigue is relatively modest (accounting for ~25% of the force loss) for 2-min sustained MVC (7,8).

Group III–IV muscle afferents are known to be sensitive to mechanical and metabolic stimuli associated with muscle contractions (36). After a fatiguing exercise, the activity of group III–IV muscle afferents reduces VA of the fatigued muscle (11). However, the action of these afferent fibers does not seem to mediate the reduction of the excitability of the motor cortex or the corticospinal pathway after fatiguing exercises in the lower limbs (9), even though the contribution of factors "upstream" of the output of the motor cortex cannot be completely ruled out (14). Interestingly, group III–IV muscle afferents likely play a facilitatory role in EF spinal motoneurons (16) with negligible effects on KE motoneurons (9). A recent study looked at the role of group III–IV muscle afferents during a whole-body endurance exercise in subjects where feedback from these afferents was temporarily blocked with intrathecal fentanyl (37). When considering the changes from fentanyl to control conditions, the authors found a positive relationship between increasing levels of intramuscular metabolite concentrations and decrease in the potentiated KE twitch torque (as an index of peripheral fatigue). This relationship suggests a progressive activation of these afferents that limits descending central drive, acting as a protective mechanism against peripheral fatigue (37). At the end of the 2-min MVC, VA_{TMS} decreased to 42% and 66% of the initial values for KE and EF, respectively, and this is supported by the decrease in RMS/M_{max} (to 42% and 65% of initial values for KE and EF, respectively). Because this decrease was significantly larger in KE than EF, we suggest that group III–IV muscle afferents may have a lesser role in the upper limbs compared with the lower limbs, explaining the smaller decrease in the neural drive during the 2-min MVC in EF muscles that may have led to greater peripheral fatigue.

CONCLUSIONS

The present study is the first to show that when the mechanisms of fatigue and recovery after a sustained maximal isometric exercise model are compared in the same subjects, upper and lower limbs present different magnitudes of total, central, and peripheral fatigue. Maximal force loss, as an index of global fatigue, was greater in the lower limbs compared with the upper limbs. Although there were no between-limb differences in corticospinal excitability changes, VA assessed after the 2-min MVC, as well as neural drive assessed at the end the 2-min MVC, decreased significantly more in the lower limbs, whereas the upper limbs presented a greater increase in corticospinal inhibition and peripheral fatigue, the latter factor probably being related to the attenuated reduction of neural drive in the upper limbs. Thus, neuromuscular fatigue after a maximal exercise is specific to the limb performing the exercise from both functional and neurophysiological points of view.

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No conflicts of interest, financial or otherwise, are declared by the authors. Further, the authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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