Performance fatigability and recovery after dynamic multi-joint maximal exercise in elbow flexors versus knee extensors

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Abstract

Elbow flexors (EF) and knee extensors (KE) have shown differences in performance fatigability and recovery of neuromuscular function after isometric and isotonic single-joint fatiguing contractions. However, dynamic multi-joint movements are more representative of real-world activities. The aim of the study was to assess central and peripheral mechanisms of fatigability after either arm-cranking or cycling. Ten physically-active men performed maximal incremental arm-cranking and cycling until task-failure. Maximal voluntary isometric contraction (MVIC) and electrically-evoked forces of both EF and KE were assessed before (PRE) and 1 (POST) and 20 (POST20) min after exercise. At POST, MVIC decreased similarly to 76 ± 8% and 81 ± 7% (both $P < 0.001$) of PRE for EF and KE, respectively. MVIC force remained lower than PRE at POST20 for both EF and KE (85 ± 8% vs. 95 ± 3% of PRE, $P \leq 0.033$), having recovered less in EF than KE ($P = 0.003$). Electrically-evoked forces decreased similarly from PRE to POST in EF and KE (all $P > 0.05$). At POST20, the ratio of low-to-high frequency doublets was lower in EF than KE (75 ± 13% vs. 85 ± 10% of PRE; $P \leq 0.034$). Dynamic maximal incremental exercise acutely induced similar magnitudes of MVIC and evoked forces loss in EF and KE. However, at POST20, impaired MVIC recovery and lower ratio of low-to-high frequency doublets in EF compared to KE suggests the recovery of neuromuscular function after dynamic maximal exercises is specific to and dependent on changes within the muscles investigated.

Key words: arm cranking, cycling, incremental maximal exercise, fatigue, recovery

Running Head: Neuromuscular function after dynamic multi-joint exercise
INTRODUCTION

Performance fatigability is a decline in an objective measure of performance over a discrete period of time due to changes within the neuromuscular system (1). These changes often manifest by reducing the maximal voluntary isometric contraction (MVIC) force that can be produced (2). Impairments in force production can originate from one or more sites in the neuromuscular system and can be classified as central (i.e. proximal to the neuromuscular junction and encompassing the brain and upper and lower motoneurons) (2) or peripheral (i.e. within the skeletal muscle) (3).

Fatigability of different muscle groups is of interest since daily-living (e.g. climbing stairs, carrying bags) and sporting (e.g. cycling, rowing) activities have different physical requirements. As a result, comparisons of the fatigability of upper- (UL) and lower-limb (LL) muscles have been investigated, most commonly comparing the elbow flexors (EF) and knee extensors (KE) during maximal and submaximal single-joint isometric contractions (4, 5). For example, Neyroud et al. (5) showed that MVIC force loss after sustained submaximal isometric contractions at 50% MVIC until task failure in EF and KE were not different (-40% versus -34% for EF and KE, respectively), with voluntary activation (VA) [i.e. the level of voluntary drive to the muscle during an exercise (6)] unchanged in either muscle group. Meanwhile, the decrease in the amplitude of high-frequency doublets was greater in EF than KE (-59% versus -28%, respectively). Vernillo et al. (4) showed that after a 2-min sustained MVIC, the decreases in MVIC force and VA were ~12% and ~25% greater in KE than EF, respectively, while the decrease in the potentiated twitch amplitude was greater in EF than KE (-86% versus -74%, respectively).

While these comparisons provide a foundation for understanding differences in fatigability between muscle groups, they lack applicability to the real world where dynamic exercises are usually performed (7).
To elucidate the potential differences in the mechanisms of fatigability between UL and LL during dynamic exercises, Senefeld et al. (8, 9) investigated fatigability after 90 submaximal isotonic EF or KE contractions at maximal voluntary shortening velocity and observed the loss in MVIC torque was ~15% greater in EF than KE. However, these results from dynamic single-joint contractions have not been confirmed by exercise comprising multi-joint contractions such as arm-cranking and cycling. Specifically, to the best of our knowledge, only Halperin et al. (10) investigated the fatigability induced by ten 10-s arm-cranking and cycling sprints (with 30 s or 180 s of rest between sprints) and reported that MVIC decreased ~9.5% less in EF than KE with recovery conditions pooled. However, as previously suggested (11, 12), effects of muscle group on performance fatigability may arise from different characteristics of the fatiguing exercise task (e.g. intermittent sprint exercise vs repeated isotonic contractions; multi-joint vs single-joint). Since the metabolic responses to exercise are quantitatively different between UL and LL across different exercise intensities (13–15), it is of interest to investigate whether dynamic multi-joint incremental exercise may affect performance fatigability differently in EF and KE.

The ability for neuromuscular function to recover after a bout of exercise may impact the ability to perform subsequent exercise bouts even when interspersed with periods of rest. Thus, to understand beyond immediately post-exercise, MVIC force recovery must be considered. Previous studies have shown that the magnitude and mechanisms of recovery after fatiguing exercise are related to the characteristics of the preceding exercise bout (12) and may be different between muscle groups (4, 9, 16). For example, Vernillo et al. (16) showed that after a sustained 2-min MVIC, MVIC force gradually recovered and returned to baseline values for both EF and KE within 4 min of recovery; whereas Senefeld et al. (9) reported that EF MVIC force loss was ~15% lower than KE MVIC force 10 min after completing 90 submaximal isotonic contractions at maximal voluntary shortening velocity.
The recovery of performance fatigability resulting from single-joint isometric versus multi-joint isotonic and dynamic intermittent exercise cannot be interchangeable (12). However, there is a lack of information about MVIC force recovery following dynamic multi-joint exercise that regularly presents in daily-living activities. Therefore, it is of scientific and practical interest to investigate whether differences in cardiorespiratory demand and muscles involved in this type of exercise differently affect recovery of neuromuscular function in EF and KE. Therefore, the primary aim of this study was to evaluate the magnitude and aetiology of neuromuscular function changes in EF and KE from a dynamic multi-joint maximal incremental exercise on either an arm-cranking or a cycle ergometer. We hypothesized there would be a larger MVIC force decrease in EF than KE after dynamic multi-joint maximal exercise due to greater contractile function impairment in EF. This is because UL muscles have a lower oxidative capacity than LL muscles, resulting in a higher reliance on anaerobic metabolism (11, 28, 35), lower lactate handling capacity and, consequently, higher lactate production at a similar relative exercise intensity (22, 27). This hypothesis contrasts with the findings of Halperin et al. (11), who used repeated sprint exercise involving much shorter exercise bouts. A secondary aim was to investigate the recovery in neuromuscular function 20 minutes after termination of maximal incremental exercise in UL and LL. We hypothesized that there would be less recovery of neuromuscular function in EF than KE since recovery depends on the characteristics of the fatiguing exercise task (12) and the expected greater contractile function impairment in EF, compared to KE, may delay recovery of neuromuscular function (9).

MATERIALS AND METHODS

Participants

After a maximal incremental exercise on either an arm-cranking ergometer or a cycling ergometer performed by the same participants during pilot testing, the effect size of the
difference between EF and KE for the pre-to-post change in the main outcome (MVIC force) was 1.70. Using this value, an $\alpha$ [threshold probability for rejecting the null hypothesis (type I error)] at 0.05 and a $\beta$ [probability of failing to reject the null hypothesis under the alternative hypothesis (type II error)] at 0.2, a sample size of five participants was determined to be sufficient to detect statistical changes. Accounting for potential dropouts, ten young, healthy, and physically active men volunteered to participate in the study (age: 24 ± 2 years; body mass: 72 ± 8 kg; height: 177 ± 6 cm). Participants were not involved in any structured training program either for UL or LL, had no history of neuromuscular or cardiovascular disease, and had not suffered a recent UL or LL injury. They were informed about the experimental protocol and all associated risks before providing written informed consent. All procedures conformed to the Declaration of Helsinki and were approved by the local Ethics Committee (BESTA/IBFM, Report #43, 8/11/2017).

**Experimental design**

Each participant completed one familiarization session and two experimental sessions. All sessions were separated by 3 to 7 days and performed at the same time of day. Participants were instructed to avoid the consumption of caffeine on the day of the experiment and avoid performing any strenuous exercise during the 48 h prior to testing. During the familiarization session, participants performed anthropometric measurements, and maximal/submaximal isometric contractions of EF and KE of the dominant limb on customized ergometers, with and without peripheral nerve (EF and KE) and muscle (EF) stimulation. Participants’ limb dominance was assessed using the Revised Waterloo Footedness Questionnaire (17). All participants were right limb dominant for both arms and legs. The two experimental sessions were performed in a pseudo-randomized and counterbalanced order and consisted of a maximal incremental exercise to task failure on either an arm-cranking ergometer or a cycle ergometer. Cardiorespiratory and metabolic...
responses to exercise were monitored during the incremental exercise. Before (PRE), exactly 1 min (POST) and 20 min (POST20) after exercise cessation, neuromuscular function evaluation of either EF or KE muscles was conducted (Figure 1A). The cycle and arm-crank ergometers were positioned beside the custom-built ergometers utilized for neuromuscular function evaluation to enable the quickest transition possible at the end of the incremental exercise. The 1-min delay to POST measurements was the shortest that was consistently feasible in pilot testing.

**Anthropometric measurements**

With the participant standing erect and the feet slightly apart, the height above the floor and the circumference were taken at seven sites on the right leg and arm. The levels were marked with a dermatograph pencil; the circumferences measured with a flexible steel metric tape and the distance from the floor level measured with a digital reading anthropometer (3.0, Itiesse s.a.s, Verona, Italy). Skin-fold thicknesses were also measured at the same sites with a skinfold caliper (Holtain Tanner/Whitehouse Skinfold Caliper, Crymych, United Kingdom). The following formula to calculate the volume of a truncated cone was applied to the six truncated cones:

\[
\frac{1}{3} h(a + \sqrt{ab} + b) \quad [\text{Equation 1}]
\]

where \(a\) and \(b\) are the areas of two parallel surfaces derived from circumference measurements. Then, muscle mass was calculated according to Jones and Pearson (18) and a muscle density of about 1.0597 g/cm\(^3\). UL (i.e. two upper limbs) estimated muscle mass resulted in 9.0 ± 1.0 kg and LL (i.e. two lower limbs) estimated muscle mass resulted in 16.2 ± 1.8 kg.

**Maximal ramp-incremental exercise**

UL maximal incremental exercise was conducted on an arm-cranking ergometer (Cardio Rehab 891E, Monark, Vansbro, Sweden) with the hands in a pronated position. The warm-
up was set at 35 W for 1 min and power output increased thereafter by 9 ± 4 W every minute (depending on the participant’s fitness level) until task failure. During the test, the participants were instructed to “pull more than push” to preferentially target the biceps brachii (BB). LL maximal incremental exercise was conducted on a cycle ergometer (Corival V2, Lode, Groningen, Netherlands). The warm-up was set at 60 W for 1 min and power output increased thereafter by 23 ± 11 W every minute (depending on the participant’s fitness level) until task failure. The exercise protocols were designed to match the time to task failure in both arm-cranking and cycling tests (19). Tests were terminated when participants were no longer able to maintain the arm-cranking or pedalling cadence required (60 ± 2 rpm) for at least 10 s, despite vigorous verbal encouragement.

Neuromuscular function evaluation

During the neuromuscular function evaluation (Figure 1B for EF; Figure 1C for KE) participants contracted to maximal force (for 5 s) and once the maximal force was attained and plateaued a high-frequency (100 Hz) paired pulse was delivered. At the end of the MVIC, a set of high- and low-frequency (100 and 10 Hz) paired pulses followed by a single pulse, all separated by 2 s, were delivered to the relaxed muscle (20). Electrical stimuli were delivered to the right femoral nerve for KE and BB motor point for EF (since stimulation of the brachial plexus leads to contraction of both agonist and antagonist muscles). For EF only, an additional single supramaximal stimulus was delivered to the brachial plexus 2 s later with the muscle relaxed to elicit maximal M-waves (M\text{max}) (4, 5). Visual feedback of the force produced was provided to the participants by means of a real-time display on a computer screen.

****Figure 1 about here****

Data Collection
Force and Electromyographic (EMG) Recordings

Muscle force data were obtained from voluntary and evoked isometric contractions. EF force was assessed by a calibrated force transducer (SML load cell, Interface, Scottsdale, AZ, USA) attached by a noncompliant strap to the wrist and to the rigid dynamometer (Figure 1B). Participants were seated upright in a custom-built dynamometer with both right shoulder and elbow joints at 90° of flexion, and the forearm in a supinated position. KE force was measured by a calibrated force transducer (SML load cell, Interface) attached by a noncompliant strap to the right leg immediately proximal to the malleoli of the ankle joint and to the rigid dynamometer (Figure 1C). Participants were seated upright in a custom-built dynamometer with knee and hip angles of 120° (180° corresponding to full extension) (21), and secured by chest and hip straps. Force was collected at a sampling rate of 2000 Hz and analog-to-digitally converted (Load Cell Adapter, Delsys, Natick, MA, USA).

During isometric contractions, EMG signals of EF (BB) and KE [vastus lateralis (VL)] were recorded with pairs of self-adhesive surface electrodes in a bipolar configuration (Trigio EMG sensor, Delsys) positioned over the muscle belly (22). EMG signals were digitalized at a sampling rate of 2000 Hz and band-pass filtered (20-450 Hz, 40/80 dB/dec).

Peripheral stimulation

All single and paired-pulse electrical stimuli (200-µs duration) were delivered via constant-current stimulator (DS7AH, Digitimer, Welwyn Garden City, Hertfordshire, UK). During EF evaluation, the intramuscular nerve fibres of BB were stimulated using a cathode (H135SG, Covidien, Mansfield, USA) located over the BB muscle belly and anode (H135SG, Covidien,) over the bicipital tendon. This site was selected since stimulation of the brachial plexus leads to contraction of both the agonist and antagonist muscles. The brachial plexus was also stimulated for M-wave measurement using a
cathode (H135SG, Covidien) securely taped into the supraclavicular fossa and rectangular anode (50 × 90 mm Durastick, DJO Global, USA) placed over the acromion. During KE evaluation, stimuli were delivered to the right femoral nerve using a surface cathode securely taped into the femoral triangle (H135SG, Covidien) and rectangular anode (50 × 90 mm Durastick, DJO Global) in the gluteal fold. Stimulus intensity was always determined by single stimuli delivered with increasing intensity in the relaxed muscle state until M-wave and twitch amplitudes plateaued. A stimulus intensity of 120% of the maximal intensity was used for the evaluation of neuromuscular function (153 ± 51 mA for BB muscle belly stimulation; 125 ± 29 mA for brachial plexus stimulation; 149 ± 34 mA for femoral nerve stimulation).

**Cardiorespiratory and metabolic responses to exercise**

To determine that participants reached maximal effort as well as the amount of work performed, cardiorespiratory and metabolic data were captured during incremental exercise. Pulmonary ventilation (VE), O2 consumption (VO2) and CO2 output (VCO2) were continuously assessed breath-by-breath via a metabolic cart (Vyntus CPX, CareFusion, Germany). Respiratory exchange ratio (RER) was calculated as VCO2/VO2. Before each test, gas analysers and turbine flowmeter were calibrated. Heart rate (HR) was recorded using a HR chest band (H7; Polar, Finland) throughout each test. At the end of each incremental exercise, the rate of perceived exertion (RPE) was determined using the Borg 6-20 scale (23). At rest and at discrete time intervals during the recovery period (3, 5, 7 min), 20 μL of capillary blood was collected from pre-heated earlobe for the determination of blood lactate concentration ([La]b) by electroenzymatic analyser (Biosen C-line, EKF, Germany). The test was considered maximal when at least two of the following criteria were observed: (i) RPE > 15; (ii) peak HR (HRpeak) > 95% of the age-predicted maximum; (iii) RER ≥ 1.1; and (iv) peak [La]b > 8 mmol·L⁻¹ (24). The gas
exchange threshold (GET) was visually, individually and independently determined by two blinded expert investigators using both the V-slope method and secondary criteria (24).

**Data Analysis**

**Cardiorespiratory and metabolic responses to exercise**

Data analyses were performed using Prism 8.0 (GraphPad, Software Inc., San Diego, CA, USA) and Excel (Office 365, Microsoft Inc., Redmond, WA, USA). Peak power output ($P_{peak}$) was defined as the highest power output recorded before task failure. Data obtained during the last 20 s of the incremental tests were considered peak values. The highest $[La]_b$ value obtained during the recovery was considered as the peak value ($[La]_{peak}$) and retained for further analysis. The amount of total work performed during each test was calculated as:

$$Work = \sum_1^0 W_i \times t_i \quad [Equation \ 2]$$

where $W$ is the power output of each step (i) during the incremental exercise and $t$ is the duration of each step (i) at that power output. Then the total amount of work was normalized per the estimated muscle mass involved in the exercise (see “anthropometrics” paragraph).

**Neuromuscular Function**

Data were analysed offline using EMGworks (version 4.5, Delsys). MVIC force was considered as the greatest force before the delivery of electrical stimulation. To quantify impairments to central nervous system drive, EF and KE VA was assessed by twitch interpolation (Figure 2) using the superimposed (sDb100) and potentiated high-frequency doublets (Db100) during and after MVIC and calculated from the equation (25):

$$VA (\%) = \left[ 1 - \left( \frac{\text{sDb100}}{\text{Db100}} \right) \right] \times 100 \quad [Equation \ 3]$$

****Figure 2 about here****
where $sDb_{100}$ was calculated as the difference between the voluntary force pre-stimulus and the peak force immediately after.

Changes to skeletal muscle function were assessed by changes in the amplitudes of potentiated twitch ($Tw_{pot}$; muscle contractile properties), $Db_{100}$ and the ratio of low- and high-frequency doublets ($Db_{10:100}$) to assess changes in excitation-contraction coupling (26). Maximal rate of force development from $Tw_{pot}$ ($RFDTw$) was calculated as the instantaneous slope from the ascending part of the force-time curve. Peak-to-peak amplitude, area and duration of $M_{max}$ elicited by brachial plexus or femoral nerve electrical stimulation for BB and VL, respectively, were measured to assess action potential propagation along the sarcolemma. Area and duration were determined from the initial deflection from baseline to the second crossing of the horizontal axis (27). All data at POST and POST20 were normalized as a percentage of the PRE evaluation except for VA, for which the raw data are presented.

**Statistical analysis**

Results are presented as means ± SD. Standardized Cohen's effect size ($ES$) with Hedges’ $g$ correction and [95% confidence interval] were also computed (28). The data were tested for normality using a Shapiro-Wilk $W$-test. Student’s paired $t$-tests were used to determine differences in cardiorespiratory and metabolic responses to maximal incremental exercise between arm cranking and cycling. Repeated-measures ANOVAs with time (PRE, POST, POST20) and muscle (EF, KE) as within-participant factors were used to evaluate changes in neuromuscular function parameters. Sphericity was checked using Mauchly's test. For all parameters, Mauchly’s test of sphericity indicated that the assumption of sphericity had not been violated (all $P \geq 0.184$). When significant main effects or interactions were observed, Bonferroni's test was used for *post-hoc* analysis. Pearson product moment correlation coefficient ($r$) was used to examine the relationship between EF and KE MVIC.
force loss after exercise. Precision of estimates is indicated as [95% confidence intervals] (29). Statistical analyses were conducted using IBM\textsuperscript{TM} SPSS\textsuperscript{TM} Statistics (version 26.0.0; IBM Corp., Somers, New York, NY) with the criterion $\alpha$-level set to 0.05.

**RESULTS**

**Maximal incremental exercise**

Table 1 shows the cardiorespiratory and metabolic variables during the incremental exercises. Time to task failure for the incremental exercise was not different between UL and LL ($P = 0.190$, $ES = 0.5 [-1.2; 0.1]$). Peak $\dot{V}E$, $\dot{V}O_2$, $\dot{V}CO_2$ and RER and $P_{peak}$ values were lower in UL compared to LL (all $P \leq 0.039$). Normalized $\dot{V}O_{2peak}$ values were lower in UL than LL ($36.0 \pm 8.1 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ versus $48.4 \pm 6.3 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P < 0.001$, $ES = 2.4 [-4.0; -1.3]$). Time spent above GET was ~19% longer in UL than LL ($391 \pm 86$ s versus $307 \pm 71$ s, respectively, $P = 0.043$, $ES = 0.8 [-0.1; 1.8]$). Both $[\text{La}]_b$ ($9.0 \pm 2.7 \text{ mmol} \cdot \text{L}^{-1}$ versus $10.3 \pm 2.1 \text{ mmol} \cdot \text{L}^{-1}$, $P = 0.163$, $ES = -0.7 [-1.6; 0.2]$) and RPE ($17 \pm 1$, $P = 0.351$, $ES = -0.3 [-1.0; 0.3]$) were not different between UL and LL. The total work, normalized per unit of estimated muscle mass, performed during UL and LL incremental tests was not different ($7945 \pm 2190 \text{ J} \cdot \text{kg}^{-1}$ of estimated muscle mass versus $9017 \pm 1443 \text{ J} \cdot \text{kg}^{-1}$ of estimated muscle mass, $P = 0.138$, $ES = 0.6 [-0.2; 1.3]$).

**Performance fatigability**

MVIC force (Figure 3) showed time ($F_{(2,36)} = 83.7$, $P < 0.001$) and muscle ($F_{(1,18)} = 6.9$, $P = 0.017$) effects and a muscle $\times$ time interaction ($F_{(2,36)} = 2.7$, $P = 0.030$). MVIC force decreased to $76 \pm 8\%$ and $81 \pm 7\%$ of PRE values at POST for EF and KE (both $P < 0.001$), respectively, and there was no difference between muscles ($P = 0.238$, $ES = 0.5 [0.1; 1.1]$). At POST20, MVIC force remained lower than at PRE for both EF and KE.
although MVIC force increased from POST for both EF ($P = 0.008$, $ES = 1.1 \ [0.6; 1.7]$) and KE ($P < 0.001$, $ES = 1.8 \ [1.0; 3.0]$). MVIC force, as a percentage of PRE, was lower in EF than KE ($P = 0.003$, $ES = -1.3 \ [0.4; 2.4]$) at POST20.

A significant correlation (Figure 4) was found between force loss, expressed as percentage of the PRE evaluation, in EF and KE at POST ($r = 0.66 \ [0.1; 0.9]$, $P = 0.038$). No relationship was found for the same variables at POST20 ($r = 0.24 \ [-0.5; 0.8], P = 0.514$).

Voluntary activation

VA showed a muscle effect ($F_{(1,18)} = 7.6$, $P = 0.013$) where VA was higher in EF than KE. No time effect ($F_{(2,36)} = 3.2$, $P = 0.054$) or muscle $\times$ time interaction ($F_{(2,36)} = 0.1$, $P = 0.866$) were observed. VA was $97 \pm 2\%$ and $91 \pm 7\%$ at PRE for EF and KE, respectively, $93 \pm 5\%$ and $86 \pm 8\%$ at POST for EF and KE, respectively, and $93 \pm 7\%$ and $89 \pm 8\%$ at POST20 for EF and KE, respectively.

Electrically-evoked forces and M waves

$Tw_{pot}$ showed a time effect ($F_{(2,36)} = 110.2$, $P < 0.001$) but not a muscle effect ($F_{(1,18)} = 0.5$, $P = 0.500$) or a muscle $\times$ time interaction ($F_{(2,36)} = 0.3$, $P = 0.758$) (Figure 5A). $Tw_{pot}$ decreased to $51 \pm 12\%$ ($P < 0.001$, $ES = -4.2 \ [-6.6; -2.4]$) of PRE values at POST. At POST20, $Tw_{pot}$ remained lower than PRE ($63 \pm 11\%$ of PRE, $P < 0.001$, $ES = -3.6 \ [-5.7; -3.0]$) but greater than POST ($P = 0.008$, $ES = 0.6 \ [0.4; 0.8]$). $RFD_{Tw}$ showed a time ($F_{(2,36)} = 66.6$, $P = 0.021$) effect whereas no muscle ($F_{(1,18)} = 0.2$, $P = 0.688$) effect or muscle $\times$ time interaction ($F_{(2,36)} = 0.8$, $P = 0.466$) were observed. At POST, $RFD_{Tw}$ decreased to

****Figure 3 about here****

****Figure 4 about here****
54 ± 15% of PRE \((P < 0.001, \text{ES} = -3.3 [-5.5; -1.7])\). At POST20, RFDTw remained lower than PRE \((59 ± 20 \% \text{ of PRE}, \ P < 0.001, \text{ES} = -2.5 [-4.4; -1.2])\). \(\text{Db}_{100}\) showed a time effect \( (F(2,36) = 50.2, \ P < 0.001)\) but not a muscle effect \( (F(1,18) = 0.0, \ P = 0.963)\) or a muscle × time interaction \( (F(2,36) = 0.0, \ P = 0.998)\) (Figure 5B). \(\text{Db}_{100}\) decreased to 70 ± 11% \((P < 0.001, \text{ES} = -2.4 [-4.0; -1.2])\) of PRE values at POST. At POST20, \(\text{Db}_{100}\) remained significantly lower than PRE \((84 ± 8\% \text{ of PRE}, \ P < 0.001, \text{ES} = -1.8 [-3.1; -0.7])\) but greater than POST \((P < 0.001, \text{ES} = 1.0 [0.7; 1.4])\). \(\text{Db}_{10:100}\) showed a time effect \( (F(2,36) = 61.8, \ P < 0.001)\) and a muscle × time interaction \( (F(2,36) = 5.0, \ P = 0.012)\) but not a muscle effect \( (F(1,18) = 1.2, \ P = 0.282)\) (Figure 5C). \(\text{Db}_{10:100}\) decreased to 75 ± 11% and 72 ± 10% of PRE values at POST for EF and KE (both \(P < 0.001\)), respectively, and there was no difference between muscles \((P = 0.541, \text{ES} = 0.2 [-1.2; 0.7])\). At POST20, \(\text{Db}_{10:100}\) remained significantly lower than PRE for both EF \((75 ± 13\% \text{ of PRE}, \ P < 0.001, \text{ES} = -2.5 [-4.1; 1.2])\) and KE \((85 ± 10\% \text{ of PRE}, \ P = 0.002, \text{ES} = -2.1 [-3.5; -0.9])\). \(\text{Db}_{10:100}\) increased from POST to POST20 for KE \((P = 0.011, \text{ES} = 1.2 [0.7; 1.9])\) but not for EF \((P = 1.000, \text{ES} = 0.0 [-0.1; 0.1])\), resulting in \(\text{Db}_{10:100}\) greater in KE than EF \((P = 0.034, \text{ES} = 0.7 [-0.1; 1.6])\) at POST20. \(\text{M}_{max}\) peak-to-peak amplitude did not show a time \( (F(2,36) = 0.8, \ P = 0.441)\) or muscle \( (F(1,18) = 0.5, \ P = 0.496)\) effect or a muscle × time interaction \( (F(2,36) = 0.2, \ P = 0.817)\). \(\text{M}_{max}\) area did not show a time \( (F(2,36) = 2.9, \ P = 0.070)\) or muscle \( (F(1,18) = 0.0, \ P = 0.997)\) effect or a muscle × time interaction \( (F(2,36) = 0.3, \ P = 0.757)\). \(\text{M}_{max}\) duration also did not show a time \( (F(2,36) = 0.2, \ P = 0.799)\) or muscle \( (F(1,18) = 2.5, \ P = 0.129)\) effect or a muscle × time interaction \( (F(2,36) = 1.0, \ P = 0.364)\).

****Figure 5 about here****
This study compared the magnitude and aetiology of changes in neuromuscular function following maximal incremental exercise of the upper and lower limbs in the same participants. The results show that both MVIC force loss and decreases in evoked forces were not different between EF and KE 1 min after task failure. However, 20 min after task failure, MVIC force and \( \text{Db}_{10;100} \), as a percentage of PRE, were greater in KE than EF. The present findings suggest that the recovery of neuromuscular function after dynamic multi-joint maximal exercises is specific to the muscle group investigated.

**Incremental exercise**

Performance fatigability is a reversible and acute exercise-induced reduction in force caused by changes within the central nervous system and/or muscles. Exercise characteristics (e.g. type, duration, intensity) affect the magnitude and aetiology of performance fatigability (30). As such, similar characteristics of fatiguing exercise are important pre-requisites to investigate the magnitude and aetiology of neuromuscular changes between muscles. In the present study, \( \dot{\text{VO}}_{2\text{peak}} \) and \( \dot{\text{V}}_{\text{E}_{\text{peak}}} \) values were lower in UL compared to LL, as previously observed (15, 31). However, the exercise duration was similar between arm-cranking and cycling tests. Moreover, all participants reached task failure and the cardiorespiratory data at the end of each test met the secondary criteria (e.g. HR, [La]_b, RER and RPE) for the determination of \( \dot{\text{VO}}_{2\text{peak}} \) (24), suggesting that a maximal effort was achieved in both conditions. Importantly, the calculated amount of work normalized per estimated muscle mass was not different between UL and LL exercise. Therefore, the presented similarities in duration, intensity and amount of work allowed us to compare the effects of arm-cranking and cycling incremental exercise tests on performance fatigability and recovery. However, it should be acknowledged that the time spent above GET was significantly different between UL and LL. This may limit the interpretation of the fatigue recovery across EF versus KE since time of exercise.
performed in a specific intensity domain affects fatigability and the subsequent recovery (12).

**Magnitude of fatigability and recovery**

Performance fatigability is often investigated by changes in the MVIC force (2). In the present study, MVIC force decreased by 23% and 19% from PRE to POST in EF and KE, respectively (Figure 3). This suggests that the capacity for EF and KE muscles to produce force is similarly impaired 1 min after dynamic incremental exercise to task failure of similar duration, intensity, and amount of performed work normalised to estimated muscle mass. The magnitudes of MVIC force loss in EF and KE at POST in the present study (-23% and -19% for EF and KE, respectively) were generally comparable with Senefeld et al. (8) for KE (-18%) but lower for EF (-30%). The MVIC force loss was also different than previously reported immediately after either isometric (4, 5) or dynamic (10) exercise tasks. More specifically, sustained submaximal (-40% and -34% for EF and KE, respectively) (5) and maximal (-58% and -70% for EF and KE, respectively) (4) isometric tasks reported higher MVIC force losses; while Halperin et al. (10) reported lower MVIC force loss of ∼15% in EF but comparable MVIC force loss of ∼24% in KE following repeated intermittent sprints. Although the delay to MVIC force evaluation after exercise cessation influences the results, these findings collectively suggest that sustained isometric tasks elicit a greater MVIC loss than dynamic intermittent tasks. Moreover, intermittent exercise with repeated cycles of contraction and relaxation elicit different magnitude of fatigue than continuous exercise sustaining a contraction, reinforcing that performance fatigability depends on the characteristics of the fatiguing exercise task (30). To better understand the individual response to exercise we assessed the relationship between MVIC force decrements in EF and KE. There was a significant correlation whereby participants with greater EF MVIC loss also had greater KE MVIC loss at POST (Figure 4).
Twenty minutes after exercise cessation, the recovery in MVIC force was lower for EF (85% of PRE) than KE (95% of PRE), indicating that EF force recovered slower than in KE. Although the exact mechanisms involved in neuromuscular function recovery to specific exercise tasks are still to be definitively elucidated (12), the difference in force recovery rate may be due to differences in muscle fiber composition between EF (larger proportion of type II muscle fibres) and KE (larger proportion of type I muscle fibers) (32). Indeed, the smaller mitochondrial volume and lower activity of oxidative enzymes in type II muscle fibers (compared to type I fibers) (33) may have delayed clearance of waste products of muscle contraction in EF, compared to KE, potentially hindering the recovery process (3).

**Etiology of performance fatigability and recovery**

The observed MVIC loss can be attributed to changes in neuromuscular function, whether proximal [i.e. within the brain and motoneurons (2)] or distal [i.e. within the skeletal muscle (3)] to the neuromuscular junction. The lack of change in VA from PRE to POST and POST20 suggests that central nervous system impairment did not contribute to the magnitude and aetiology of MVIC force loss either 1 or 20 minutes after exercise. These results are comparable to previous findings obtained during cycling for a similar duration in the heavy-intensity domain (34) and they are in line with the studies that have observed that prolonged endurance exercise causes greater impairment of the central nervous system than short high-intensity exercise (35, 36).

On the other hand, contractile muscle properties were impaired in both arm-cranking and cycling, as demonstrated by the decreases in $T_{w_{pot}}$, $RFDT_{w}$, $DB_{100}$ and $DB_{10:100}$. Meanwhile, the lack of change in $M_{max}$ properties suggests that action potential propagation along the sarcolemma and t tubules and/or muscle membrane excitability was unaffected by the exercise bouts. These results support the results of previous cycling studies (36, 37) that showed muscle contractile impairments without changes to the $M$-
wave and suggest that the observed MVIC force loss in EF and KE was due to changes in muscle contractile properties changes. It is likely that during exercise, intramuscular Pi accumulation reduced the free Ca$^{2+}$ available for release from the sarcoplasmic reticulum (38) (coupled with increasing recruitment of muscle fibers) leading to disrupted skeletal muscle contractile processes (3). Indeed, Db$_{10:100}$ represents the preferential loss of force at low frequencies of electrical stimulation and is believed to occur due to a reduction in the release of Ca$^{2+}$ from the sarcoplasmic reticulum, leading to excitation-contraction coupling failure (26). On the other hand, the lack of changes in $M_{\text{max}}$ suggest that muscle relaxation during each revolution of the contralateral limb may have prevented an excessive increase in extracellular [K$^+$] during both arm-cranking and cycling, further suggesting that that muscle excitability changes did not contribute to MVIC force loss (39).

Twenty minutes were insufficient for Tw$_{\text{pot}}$, RFDtw or Db$_{100}$ to fully recover after task failure for either EF or KE. These results agree with previous observations from Krüger et al. (36), who failed to observe complete recovery in KE after 8 min following constant work-rate cycling, suggesting that EF and KE muscle contractile properties are still compromised for an extended period after exercise cessation. Interestingly, Db$_{10:100}$ also did not recover after 20 min and was lower in EF (75% of PRE) than KE (85% of PRE) at POST20. This result could be explained by a delayed restoration of metabolic homeostasis induced by the dynamic maximal exercise in EF compared to KE. Thus, we can speculate that MVIC force loss was similar 1 min after exercise cessation due to comparable intracellular metabolic perturbations after arm-cranking and cycling (3). However, after 20 min of recovery, we can hypothesize that removal of lactate, H$^+$ and Pi was slower, and intracellular Ca$^{2+}$ handling impaired, in EF compared to KE. This resulted in impaired MVIC and Db$_{10:100}$ recovery 20 min after exercise. A possible explanation is that the higher percentage of type II fibers in UL muscles, compared to LL muscles (40–42), may have affected muscle oxidative function, lactate extrusion rate, and sarcoplasmic reticulum
Ca\textsuperscript{2+} uptake, leading to delayed restoration of metabolic homeostasis in EF, compared to KE. Further studies are required to determine how different physiological characteristics in UL and LL muscles influence performance fatigability recovery.

**Limitations**

It has been observed that handgrip position affects the neuromuscular responses to arm-cranking exercise (43). In this study participants arm-cranked with their hands pronated, rather than in neutral or supinate positions. However, it has been demonstrated that BB and \textit{brachioradialis} EMG activity is similar during arm-cranking for these three handgrip positions (43). Additionally, a pronated handgrip showed (i) greater EF change in intramuscular oxygen status (44) and (ii) higher power output than the supinated position (45) and it is also the most similar position to those utilized during other exercises with upper limbs such as rowing and kayaking (46). Another limitation is that time above GET was different between UL and LL during incremental exercise performed to task failure. Although this exercise protocol was selected to replicate functional evaluations tests routinely used on healthy participants and patients, we cannot exclude those neuromuscular changes observed in EF \textit{versus} KE would have been different with other exercise paradigms. However, we decided to control for duration of exercise to reduce as much as possible the influence of one potential confounder affecting neuromuscular fatigue (i.e. time of exercise) whereas controlling the protocol for something other than time of exercise (e.g. time spent above GET) would have created further methodological issues. Furthermore, it should be considered that our study design was pseudorandomized and counterbalanced, and a different study design is needed to control for time above GET. Finally, neuromuscular evaluations were performed exactly 1 min after exercise cessation. This was the shortest delay possible to consistently assess the neuromuscular function in our experimental setting. The reported neuromuscular impairments have likely been
underestimated since MVIC and electrically-evoked force recovery begins immediately when exercise ceases and measures of muscle activation are affected if a short time delay exists between task failure and measurement (47). Thus, caution should be taken when comparing the changes observed in this study with other experimental designs and settings.

**Conclusion**

When mechanisms of performance fatigability after dynamic multi-joint maximal exercise are compared in the same participant, EF and KE present a similar magnitude of neuromuscular function impairment which is not from central determinants (i.e. proximal to the neuromuscular junction and encompassing the brain as well as upper and lower motoneurons). Instead, impairments are due to peripheral (i.e. within the skeletal muscle) factors. The recovery in MVIC and Db10:100 20 minutes after exercise was lower in EF, suggesting that exercise-induced recovery is muscle-specific. The differences in MVIC and electrically-evoked force recovery 20 min after exercise between arm-cranking and cycling pave the way for further studies investigating whether the delayed restoration of metabolic homeostasis in EF, compared to KE, is responsible for differences in recovery of neuromuscular function.

**Perspectives and Significance**

The results of the present study extend the current knowledge about performance fatigability and recovery characteristics in EF and KE muscles after dynamic multi-joint exercise, highlighting muscle-specific neurophysiological differences. These results have direct implications for daily-life (e.g. climbing stairs, carrying bags) and sporting activities (e.g. cycling, rowing) involving dynamic contractions of UL and/or LL. Furthermore, the differences in recovery in EF and KE suggest coaches and physicians should monitor recovery between bouts when prescribing multi-joint UL exercises for training and/or rehabilitation of athletes and/or patients.
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**Author contributions**
MC, LR, MM and SP conceived of and designed the research. MC, LR, and SP performed the experiment. MC, LR, GB and GV analysed the data. MC, LR, GB, JT, GV, MM and SP interpreted the data of the experiment. MC, LR, GB, JT, GV, MM and SP edited and revised the manuscript. MC, LR, GB, JT, GV, MM and SP approved the final version of the manuscript.

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**Availability of data and materials**
Available under motivated request to SP.

**Code availability**
Not applicable.

**Declarations**

**Conflict of interest**
The authors declare that they have no conflict of interest. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

**Ethical approval**
This study was conducted in accordance with the recommendations of the 1964 Declaration of Helsinki and its later amendments. The research plan was examined and approved by the local ethical committee (BESTA/IBFM, Report #43, 8/11/2017).

Consent to participate

Prior to testing, all participants gave a voluntary written informed consent which indicated the purpose, the benefits and the risks of the investigation and the possibility stopping their participation at any time.

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References


41. Ørtenblad N, Nielsen J, Boushel R, Söderlund K, Saltin B, Holmberg HC. The muscle fiber profiles, mitochondrial content, and enzyme activities of the
exceptionally well-trained arm and leg muscles of elite cross-country skiers. Front Physiol. 2018;9(8):1031.


**FIGURE CAPTIONS**

**Figure 1.** Schematic representation of the experimental protocol. Neuromuscular function evaluation was performed before (PRE), exactly 1-min (POST) and 20-min (POST20) after maximal incremental exercises performed by either an arm-cranking or a cycle ergometer (Panel A). A single-participant’s data is shown for the neuromuscular...
evaluation of either the elbow-flexor (EF, Panel B) or knee-extensor (KE, Panel C) muscles at PRE. For both muscles, the neuromuscular function evaluation consisted of a sustained 5-s isometric contraction during which a high-frequency paired pulse ($sDb_{100}$) was delivered at maximal force. Immediately after, a set of high- and low-frequency (100 and 10 Hz) paired pulses followed by a single pulse, separated by 2 s each, were delivered to the relaxed muscle. For EF evaluation (Panel B), an additional stimulus was delivered to the brachial plexus 2 s later to the relaxed muscle. Peripheral nerve stimulation (brachial plexus or femoral nerve) is indicated by black arrows and *biceps brachii* motor point stimulation by grey arrows. The responses are indicated as $Db_{100}$ (high-frequency doublet), $Db_{10}$ (low-frequency doublet), $Tw_{pot}$ (potentiated twitch), and $M_{max}$ (maximal M wave).

**Figure 2.** Typical example of voluntary activation (VA) assessment by interpolated twitch technique. A single-participant’s force data obtained during a sustained 5-s isometric contraction with a high-frequency (100 Hz) pulse delivered at maximal force and to the relaxed muscle are shown. The square highlights the force-time trace of the superimposed 100-Hz doublet ($sDb_{100}$) that is magnified in the upper-right corner. Arrows indicate the time points when stimuli were delivered. Capped lines indicate the forces for $sDb_{100}$ and potentiated high-frequency doublet ($Db_{100}$). The VA calculation for this participant, using equation 3, is reported in the figure (see text for further details).

**Figure 3.** Maximal voluntary isometric contraction (MVIC) force before (PRE) and after the incremental tests for both elbow-flexor (EF) and knee-extensor (KE) muscles. At the end of the incremental tests, a neuromuscular function evaluation was performed 1 min (POST) and 20 min (POST20) after exercise cessation. Values are presented as means and standard deviations and normalized as a percentage of PRE evaluation. Asterisks (*) denote within-limb differences compared to PRE by means of ANOVA: $P < 0.05$. Dollar signs ($) denote within-limb differences compared to POST by means of ANOVA: $P < 0.05$. Number sign (#) denotes between-limb differences by means of ANOVA: $P < 0.05$. 

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Figure 4. Relationship between maximal voluntary isometric contraction force loss decrement after the incremental test (POST) as percentage of before exercise (PRE) values from knee extensors (KE) and elbow flexors (EF) muscles. The paired dashed line represents the 95% confidence interval.

Figure 5. Potentiated twitch ($T_{w_{pot}}$, Panel A), high-frequency doublet ($D_{b100}$, Panel B) and ratio between low and high frequency doublets ($D_{b10}:D_{b100}$, Panel C) before (PRE) and after the incremental tests for both elbow-flexor (EF) and knee-extensor (KE) muscles. At the end of the incremental tests, a neuromuscular function evaluation was performed 1 (POST) and 20 (POST20) min after exercise cessation. Values are presented as means and standard deviations and normalized as a percentage of the PRE evaluation. Asterisks (*) denote within-limb differences compared to PRE by means of ANOVA: $P < 0.05$. Dollar signs ($) denote within-limb differences compared to POST by means of ANOVA: $P < 0.05$. Number sign (#) denotes between-limb differences by means of ANOVA: $P < 0.05$. 


Table 1. Means ± SD of peak values for the respiratory, cardiovascular, and metabolic variables determined during the maximal incremental exercises on either an arm-cranking [for upper limbs (UL)] or a cycle [for lower limbs (LL)] ergometer.

<table>
<thead>
<tr>
<th></th>
<th>UL</th>
<th>LL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to exhaustion (min)</td>
<td>13 ± 2</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>$P_{peak}$ (W)</td>
<td>135.8 ± 25.4*</td>
<td>290.0 ± 45.3</td>
</tr>
<tr>
<td>$\dot{V}O_{2peak}$ (L·min$^{-1}$)</td>
<td>2.49 ± 0.57*</td>
<td>3.46 ± 0.51</td>
</tr>
<tr>
<td>$\dot{V}CO_{2peak}$ (L·min$^{-1}$)</td>
<td>3.00 ± 0.72*</td>
<td>4.40 ± 0.62</td>
</tr>
<tr>
<td>RER</td>
<td>1.2 ± 0.1*</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>$\dot{V}E_{peak}$ (L·min$^{-1}$)</td>
<td>117.6 ± 28.9*</td>
<td>158.1 ± 30.5</td>
</tr>
<tr>
<td>GET (%$\dot{V}O_{2peak}$)</td>
<td>51.9 ± 6.8*</td>
<td>70.2 ± 5.8</td>
</tr>
<tr>
<td>$HR_{peak}$ (beats·min$^{-1}$)</td>
<td>170 ± 8*</td>
<td>184 ± 9</td>
</tr>
<tr>
<td>Total work (J·kg$^{-1}$)</td>
<td>7945 ± 2190</td>
<td>9017 ± 1443</td>
</tr>
</tbody>
</table>

Note: Time to exhaustion, peak power output ($P_{peak}$); peak O$_2$ consumption ($\dot{V}O_{2peak}$); peak CO$_2$ output ($\dot{V}CO_{2peak}$); respiratory exchange ratio (RER); peak pulmonary ventilation ($\dot{V}E_{peak}$); gas exchange threshold (GET), peak heart rate ($HR_{peak}$), total work normalized per unit of estimated muscle mass (Total work). Asterisks denote between-limb differences by means of Student’s paired t test: * $P < 0.05$
VA (%) = \( 1 - \left( \frac{7.5}{87} \right) \) \times 100 = 91.4 %

\( sDb_{100} = 7.5 \text{N} \)

\( Db_{100} = 87 \text{N} \)
KE Force loss (%PRE)

-30  -20  -10  0

EF Force loss (%PRE)

-30  -20  -10  0

r = 0.66
P = 0.038

POST