Effect of race distance on performance fatigability in male trail and ultra-trail runners

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ABSTRACT

The etiology of changes in lower-limb neuromuscular function, especially to the central nervous system, may be affected by exercise duration. Direct evidence is lacking as few studies have directly compared different race distances. This study aimed to investigate the etiology of deficits in neuromuscular function following short versus long trail-running races. Thirty-two male trail runners completed one of five trail-running races as LONG (>100 km) or SHORT (<60 km). Pre- and post-race, maximal voluntary contraction (MVC) torque and evoked responses to electrical nerve stimulation during MVCs and at rest were used to assess voluntary activation and muscle contractile properties of knee-extensor (KE) and plantar-flexor (PF) muscles. Transcranial magnetic stimulation (TMS) was used to assess evoked responses and corticospinal excitability in maximal and submaximal KE contractions. Race distance correlated with KE MVC (ρ = -0.556) and twitch (ρ = -0.521) torque decreases ($P \leq$ 0.003). KE twitch torque decreased more in LONG (-28 ± 14%) than SHORT (-14 ± 10%, P = 0.005); however, KE MVC time × distance interaction was not significant (P = 0.073). No differences between LONG and SHORT for PF MVC or twitch torque were observed. Maximal voluntary activation decreased similarly in LONG and SHORT in both muscle groups ($P \geq$ 0.637). TMS-elicited silent period decreased in LONG (P = 0.021) but not SHORT (P = 0.912). Greater muscle-contractile property impairment in longer races, not central perturbations, contributed to the correlation between KE MVC loss and race distance. Conversely, PF fatigability was unaffected by race distance.

Keywords: fatigue, knee extensors, plantar flexors.

1. INTRODUCTION

Performance fatigability is an acute exercise-induced decrease in torque or power output of the involved muscles[1] and is mediated by reductions in neuromuscular function. This often presents as decreased maximal voluntary contraction (MVC) torque of a muscle or muscle group, regardless of the ability to sustain a given task.[2] Previous research has shown that endurance running elicits impairments in neuromuscular function.[3-6] While a decrease in MVC torque is indicative of impairments throughout the neuromuscular system, these deficits can occur via central and/or peripheral processes which can be assessed by neurostimulation methods.[7] Both central and peripheral processes contribute to MVC loss after endurance running.[4,8]

Using data from studies assessing neuromuscular responses to prolonged running bouts (≥ 2 h), previous reviews[9,10] have identified a curvilinear relationship between exercise duration and the decrease in knee-extensor (KE) MVC, specifically that pre-post MVC loss rapidly increases as exercise duration increases before MVC loss plateaus around -35 to -40% after races of 1000-2500 min. Plantar-flexor (PF) MVC loss was also observed to increase with increasing exercise duration (500-2500 min).[9] The magnitude of impairments to central nervous system function (i.e., decrease in maximal voluntary activation, VA) in KE following ultra-endurance running bouts (i.e., 24 h or ≥ 110 km) was greater than after shorter running bouts. This difference was not observed for peripheral processes (i.e., decrease in evoked torques),[9] suggesting that a greater magnitude of central but not peripheral impairments develop with increasing exercise distance or duration. While these observations have been made by examining the literature, few studies have directly assessed the effect of exercise duration on neuromuscular responses to running. To better understand the effect of exercise duration on neuromuscular function, investigation of maximal strength, voluntary activation, and contractile function changes following races of different distances run under similar

conditions and on similar types of terrain is warranted. Another useful tool, specifically for assessing central neuromuscular function, is transcranial magnetic stimulation (TMS); however, the use of this technique has been limited in ultra-endurance running.[3,8]

Trail running is an increasingly popular endurance sport with races over a variety of distances, topographies, and terrains. Races range from shorter races where the winners finish in 1-2 h to longer events lasting > 24 h. Given the diversity of events, trail running provides a unique opportunity to assess the effect of exercise distance on neuromuscular function following competitive races.

Previously, our group investigated the effect a 169-km trail-running race performed as a single stage or the same race run across four stages over consecutive days.[11] KE MVC decreased similarly during the single-stage (-32%) and four-stage (-24%) races. Interestingly, there was no difference in KE MVC after any stage of the four-stage race, suggesting that after the initial race stage, subsequent stages did not contribute to the magnitude of KE MVC loss. Similar results were observed in PF. These findings are consistent with the aforementioned curvilinear relationship between race duration and strength loss.[9,10] While this suggests that race distance and duration have limited effects on the magnitude of MVC loss induced by trail running, the two events are not directly comparable. Single-stage runners completed the initial 40 km ~10% slower than multi-stage runners. By the end of the race, total race time for the single-stage runners was ~35% longer than for multi-stage runners. While all runners are expected to attempt to maximize performance, being able to run a race, sleep, eat, and recover for the following day undoubtedly contributed to differences in pacing strategy. Martinez-Navarro et al [12] compared changes in neuromuscular function after trail-running races of 65 and 107 km. They observed a larger decrease in squat jump height after the longer race (~-10% versus ~-25%). Thus, the effect of race duration on neuromuscular function following a bout of continuous running remains unclear.

The series of races at Ultra-Trail du Mont-Blanc® that attract the world's best trail runners provide an opportunity to investigate the effects of race distance. Therefore, the study aim was to investigate the effect of trail-running race distance on measures of neuromuscular function and fatigability and corticospinal excitability in male trail runners. Specifically, we aimed to determine whether the etiology of change in neuromuscular function is different between shorter trail and longer ultra-trail running races. It was hypothesized that reduced neuromuscular function, particularly within the central nervous system, would be greater for longer races.

2. METHODS

2.1 Participants

This study forms part of a larger study investigating the effects of race distance and sex on fatigability in trail runners. Data for female participants and sex differences will be presented elsewhere. Forty-six healthy experienced male ultra-endurance trail runners were recruited for the current study (for study flow diagram, see Figure 1) and 35 participants performed assessments both before (PRE) and after (POST) their race. Thirty-two participants were included in data analyses (i.e., three participants were excluded because they completed the race with a slower competitor and indicated they did not push themselves at the end of the race). Participant characteristics are presented in Table 1. Only male participants are reported here since sex differences in neuromuscular function have previously been observed in long-distance trail running.[8]

Participants were informed of the experimental protocol and all associated risks prior to giving written informed consent as part of a medical inclusion. All procedures conformed to the Declaration of Helsinki and were approved by the research ethics committee (Study ID 2019-A00736-51, Ethics Committee Agreement #19.03.14.41740, ClinicalTrials,org:

NCT04025138). The University Hospital of Saint-Etienne (France) was the sponsor of this study.

2.2 Experimental design

Each participant completed a medical inclusion/familiarization and two sessions. The medical inclusion/familiarization was conducted in July 2019 in Saint-Etienne. The PRE session occurred > 24 h and < 128 h before each participant started their race at Ultra-Trail du Mont-Blanc® 2019 and the POST session as soon as possible after race completion (Table 1). PRE and POST sessions were conducted in a laboratory at École Nationale de Ski et d'Alpinisme near the finish line in Chamonix for all races.

Participants completed one of five individual races at Ultra-Trail du Mont-Blanc® 2019 (Table 2) categorized as either LONG (> 100 km) or SHORT (< 60 km). Individual race times by distance and category are presented in Supplementary File 1. Temperatures in Chamonix ranged from a low of 11°C at night to a daytime high of 31°C.[13]

2.3 Familiarization

The familiarization began with a medical inclusion and skinfold measurement to determine body fat percentage.[14] Then participants were familiarized with neuromuscular testing procedures conducted on the right leg. This consisted of maximal and submaximal KE voluntary contractions with and without femoral nerve electrical stimulation (FNES) and TMS and PF MVCs with and without tibial nerve electrical stimulation (TNES). Finally, a maximal incremental running test to task failure was conducted on a treadmill with 12% slope to determine peak oxygen consumption ($\dot{V}O_{2PEAK}$).

2.4 Neuromuscular testing protocol

Neuromuscular testing was conducted in the right KE and PF. POST evaluations were conducted as soon as possible after race completion. To optimize testing stations, participants performed POST KE evaluations and then POST PF evaluations or vice versa, depending on station availability. Testing order POST was not counterbalanced. All neuromuscular measures were assessed with real-time visual feedback of the torque produced.

For KE and PF, a brief ~3-s MVC was followed by two ~5-s MVCs with electrical nerve stimulation (100-Hz paired pulse) delivered at peak torque and immediately after in the relaxed state (100- and 10-Hz paired pulses and single pulse separated by 3 s). MVCs were separated by 30 s. Supplementary File 2 shows raw traces of PRE-POST torque responses in LONG and SHORT. For KE only, two series of three ~3-s contractions separated by 5 s were then performed. After an MVC with TMS delivered at maximal torque, target guidelines at 75 and 50% MVC appeared on a computer screen for subsequent contractions (75% MVC with TMS and then 50% MVC with TMS followed by FNES). Series were separated by ~30 s at PRE and ~10 s at POST.

2.5 Torque and electromyographic recordings

KE torque was measured during voluntary and evoked contractions by isometric knee dynamometer (ARS dynamometer, S2P, Ljubljana, Slovenia) with the right leg securely attached proximal to the malleoli. Participants were seated upright with both right knee and hips at 90° of flexion and secured by hip straps. PF torque was assessed by instrumented pedal (CS1060 300 Nm, FGP Sensors, Les Clayes Sous Bois, France) with participants seated upright in a custom-built chair with right ankle, knee, and hip joints at 90°. Non-compliant straps secured the chest, heel, and forefoot. Electromyographic activity (EMG) of the right KE (*vastus lateralis*, VL) and PF (*gastrocnemius medialis*, GM and *soleus*, SOL) was recorded with pairs of self-adhesive surface (10-mm recording diameter) electrodes

(Meditrace 100, Covidien, Mansfield, USA) in bipolar configuration with a 30-mm interelectrode distance and the reference on the patella for KE and medial malleolus for PF. Low impedance (<5 kΩ) between electrodes was obtained by shaving, gently abrading the skin and then cleaning it with isopropyl alcohol. Signals were analog-to-digitally converted at a sampling rate of 2000 Hz by PowerLab system (16/30-ML880/P, ADInstruments, Bella Vista, Australia) and octal bio-amplifier (ML138, ADInstruments; common mode rejection ratio = 85 dB, gain = 500) with bandpass filter (5-500 Hz) and analyzed offline using Labchart 8 software (ADInstruments).

2.6 Electrical nerve stimulation

Single electrical stimuli (1-ms duration) were delivered via constant-current stimulator (DS7A or DS7R, Digitimer, Welwyn Garden City, Hertfordshire, UK) to the right femoral nerve and right tibial nerve. Femoral nerve stimuli were delivered via 10-mm diameter surface cathode manually pressed into the femoral triangle (Meditrace 100) and 50×100-mm rectangular anode (Medicompex SA, Ecublens, Switzerland) in the gluteal fold. Tibial nerve stimuli were delivered via stimulating bar electrode with 30-mm anode-cathode spacing (E.SB020/4 mm Bipolar Felt Pad Stimulating Electrode, Digitimer) placed in the popliteal fossa parallel to the nerve. Single stimuli were delivered incrementally in relaxed muscle until maximal M-wave (M_{MAX}) and twitch amplitudes plateaued. Stimulus intensity of 130% of the intensity to produce M_{MAX} and maximal twitch responses was employed to ensure supramaximality and determined at the start of each session. Supramaximal FNES intensity decreased from PRE (122 ± 65 mA) to POST (104 ± 62 mA) (P = 0.025) and supramaximal TNES intensity was unchanged between PRE (59 ± 29 mA) and POST (62 ± 23 mA) (P = 0.636).

2.7 Transcranial magnetic stimulation

Single TMS pulses were manually delivered to elicit motor-evoked potentials (MEP) and superimposed twitches (SIT) during voluntary isometric knee extension. The left motor cortex was stimulated by a magnetic stimulator (Magstim 200², The Magstim Company Ltd, Whitland, UK) with 110-mm concave double-cone coil inducing a postero-anterior current. Participants wore a lycra swim cap and the hotspot was determined as presented elsewhere.[3] The TMS intensity was the lowest stimulus intensity eliciting maximal MEP amplitude during brief 20% MVC voluntary contractions.[15] Mean stimulus intensity PRE (n = 30) was 64 ± 12% maximal stimulator output. Identical coil position and TMS intensity were utilized PRE and POST. TMS was always delivered once the torque had stabilized at the appropriate level during voluntary contractions. Participants were instructed to re-contract to the pre-stimulus torque immediately after TMS delivery.[16]

2.8 Blood parameters

Venous blood samples were taken from an antecubital vein PRE and POST (immediately prior to the neuromuscular testing protocol). A complete blood count (CBC) was performed using a hematological analyzer (XN2000, Sysmex, Kobe, Japan) to determine leukocyte concentration. A Cobas C501 integrated system (Roche, Basel, Switzerland) was used for simultaneous assay of C-reactive protein (CRP) and creatine kinase (CK) with reagents from the manufacturer.

2.9 Data analysis

2.9.1 Voluntary and evoked torques

Maximal torque corresponds to the highest peak pre-stimulus torque from all MVCs at each time point. Potentiated peak twitch (TwPot) and doublet (100-Hz paired pulse, Db100; 10-Hz

paired pulse, Db10) torque amplitudes were determined for the MVC where torque was highest when FNES or TNES was delivered. The presence of low-frequency fatigue POST was evaluated as $\Delta Db10 \cdot Db100^{-1}$.[17] Voluntary activation was assessed from the same MVC by twitch interpolation from evoked FNES (VA_{FNES}) or TNES (VA_{TNES}) responses. The superimposed and potentiated Db100 amplitudes during and after MVCs with both muscle groups permitted VA to be calculated as $VA_{FNES/TNES}$ = ((1 – superimposed Db100)/potentiated Db100) × 100.

2.9.2 EMG and M waves

M-wave amplitude was measured in the relaxed (M_{MAX}) muscle for KE and PF and during voluntary contractions at 50% MVC (M_{SUP}) for KE. KE M_{SUP} area[18] was also measured. EMG root mean square (RMS) was calculated from the 500-ms period after torque had reached a plateau and before electrical nerve stimulation delivery during the highest MVC at each time point. RMS was normalized to M_{MAX} amplitude to account for PRE-POST changes in sarcolemmal excitability or electrode position and differences between participants.

2.9.3 Transcranial magnetic stimulation

Voluntary activation was assessed with TMS (VA_{TMS}) by modified twitch interpolation. For each series of contractions, estimated resting twitch amplitude was determined by extrapolation of the linear regression of the relation between SIT amplitude elicited at 100, 75, and 50% MVC and the corresponding voluntary torque.[19] The regression was linear (r > 0.9) in most participants (n = 28) for at least one series at both PRE and POST, permitting determination of VA_{TMS}.[20] VA_{TMS} was assessed using the series with highest MVC and regression linear as VA_{TMS} = ((1 – SIT)/estimated resting twitch) × 100.[19]

MEP amplitude and area were measured in accordance with Martin et al [18] and normalized to the M_{SUP} amplitude or area at the same time point. SP duration was determined visually as the duration from the stimulus to the return of continuous voluntary EMG.[20] Mean MEP amplitude and area and SP duration at each time point and contraction intensity was considered for each participant. Since comparable changes were observed for MEP amplitude and area, only MEP area is reported.

2.10 Statistics

Statistical analyses were performed with Statistica (version 8, Statsoft, Tulsa, USA). Shapiro-Wilk and Levene's tests were used to examine data normality and homogeneity of variances. Non-parametric tests were performed when assumptions of normality or homogeneity of variance were violated. Mauchly's test of sphericity was used to confirm the assumption of sphericity for ANOVAs where contraction intensity was a within-participant factor. When the sphericity assumption was violated, a Greenhouse-Geisser correction was applied since all $\varepsilon < 0.75$.

Independent-sample *t* tests and Mann-Whitney *U* tests were performed to evaluate differences between SHORT and LONG at PRE for all parameters, participant characteristics, and race performance. Spearman rank-order correlation coefficient (ρ) was employed to examine the direction and strength of the relationships between distance and the percentage PRE-POST change (Δ) in neuromuscular parameters and leukocyte concentration or POST CK and CRP concentrations. Spearman rank-order correlation coefficient was also used to examine the direction and strength of between race time and the percentage PRE-POST change (Δ) in neuromuscular parameters and leukocyte concentration or POST CK and CRP concentrations (Supplementary File 3). The *P* values for PRE comparisons and Spearman rank-order correlation coefficient were corrected for multiple comparisons using

the procedures described by Benjamini and Hochberg[21] with the false discovery rate set at 5%. Repeated-measures ANOVAs for time (PRE, POST) and voluntary contraction intensity (100, 75, 50% MVC) as within-participant factors with distance (LONG, SHORT) as a between-participant factor were used to evaluate changes in MEP and SP. When significant main effects or interactions were observed for MEP or SP, Tukey's test was used for *post-hoc* analysis. Repeated-measures ANOVAs for time (PRE, POST) as a within-participant factor with distance (LONG, SHORT) as a between-participant factor were used to evaluate all other normally-distributed parameters. When a significant time × distance interaction was found, an independent-sample *t* test or Mann-Whitney *U* test was conducted as *post-hoc* analysis to determine whether the percentage PRE-POST change (Δ) between LONG and SHORT was different.

Where the assumption of normality or heterogeneity of equal variances were violated, Wilcoxon signed-rank test was employed to assess PRE-POST changes for both LONG and SHORT. If the PRE-POST change of at least one of LONG and SHORT was significant, an independent-samples *t* test or Mann-Whitney *U* test was conducted to determine whether the percentage PRE-POST change (Δ) between LONG and SHORT was different. POST concentrations of CK and CRP were assessed by Mann-Whitney *U* test to compare LONG and SHORT. Effect size is presented for significant findings only as partial eta-squared (η^2_p) for ANOVAs and Cohen's *d* for *t* tests.[22] Effect sizes were not calculated for non-parametric data. Statistical significance was set at $P < 0.05$. All data are presented as mean ± standard deviation (SD) for normally-distributed data or median [inter-quartile range (IQR)] for non-normal data.

3. RESULTS

3.1 Race performance and pre-race torque and EMG measures

Race performance and PRE torque measures are presented in Table 1. There were no differences between LONG and SHORT for pre-race MVC (both $P \geq 0.479$), evoked torques (all $P \geq 0.186$), or voluntary activation (all $P \geq 0.695$) for KE or PF. There were also no pre-race differences in any EMG or TMS parameter between LONG and SHORT (all $P \geq 0.112$).

3.2 Maximal voluntary and evoked torques and M waves

There was a negative relationship between distance and both ΔKE MVC ($\rho(30) = -0.556$, $P < 0.001$) and ΔKE TwPot ($\rho(29) = -0.521$, $P = 0.003$) such that as distance increased, the decrease in KE MVC and TwPot was larger (Supplementary File 4). There was no relationship between distance and ΔKE Db10·Db100^{-1}, ΔPF MVC, ΔPF TwPot, or ΔPF Db10·Db100^{-1} (all $P \geq 0.195$).

MVC decreased PRE to POST in KE (Figure 2A; $F(1,30) = 101.18$, $P < 0.001$, $\eta^2_p = 0.771$) and PF (Figure 2B; $F(1,27) = 95.59$, $P < 0.001$, $\eta^2_p = 0.780$). There were also PRE to POST decreases in KE TwPot and Db10·Db100^{-1} (Figure 2A; both $P \leq 0.001$).

A time × distance interaction was observed for KE TwPot ($F(1,29) = 8.00$, $P = 0.008$, $\eta^2_p = 0.216$). The subsequent independent-samples *t* test found a greater KE TwPot decrease in LONG than SHORT (-28 ± 14% versus -14 ± 10%; $t(29) = -3.04$, $P = 0.005$, $d = 1.105$). However, the time × distance interaction did not reach significance for KE MVC ($P = 0.073$) or any other KE torque parameter (all $P \geq 0.096$), nor any distance effects (all $P \geq 0.126$). PF TwPot and Db10·Db100^{-1} violated the assumption of heterogeneity so were analyzed non-parametrically. PF TwPot decreased in both LONG and SHORT (both $P \leq 0.008$) while PF Db10·Db100^{-1} decreased in SHORT ($Z = 2.29$, $P = 0.022$) but not LONG ($Z = 1.81$, $P = 0.070$). There was no difference in ΔPF TwPot or Db10·Db100^{-1} between LONG and SHORT (Figure 2; both $P \geq 0.400$). There were no other effects (all $P \geq 0.506$) for any PF torque parameter.

No significant relationships were observed between distance and ΔVL M waves (all $P \geq 0.275$) or ΔGM or SOL M_{MAX} (both $P \geq 0.085$). VL M_{MAX} amplitude and M_{SUP} area were unchanged PRE-POST (all $P \geq 0.165$) and no distance effects or time × distance interactions were observed (all $P \geq 0.524$). There were also no effects for SOL or GM M_{MAX} amplitude (all $P \geq 0.060$).

3.3 Voluntary activation and EMG root mean square

There were no significant relationships between distance and ΔKE VA_{FNES}, ΔKE VA_{TMS}, or ΔPF VA_{TNES} (all $P \geq 0.535$). KE VA_{FNES} ($F(1,29) = 29.10$, $P < 0.001$, $\eta^2_p = 0.501$) and KE VA_{TMS} ($F(1,26) = 24.27$, $P < 0.001$, $\eta^2_p = 0.483$) decreased PRE to POST (Figure 2A); however, there were no other effects (all $P \geq 0.626$). PF VA_{TNES} decreased from PRE to POST in LONG ($Z = 2.77$, $P = 0.006$) and SHORT ($Z = 2.35$, $P = 0.019$) with no difference in the magnitude of decrease (Figure 2C; $U(27) = 98.0$, $P = 0.859$).

There was a significant relationship between distance and ΔVL RMS/M_{MAX} ($\rho(29) = -0.442$, $P = 0.013$). VL RMS/M_{MAX} decreased PRE-POST in LONG ($Z = 3.42$, $P < 0.001$) but not SHORT ($Z = 0.73$, $P = 0.463$), resulting in ΔVL RMS/M_{MAX} being greater in LONG than SHORT ($U(29) = 262.0$, $P = 0.031$). There was no relationship between distance and ΔGM or SOL RMS/M_{MAX} (both $P \geq 0.514$). GM RMS/M_{MAX} decreased in both LONG and SHORT (both $P \leq 0.050$); however, ΔGM RMS/M_{MAX} was not different between LONG and SHORT ($t(27) = 0.50$, $P = 0.622$). SOL RMS/ M_{MAX} decreased PRE-POST ($F(1,27) = 8.75$, $P = 0.006$, $\eta^2 p = 0.245$) while there were no other effects (both $P \geq 0.162$).

3.4 Motor-evoked potentials and silent periods

There was a contraction intensity effect ($F(2,56) = 29.90$, $P < 0.001$, $\eta^2_p = 0.516$) for MEP area (Supplementary File 5). Post-hoc analysis revealed 100% MVC MEP area was smaller

than at 75 or 50% MVC (both $P < 0.001$). There was also a time effect (F (1,28) = 12.67, P = 0.001, η^2_p =0.312) and time × contraction intensity interaction (F (1.49,41.68) = 4.62, P = 0.024, η^2_p =0.142) that showed PRE-POST increases in MEP area for all contraction intensities (all $P < 0.001$). No other effects were observed ($P \geq 0.299$).

There were no time (F (1,28) = 2.13, P = 0.156) or contraction intensity (F (1.25,35.04) = 1.64, P = 0.211) effects for SP duration (Figure 3). There was a time × distance interaction (F (1,28) = 6.15, P = 0.019, η^2_p = 0.180) with post-hoc analysis revealing a PRE-POST decrease in LONG (P = 0.021) but no change in SHORT (P = 0.912). There was also a time × contraction intensity interaction (F (2,56) = 3.31, P = 0.044, η^2_p = 0.106) where SP shortened PRE-POST at 75 and 50% MVC (both $P < 0.001$) but not at 100% MVC (P = 0.167). No other effects were observed (all $P \geq 0.418$).

3.5 Blood parameters

There were no differences between LONG and SHORT at PRE for concentrations of CK, CRP, or leukocytes (all $P \geq 0.096$). There was a positive relationship between distance and both CK ($\rho(29) = 0.810$, $P < 0.001$) and CRP ($\rho(29) = 0.762$, $P < 0.001$) concentrations at POST. There was no relationship between distance and the change in leukocyte concentration at PRE to POST ($\rho(29) = -0.391$, $P = 0.030$). All blood parameters increased PRE-POST (all $P < 0.001$). At POST, CRP (Figure 4A; $U(29) = 416.5$, $P < 0.001$) and CK (Figure 4B; $U(29) = 415.0$, $P < 0.001$) concentrations were higher in LONG than SHORT. There was a distance effect ($F(1,29) = 15.05$, $P < 0.001$, η^2_p = 0.0.342) and a time × distance interaction ($F(1,29) = 82.54$, $P < 0.001$, η^2_p = 0.345) for leukocyte concentration. The subsequent independent-samples t test showed leukocyte concentration increased by 134 ± 64% in LONG versus 192 ± 75% in SHORT (Figure 4C; $t(29) = 2.33$, $P = 0.027$, $d = 0.842$).

4. DISCUSSION

The amount of exercise-induced performance fatigability, as determined by the percentage decrease in KE MVC, has been observed to increase with increasing exercise duration to ~1000 min with no further increases thereafter across a range of exercise modalities and conditions[9,10] The aim of the present study was therefore to determine whether there is a difference in the etiology of neuromuscular changes between shorter trail and longer ultra-trail running races run on similar terrain and weather conditions. The main results are that (i) as race distance increased, the decrease in KE MVC was greater yet the decrease was not different between LONG and SHORT (P = 0.073), (ii) as race distance increased, the decrease in KE TwPot was greater such that the decrease in LONG was greater than in SHORT, and (iii) VL SP duration decreased in LONG but was unchanged in SHORT.

4.1 Maximal voluntary and evoked torques and M waves

The observed decreases in KE (-27%) and PF (-28%) MVC in SHORT are comparable to previous trail-running races of 30-65 km.[6,11,23,24] Over similar longer trail-running races, decreases in KE (-38%) and PF (-28%) MVC for LONG also compare to previously reported losses of 32-38% for KE MVC and 26-39% for PF MVC.[5,8,11] KE and PF TwPot losses were smaller than after the first 40-km stage of a 169-km trail race[11] in SHORT. In LONG, KE (-28%) and PF (-18%) TwPot losses were comparable to decreases of 14-24% and 20-23%, respectively, previously reported after longer trail-running races.[5,8,11]

From previous research [for a complete review, see Giandolini et al [9]], it was expected there would be a greater decrease in KE and PF MVC in LONG than SHORT. While there was a negative relationship between distance and ΔKE MVC (i.e., larger KE MVC loss with increasing race distance) and a decrease in KE MVC in both LONG (-38%) and SHORT (]-27%) (Figure 2A), the time × distance interaction did not reach significance (p = 0.073).

From treadmill-running studies with repeated assessments, both intensity and duration of an exercise bout influence the development of KE neuromuscular impairments.[4,25] A distance effect on KE MVC loss may have been masked (i.e., type II error) by its interaction with the various intensities of effort across the five races, inadequate sample size, or the race duration of SHORT not falling on the steep rising portion of the ΔKE MVC and exercise duration curvilinear relationship (see [9,10]). Also, despite expectations, there was no relationship between distance and ΔPF MVC or difference in ΔPF MVC between LONG and SHORT. The lack of distance effect on ΔPF MVC may have been influenced by the intensity of eccentric contractions in PF during LONG being insufficient to elicit low-frequency fatigue, as PF $Db10 \cdot Db100^{-1}$ decreased in SHORT but not LONG. This is supported by mean running speed for two long downhill sections (5 and 7.5 km) common to three of the races being respectively 45% and 18% faster in SHORT (OCC) than LONG (CCC®, UTMB®).

A review of prolonged running study findings[9] suggested there was unlikely to be a relationship between race distance and KE TwPot decrease or a difference in the magnitude of KE TwPot decrease between LONG and SHORT. However, there was both a negative relationship between distance and ΔKE TwPot and significant time × distance interaction where subsequent analysis found a larger decrease in KE TwPot in LONG (-28%) than SHORT (-14%). The decrease in KE TwPot indicates impairment of muscle contractile properties.[26] The overall difference in running duration and distance in addition to the greater total eccentric loading in KE during the downhill portions of LONG versus SHORT suggest that there would be greater KE muscle damage (i.e. changes to the muscle following eccentric contractions that are histological, systematic, or symptomatic[27]) in LONG and that this would manifest as greater impairment of the contractile properties of KE; however, the magnitude of low-frequency fatigue in KE was not different between the two race distances, possibly due to running speed being faster in SHORT. Meanwhile, the lack of change in M-wave size that

is consistent with some,[3,8] but not all,[5] previous studies on long-distance trail running, suggests that sarcolemmal propagation is maintained regardless of race distance despite the large impairments to muscle contractile properties.

The concentrations of CK, an indirect indicator of muscle damage, and CRP, an indicator of inflammation, were greater in LONG than SHORT at POST and increased with increasing distance. While these results may indicate greater muscle damage and greater inflammation in LONG than SHORT, they may also be due to the greater duration of CK and CRP release in LONG (mean race time ~26.5 h) than SHORT (mean race time ~8 h). Both CK and CRP concentrations have been observed to increase for hours following short (< 3 h) endurance running bouts[28] with peak CK and CRP occurring up to at least 24 h post-exercise. POST leukocyte concentrations were greater in SHORT than LONG, suggesting the exercise-induced increase in leukocytes is blunted with longer races. This may be from trail-running triggering a pro-inflammatory response (i.e., increase in CK and CRP concentrations) that resulted in a leukocyte response, following which there was greater leukocyte apoptosis in LONG due to the longer exercise duration.[29]

Contrary to our previous analysis,[9] performance fatigability in PF was unaffected by race distance. This may be due to the small muscle mass, function, and contribution of PF. PF muscles are not the main torque producers during graded (i.e., downhill and uphill) running[30] although PF is more active than KE during level long-distance running.[31] Our group previously reported larger increases in stride frequency and ankle flexion angle and a larger decrease in ankle range of motion were correlated with increased peripheral PF neuromuscular impairment.[32] Thus, it is possible that the external load (combination of speed, distance, and eccentric loading) reaches an individual threshold such that biomechanical changes occur in order to maintain a homeostasis of all competing demands.[10,33] These changes may offer compensatory and protective effects that preferentially preserve PF

function[9] at the expense of KE, resulting in no further impairment to PF muscle contractile properties or the resulting maximal torque production.

4.2 Voluntary activation

The decreases in KE VA_{FNES} and PF VA_{TNES} were greater in SHORT (-18% and -12%, respectively) than previously reported[6,11] while this is the first short-distance trail-running race to assess KE VA_{TMS}. Meanwhile, KE VA_{FNES} and VA_{TMS} decreased comparably in LONG (-21% and -15%, respectively) to other long-distance trail-running races.[3,5,11] The decrease in LONG for PF VA_{TNES} (-11%) was comparable to previous studies[3,5] but less than Besson et al [11] (-19%). Similar decreases in VA for LONG and SHORT are coherent with the other findings of this study and a review of running studies[9] despite previous studies reporting greater impairment of VA as exercise duration increases.[4,25]

Although KE VA_{FNES} and KE VA_{TMS} were not different between LONG and SHORT, ΔVL RMS/M_{MAX} decreased more in LONG than SHORT. While this provides some evidence for greater impairment in central nervous system function in trail-running races that are longer and more difficult, this finding must be interpreted with caution since inter-day reproducibility for VL RMS/M_{MAX} is much lower than for VA_{FNES}.[34]

The lack of difference in ΔVA between LONG and SHORT was a surprising result, especially given the changes Besson et al [11] reported between a trail-running race as a single-stage or 4-stage race. Numerous factors may contribute to reduced central drive to locomotor muscles following trail-running races. Modulation of afferent feedback via disfacilitation of Ia afferent fibers has been presented as the main contributor to central nervous system impairments in prolonged running (for a complete review, see Millet et al [35]) although hypoglycemia, decreased cerebral catecholamine concentrations, cerebral ammonia accumulation, altered brain neurotransmitter concentrations, and increased core temperature

potentially contributed. While these factors are influenced by exercise duration, exercise intensity may be an equally important consideration. For example, one may suggest that because high daily temperatures reached 27-31°C in Chamonix every day during Ultra-Trail du Mont-Blanc® 2019,[13] the participants could have experienced more thermal stress in LONG. While previous studies have found hyperthermia causes central nervous system impairments,[36,37] the high daily temperatures did not result in greater central neuromuscular impairments in LONG. In fact, the higher running speed in SHORT (Table 1) probably induced more heat stress than much longer duration events.[38]

4.3 Motor-evoked potentials and silent periods

The increase in MEP size from PRE to POST in the present study is consistent with previously reported MEP changes for females and males from a 110-km trail-running race.[3,8] This is also coherent with increased MEP size after 4 h of cycling,[39] suggesting that an exercise-related increase in MEP size is a normal response to endurance and ultra-endurance exercise. The lack of time × distance or contraction intensity × time × distance interactions suggest there may be a threshold for duration or distance that, once reached, does not lead to further increases in MEP size. The mechanisms for the increase in MEP size remain unclear with possibilities including an increase in corticospinal excitability and/or a decrease in motor unit firing that results in more motor units being recruited by TMS due to fewer motor units being in a refractory state. While no trail-running studies have directly stimulated at the spinal level to evaluate cortical and motoneuronal components of the corticospinal pathway, our group observed decreased GM V-wave and unchanged SOL H-reflex amplitudes at the same events,[40] suggesting motoneuronal impairment was limited.

Interestingly, SP shortened in LONG but was unchanged in SHORT (Figure 3). However, in a previous ultra-trail running race, both when females and males were pooled[3]

and compared,[8] there was no SP duration change PRE-POST. The SP shortening in LONG is notable since SP has rarely been observed to shorten after an exercise bout with the authors only aware of SP shortening following whole-body (in this case, cycling) exercise in one study.[41] All previous studies examining SP duration after endurance (> 3 h) running or cycling bouts reported no change in either KE[3,8,39] or *tibialis anterior*.[42] The SP is thought to reflect motoneuronal and cortical mechanisms for the first ~100-150 ms and intracortical mechanisms mediated through gamma-aminobutyric acid B-receptor ($GABA_B$) inhibition thereafter.[43] The duration of observed SPs (mean SP duration of all participants at PRE = 202 ms and POST = 195 ms, Figure 3) could indicate a reduction in $GABA_B$-mediated inhibition. The mechanisms responsible for this are not clear and require further investigation.

4.4 Limitations

This study was conducted in conjunction with a series of trail-running races held during a seven-day period in August 2019. Unlike in lab-based work, it was not possible to control how many participants completed their respective race, the effort participants put into their race, or replace participants that chose to withdraw or not to complete all study aspects. It was also not possible to control the intake of food or drink during or after the race. Participants were offered food and drink after the race for medical reasons. Another limitation is the timing of PRE and POST assessments. These often occurred at different times of day since POST was performed as soon as possible after participants completed their race. There was also a delay to both KE (35:02 [26:54-42:20]) and PF (41:45 [36:36-48:25]) evaluations that would have permitted recovery and thus underestimated the magnitude of impairment[44] and the time between series evaluating neuromuscular function at POST was shorter than at PRE (i.e. 30 s versus 10 s).

5. CONCLUSION

The magnitude of decrease in KE MVC and TwPot increased with increasing race distance. The greater loss of KE TwPot in LONG than SHORT underscores the peripheral effects of long-distance trail-running races. Maximal VA was not different between LONG and SHORT in KE, indicating that increasing KE MVC loss with increasing distance was not due to greater central impairments as hypothesized, rather greater impairment of muscle contractile properties. Conversely, changes in PF neuromuscular function were unaffected by race distance. The duration of the TMS-induced silent period in VL decreased in LONG but not SHORT, suggesting a reduction in cortical inhibition in longer races that requires further investigation to understand the mechanisms responsible.

6. PERSPECTIVE

A curvilinear relationship between the duration of an (ultra-)endurance exercise bout and the magnitude of induced maximal strength loss has been reported from aggregating multiple studies of single-distance events.[9,10] The current study is however the first to directly investigate the effect of race distance on neuromuscular function changes using multiple single-stage races under similar conditions in male trail runners. This study also builds upon previous work from our laboratory that has used ultra-endurance exercise as a model to better understand the etiology of changes in neuromuscular function. Ultra-endurance sporting events permit investigations of acute physiological, biomechanical, and psychological changes to the exercise bout and subsequent recovery in addition to adaptative responses under conditions that are challenging, if not impossible, to replicate in a laboratory. Future research must be used to identify the mechanisms and better understand the complex interplay of factors that contribute to neuromuscular and other impairments that result in reduced exercise performance. For example, from the present study, the observed reduction

in corticospinal inhibition (i.e. decrease in SP duration) in LONG but not SHORT requires further investigation to understand why this occurs and what it means from a practical perspective.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

REFERENCES

1. Enoka RM, Duchateau J. Translating fatigue to human performance. *Med Sci Sports Exerc.* 2016;48(11):2228-2238.
2. Bigland-Ritchie B, Woods JJ. Changes in muscle contractile properties and neural control during human muscular fatigue. *Muscle Nerve.* 1984;7(9):691-699.
3. Temesi J, Rupp T, Martin V, et al. Central fatigue assessed by transcranial magnetic stimulation in ultratrail running. *Med Sci Sports Exerc.* 2014;46(6):1166-1175.
4. Martin V, Kerherve H, Messonnier LA, et al. Central and peripheral contributions to neuromuscular fatigue induced by a 24-h treadmill run. *J Appl Physiol.* 2010;108(5):1224-1233.
5. Millet GY, Tomazin K, Verges S, et al. Neuromuscular consequences of an extreme mountain ultra-marathon. *PLoS One.* 2011;6(2):e17059.
6. Millet GY, Martin V, Lattier G, Ballay Y. Mechanisms contributing to knee extensor strength loss after prolonged running exercise. *J Appl Physiol.* 2003;94(1):193-198.
7. Place N, Millet GY. Quantification of neuromuscular fatigue: what do we do wrong and why? *Sports Med.* 2020;50(3):439-447.
8. Temesi J, Arnal PJ, Rupp T, et al. Are females more resistant to extreme neuromuscular fatigue? *Med Sci Sports Exerc.* 2015;47(7):1372-1382.

9. Giandolini M, Vernillo G, Samozino P, et al. Fatigue associated with prolonged graded running. *Eur J Appl Physiol.* 2016;116(10):1859-1873.
10. Millet GY. Can neuromuscular fatigue explain running strategies and performance in ultra-marathons?: the flush model. *Sports Med.* 2011;41(6):489-506.
11. Besson T, Rossi J, Mallouf TLR, et al. Fatigue and recovery following single-stage versus multistage ultramarathon running. *Med Sci Sports Exerc.* 2020;52(8):1691-1698.
12. Martinez-Navarro I, Sanchez-Gomez JM, Aparicio I, et al. Effect of mountain ultramarathon distance competition on biochemical variables, respiratory and lower-limb fatigue. *PLoS One.* 2020;15(9):e0238846.
13. Time and Date AS. Past Weather in Chamonix Mont-Blanc, Haute-Savoie, France — August 2019. https://www.timeanddate.com/weather/@7602519/historic?month=8&year=2019. Accessed May 20, 2020.
14. Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br J Nutr.* 1974;32(1):77-97.
15. Temesi J, Gruet M, Rupp T, Verges S, Millet GY. Resting and active motor thresholds versus stimulus-response curves to determine transcranial magnetic stimulation intensity in quadriceps femoris. *J Neuroeng Rehabil.* 2014;11:40.
16. Mathis J, de Quervain D, Hess CW. Dependence of the transcranially induced silent period on the 'instruction set' and the individual reaction time. *Electroencephalogr Clin Neurophysiol.* 1998;109(5):426-435.

17. Verges S, Maffiuletti NA, Kerherve H, Decorte N, Wuyam B, Millet GY. Comparison of electrical and magnetic stimulations to assess quadriceps muscle function. *J Appl Physiol.* 2009;106(2):701-710.
18. Martin PG, Gandevia SC, Taylor JL. Output of human motoneuron pools to corticospinal inputs during voluntary contractions. *J Neurophysiol.* 2006;95(6):3512-3518.
19. Todd G, Taylor JL, Gandevia SC. Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. *J Physiol.* 2003;551(2):661-671.
20. Hunter SK, Butler JE, Todd G, Gandevia SC, Taylor JL. Supraspinal fatigue does not explain the sex difference in muscle fatigue of maximal contractions. *J Appl Physiol.* 2006;101(4):1036-1044.
21. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Methodol.* 1995;57(1):289-300.
22. Cohen J. *Statistical power analysis for the behavioral sciences.* 2nd ed. Hillsdale (NJ): Lawrence Erlbaum Associates; 1988. 567 p.
23. Millet GY, Lepers R, Maffiuletti NA, Babault N, Martin V, Lattier G. Alterations of neuromuscular function after an ultramarathon. *J Appl Physiol.* 2002;92(2):486-492.
24. Gauche E, Lepers R, Rabita G, et al. Vitamin and mineral supplementation and neuromuscular recovery after a running race. *Med Sci Sports Exerc.* 2006;38(12):2110-2117.
25. Place N, Lepers R, Deley G, Millet GY. Time course of neuromuscular alterations during a prolonged running exercise. *Med Sci Sports Exerc.* 2004;36(8):1347-1356.

628 26. Edwards RH, Hill DK, Jones DA, Merton PA. Fatigue of long duration in human
629 skeletal muscle after exercise. *J Physiol.* 1977;272(3):769-778.
630 27. Hyldahl RD, Chen TC, Nosaka K. Mechanisms and mediators of the skeletal muscle
631 repeated bout effect. *Exerc Sport Sci Rev.* 2017;45(1):24-33.
632 28. Fatouros IG, Jamurtas AZ, Nikolaidis MG, et al. Time of sampling is crucial for
633 measurement of cell-free plasma DNA following acute aseptic inflammation induced
634 by exercise. *Clin Biochem.* 2010;43(16-17):1368-1370.
635 29. Kruger K, Mooren FC. Exercise-induced leukocyte apoptosis. *Exerc Immunol Rev.*
636 2014;20:117-134.
637 30. Vernillo G, Martinez A, Baggaley M, et al. Biomechanics of graded running: Part I -
638 Stride parameters, external forces, muscle activations. *Scand J Med Sci Sports.*
639 2020;30(9):1632-1641.
640 31. Novacheck TF. The biomechanics of running. *Gait Posture.* 1998;7(1):77-95.
641 32. Giandolini M, Gimenez P, Temesi J, et al. Effect of the fatigue induced by a 110-km
642 ultramarathon on tibial impact acceleration and lower leg kinematics. *PLoS One.*
643 2016;11(3):e0151687.
644 33. Thomas K, Goodall S, Howatson G. Performance fatigability is not regulated to a
645 peripheral critical threshold. *Exerc Sport Sci Rev.* 2018;46(4):240-246.
646 34. Place N, Maffiuletti NA, Martin A, Lepers R. Assessment of the reliability of central
647 and peripheral fatigue after sustained maximal voluntary contraction of the quadriceps
648 muscle. *Muscle Nerve.* 2007;35(4):486-495.
649 35. Millet GY, Martin V, Temesi J. The role of the nervous system in neuromuscular
650 fatigue induced by ultra-endurance exercise. *Appl Physiol Nutr Metab.*
651 2018;43(11):1151-1157.

36. Periard JD, Christian RJ, Knez WL, Racinais S. Voluntary muscle and motor cortical activation during progressive exercise and passively induced hyperthermia. *Exp Physiol.* 2014;99(1):136-148.
37. Nybo L, Nielsen B. Hyperthermia and central fatigue during prolonged exercise in humans. *J Appl Physiol.* 2001;91(3):1055-1060.
38. Bouscaren N, Millet GY, Racinais S. Heat stress challenges in marathon vs. ultra-endurance running. *Front Sports Act Living.* 2019;1:59.
39. Jubeau M, Rupp T, Perrey S, et al. Changes in voluntary activation assessed by transcranial magnetic stimulation during prolonged cycling exercise. *PLoS One.* 2014;9(2):e89157.
40. Espeit L, Brownstein CG, Royer N, et al. Central fatigue aetiology in prolonged trail running races. *Exp Physiol.* 2021.
41. Temesi J, Arnal PJ, Davranche K, et al. Does central fatigue explain reduced cycling after complete sleep deprivation? *Med Sci Sports Exerc.* 2013;45(12):2243-2253.
42. Ross EZ, Middleton N, Shave R, George K, Nowicky A. Corticomotor excitability contributes to neuromuscular fatigue following marathon running in man. *Exp Physiol.* 2007;92(2):417-426.
43. Škarabot J, Mesquita RNO, Brownstein CG, Ansdell P. Myths and methodologies: how loud is the story told by the transcranial magnetic stimulation-evoked silent period? *Exp Physiol.* 2019;104(5):635-642.
44. Froyd C, Millet GY, Noakes TD. The development of peripheral fatigue and short-term recovery during self-paced high-intensity exercise. *J Physiol.* 2013;591(5):1339-1346.

TABLE 1 Participant characteristics, finish time, time to POST assessment, and PRE maximal and evoked torques.

	SHORT	*n*	LONG	*n*	
Age (years)	35 ± 8	13	38 ± 10	19	
Height (cm)	180 ± 8	13	179 ± 4	19	
Body mass (kg)	74.4 [65.9-85.3]	13	69.6 [66.8-78.2]	19	
$\dot{V}O_{2PEAK}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	61.7 ± 11.2	12	62.5 ± 5.9	19	
Body fat (%)	17.7 ± 6.3	13	13.6 ± 4.5	19	*
Finish time (hh:mm:ss)	8:07:57 ± 2:44:33	13	26:27:11 ± 10:07:50	19	***
Race distance (km)	49 ± 8	13	128 ± 33	19	***
Speed ($km \cdot h^{-1}$)	6.42 ± 1.72		5.10 ± 1.00		*
Time to first KE MVC POST (mm:ss)	29:19 [24:34-37:48]	13	37:30 [27:00-49:14]	19	
Time to first PF MVC POST (mm:ss)	39:14 [35:32-45:16]	12	42:33 [37:12-49:59]	17	
KE MVC (N·m)	289 ± 71	13	290 ± 50	19	
KE TwPot (N·m)	75 ± 21	13	71 ± 16	18	
KE $Db10 \cdot Db100^{-1}$	0.98 ± 0.05	13	0.99 ± 0.09	18	
KE VA_{FNES} (%)	87 ± 8	13	86 ± 9	18	
KE VA_{TMS} (%)	92 ± 4	11	93 ± 5	17	
PF MVC (N·m)	161 ± 34	12	168 ± 24	17	
PF TwPot (N·m)	25 ± 6	12	25 ± 3	17	
PF $Db10 \cdot Db100^{-1}$	0.96 ± 0.04	12	0.98 ± 0.09	17	
PF VA_{TNES} (%)	97 [95-98]	12	95 [92-98]	17	

Note: Values are presented as mean ± SD or median [IQR].

679 Abbreviations: $Db10 \cdot Db100^{-1}$, ratio of potentiated 10-Hz doublet to potentiated 100-Hz
680 doublet; KE, knee extensors; MVC, maximal voluntary contraction; PF, plantar flexors; PRE,
681 pre-race assessment; POST, post-race assessment; TwPot, potentiated twitch; VA_{FNES},
682 voluntary activation of the knee extensors assessed by femoral nerve electrical stimulation;
683 VA_{TMS}, voluntary activation of the knee extensors assessed by transcranial magnetic
684 stimulation; VA_{TNES}, voluntary activation of the plantar flexors assessed by tibial nerve
685 electrical stimulation; $\dot{V}O_{2PEAK}$, peak oxygen consumption.
686 * Significant between-group difference: $P < 0.05$
687 *** Significant between-group difference: $P < 0.001$

688 **Table 2** Race characteristics and participants for LONG and SHORT

Race	Group	Distance (km)	Positive elevation change (m)	Negative elevation change (m)	Participants (*n*)
MCC	SHORT	40	2300	2100	6
OCC		56	3500	3400	7
CCC®	LONG	101	6100	6300	11
TDS®		145	9100	9300	2
UTMB®		171	10 300	10 300	6

689

690 Abbreviations: CCC®, Courmayeur-Champex-Chamonix; LONG, race distance > 100 km;
691 MCC, De Martigny-Combe à Chamonix; OCC, Orsières-Champex-Chamonix; SHORT, race
692 distance < 60 km; TDS®, Sur les Traces des Ducs de Savoie; UTMB®, Ultra-Trail du Mont-
693 Blanc®.

694

FIGURE LEGENDS

FIGURE 1 Consolidated Standards of Reporting Trials (CONSORT) study flow diagram. Race distance was characterized as LONG (> 100 km) or SHORT (< 60 km). The exclusion of three participants that completed PRE and POST evaluations from data analysis was because they met both of the following criteria: 1) completed the race with a slower competitor (i.e., would have been faster if not racing with a slower competitor) and 2) indicated 0 on a scale from 0 to 10 as to whether they pushed themselves at the end of the race (0 – I managed the end of the race without pushing; 10 – I pushed myself hard until the end of the race). PRE = pre-race assessment; POST = post-race assessment.

FIGURE 2 PRE to POST changes by race distance (LONG *versus* SHORT) in (A) KE for MVC, TwPot, $Db10 \cdot Db100^{-1}$, VA_{FNES}, and VA_{TMS}, (B) PF for MVC, TwPot, and $Db10 \cdot Db100^{-1}$ and (C) PF for VA_{TNES}. In panels A and B, black bars indicate LONG and white bars indicate SHORT. Values are presented as mean ± SD. In panel C, the boxplots present the median, 25^{th} and 75^{th} percentiles, range, and outlier (black circle). ** $P < 0.01$ percentage change between groups. $Db10 \cdot Db100^{-1}$, ratio of potentiated 10-Hz doublet to potentiated 100-Hz doublet; KE, knee extensors; LONG, trail-running race > 100 km; MVC, maximal voluntary contraction; PF, plantar flexors; PRE, pre-race assessment; POST, post-race assessment; SHORT, trail-running race < 60 km; TwPot, potentiated twitch; VA_{FNES}, voluntary activation assessed by femoral nerve stimulation; VA_{TMS}, voluntary activation assessed by transcranial magnetic stimulation; VA_{TNES}, voluntary activation assessed by tibial nerve electrical stimulation.

FIGURE 3 SP duration elicited by TMS during contractions at 100, 75, and 50% MVC at PRE and POST LONG and SHORT trail-running races. Black bars indicate LONG and white bars indicate SHORT. Values are presented as mean ± SD. PRE-POST SP duration decreased in LONG ($p = 0.021$) but not SHORT ($p = 0.912$). LONG, trail-running race > 100 km; MVC, maximal voluntary contraction; PRE, pre-race assessment; POST, post-race assessment; SHORT, trail-running race < 60 km; SP, silent period; TMS, transcranial magnetic stimulation.

FIGURE 4 Concentrations at POST for (A) CRP and (B) CK and (C) leukocyte concentrations at PRE and POST. In panels A and B, the boxplots present the median, 25^{th} and 75^{th} percentiles, range, and outlier (black circle). In panel C, values are presented as mean ± SD, the black bars indicate LONG, and the white bars indicate SHORT. * $P < 0.05$, percentage change between groups. *** $P < 0.001$, between groups. CK, creatine kinase; CRP, C-reactive protein; LONG, trail-running race > 100 km; PRE, pre-race assessment; POST, post-race assessment; SHORT, trail-running race < 60 km.

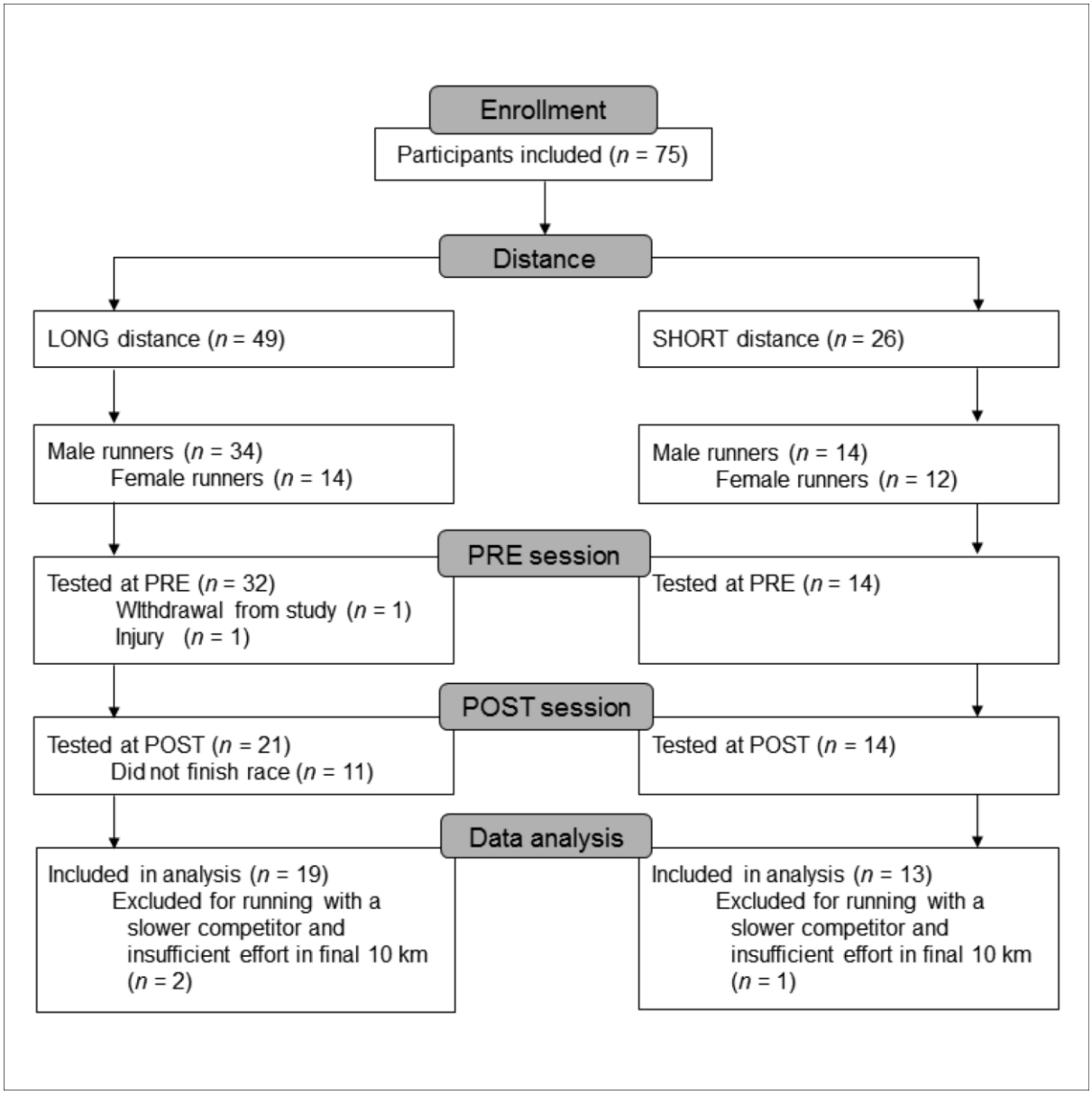

Enrollment
Participants included (n = 75)
Distance
LONG distance (n = 49)
SHORT distance (n = 26)
Male runners (n = 34)
Female runners (n = 14)
Male runners (n = 14)
Female runners (n = 12)
PRE session
Tested at PRE (n = 32)
Withdrawal from study (n = 1)
Injury (n = 1)
Tested at PRE (n = 14)
POST session
Tested at POST (n = 21)
Did not finish race (n = 11)
Tested at POST (n = 14)
Data analysis
Included in analysis (n = 19)
Excluded for running with a slower competitor and insufficient effort in final 10 km (n = 2)
Included in analysis (n = 13)
Excluded for running with a slower competitor and insufficient effort in final 10 km (n = 1)

FIGURE 1

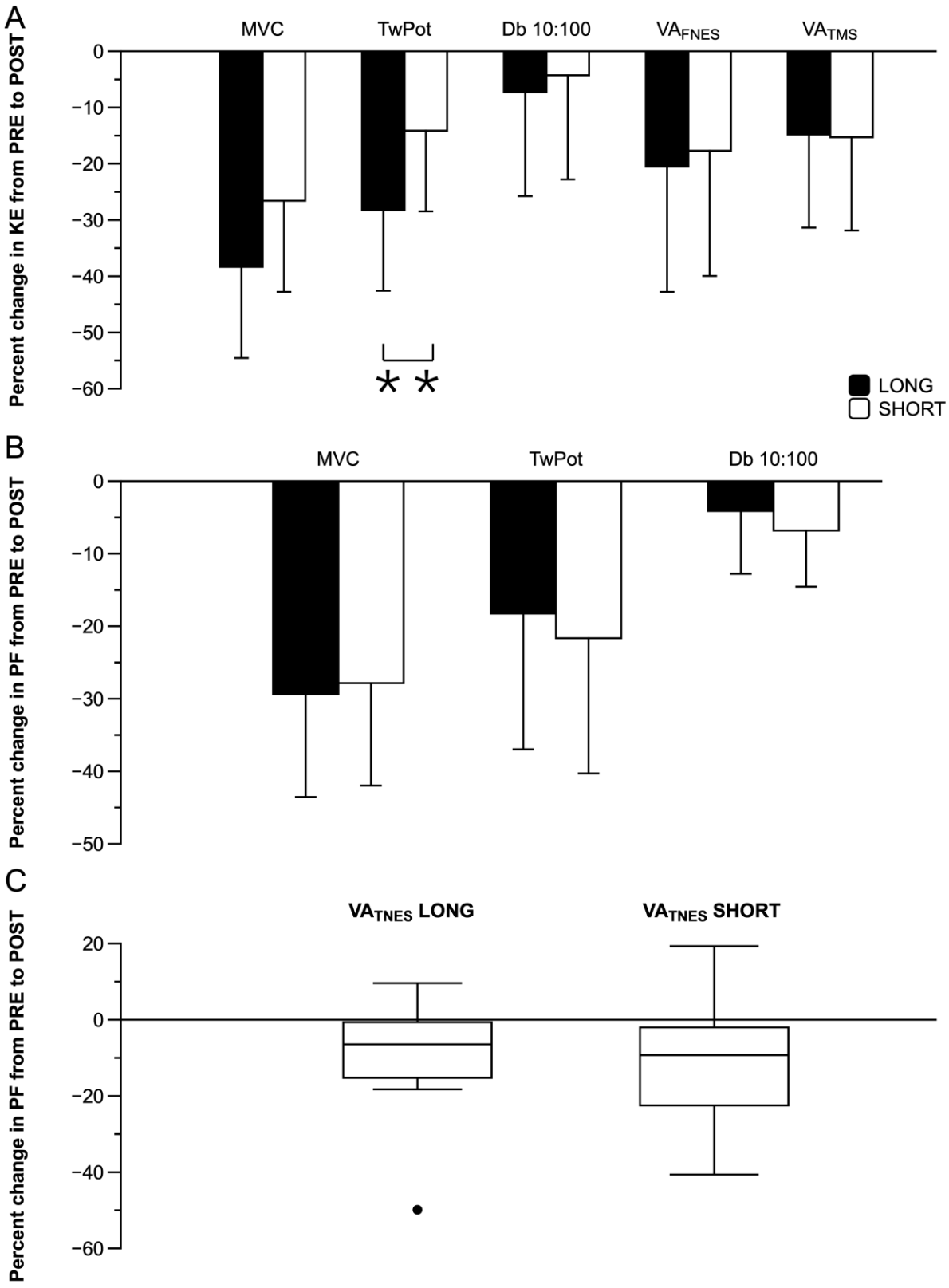

A
Percent change in KE from PRE to POST
MVC
TwPot
Db 10:100
VA$_{FNES}$
VA$_{TMS}$
0
−10
−20
−30
−40
−50
−60
* *
LONG
SHORT
B
Percent change in PF from PRE to POST
MVC
TwPot
Db 10:100
0
−10
−20
−30
−40
−50
C
Percent change in PF from PRE to POST
VA$_{TNES}$ LONG
VA$_{TNES}$ SHORT
20
0
−20
−40
−60

FIGURE 2

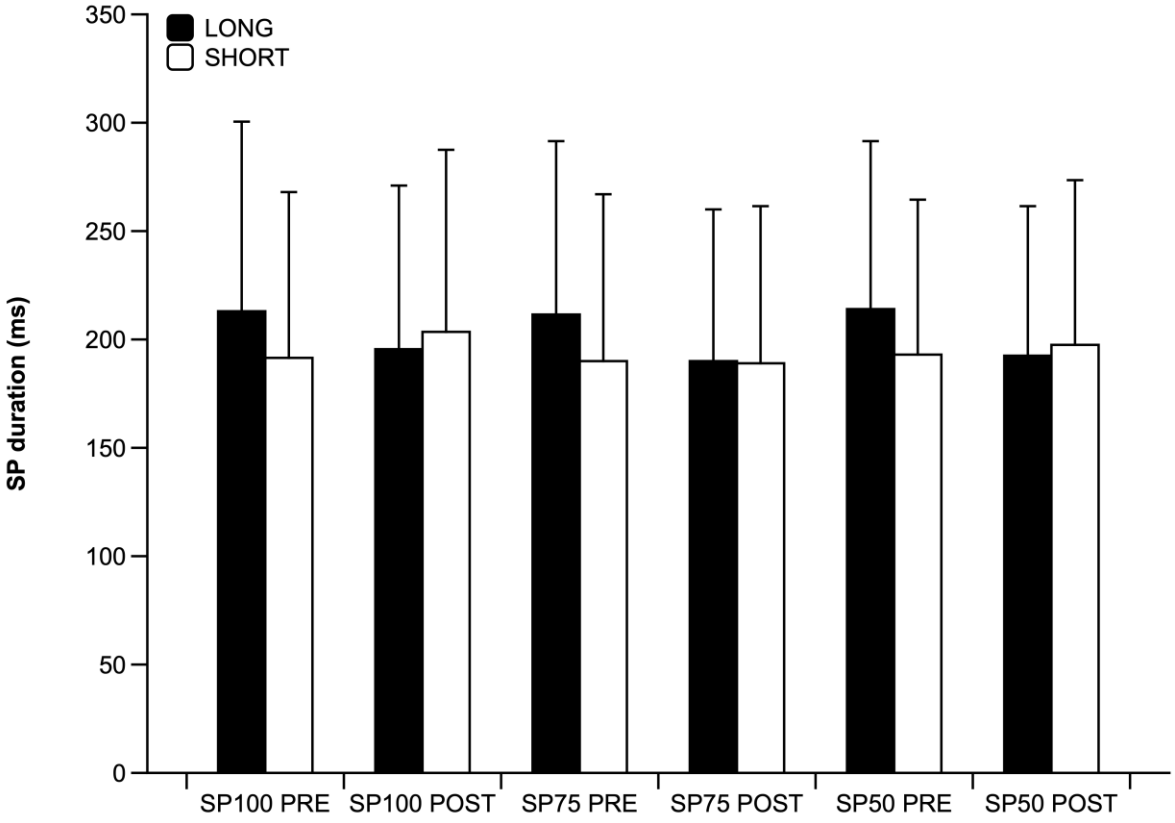

LONG
SHORT
SP duration (ms)
350
300
250
200
150
100
50
0
SP100 PRE
SP100 POST
SP75 PRE
SP75 POST
SP50 PRE
SP50 POST

FIGURE 3

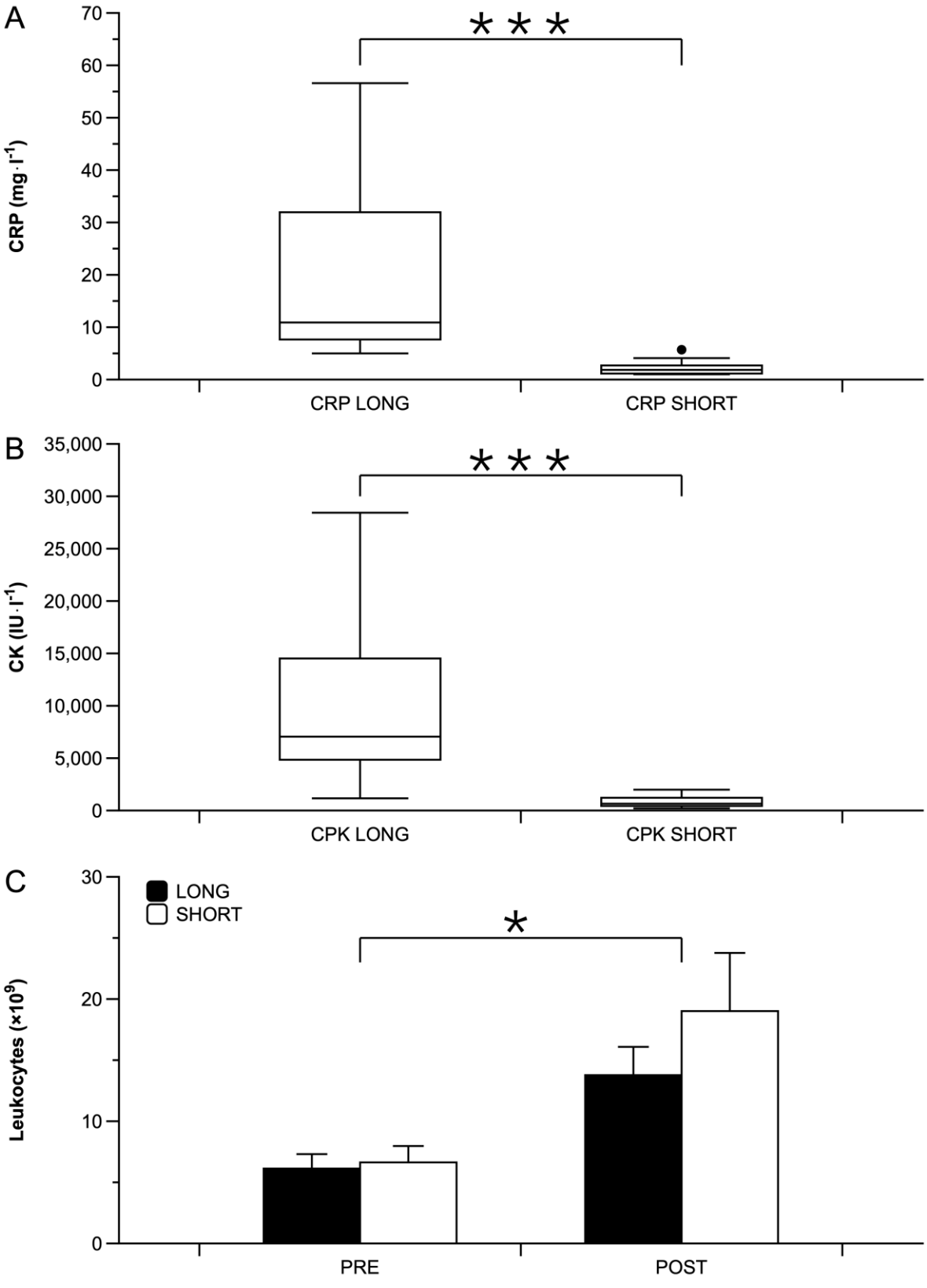
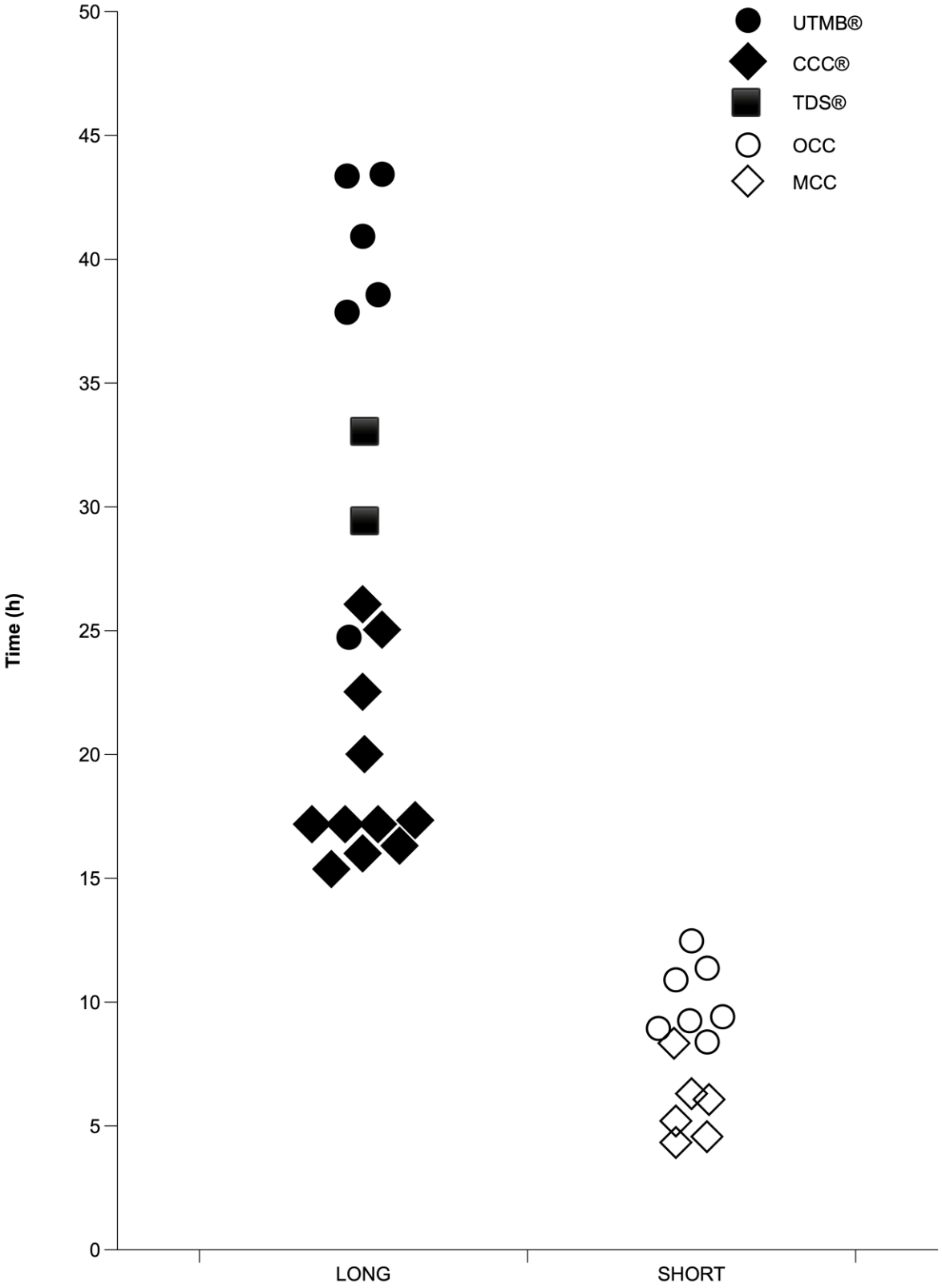
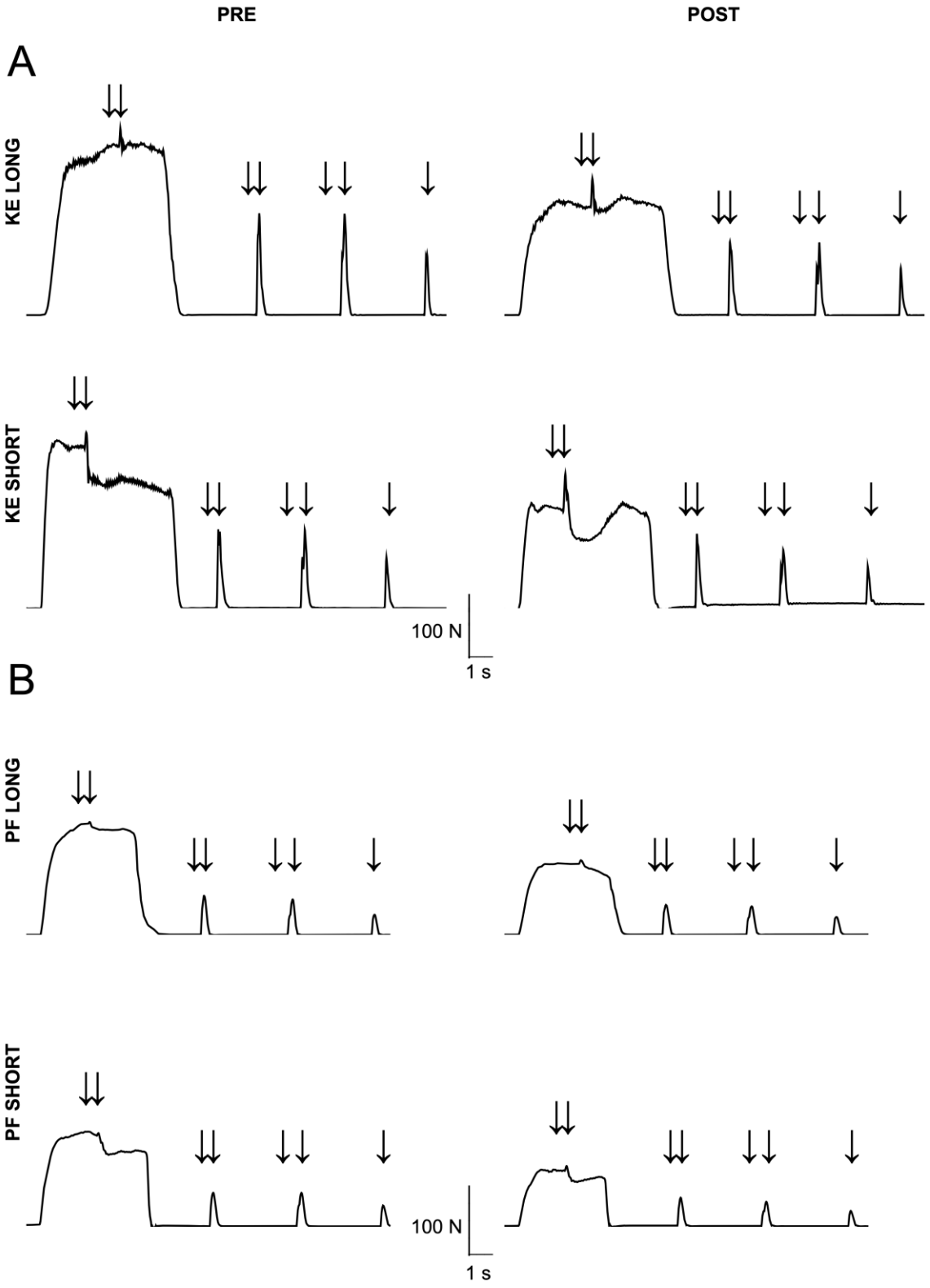

A
70
60
50
40
30
20
10
0
CRP (mg·l-1)
***
CRP LONG
CRP SHORT
B
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
CK (IU·l-1)
***
CPK LONG
CPK SHORT
C
30
20
10
0
Leukocytes (×10⁹)
LONG
SHORT
*
PRE
POST

FIGURE 4



Supplementary File 1 Individual race times of participants by race and distance category. CCC®, Courmayeur-Champex-Chamonix; LONG, race distance > 100 km; MCC, De Martigny-Combe à Chamonix; OCC, Orsières-Champex-Chamonix; SHORT, race distance < 60 km; TDS®, Sur les Traces des Ducs de Savoie; UTMB®, Ultra-Trail du Mont-Blanc®.

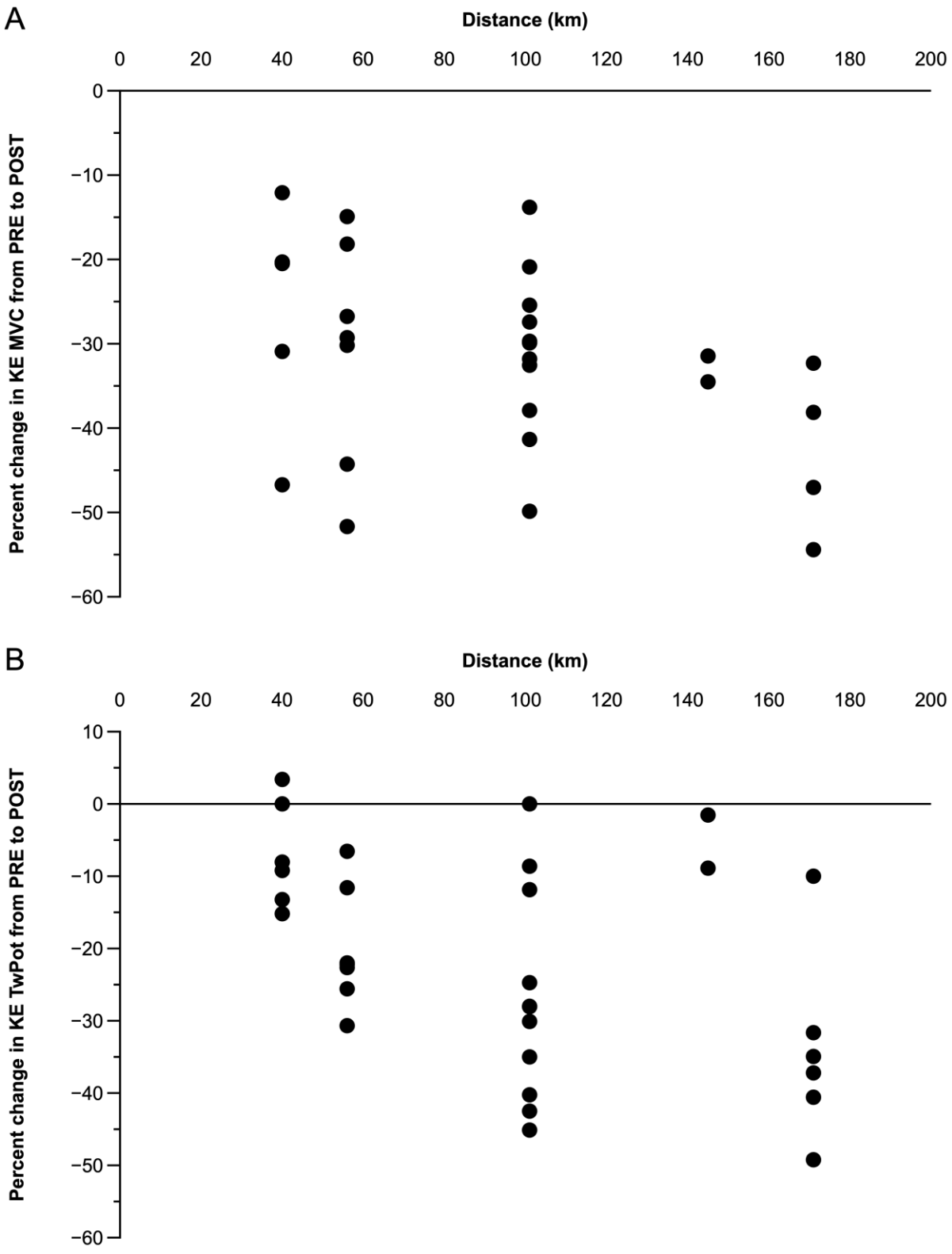


Supplementary File 2 Representative PRE and POST torque traces of MVC and electrically-evoked torques in LONG and SHORT participants in the (A) knee extensors and (B) plantar flexors. ↓↓, 100-Hz paired-pulse stimuli; ↓ ↓, 10-Hz paired-pulse stimuli; ↓, single stimulus; KE, knee extensors; LONG, trail-running race > 100 km; MVC, maximal voluntary contraction; PF, plantar flexors; PRE, pre-race assessment; POST, post-race assessment; SHORT, trail-running race < 60 km; TwPot, potentiated twitch.

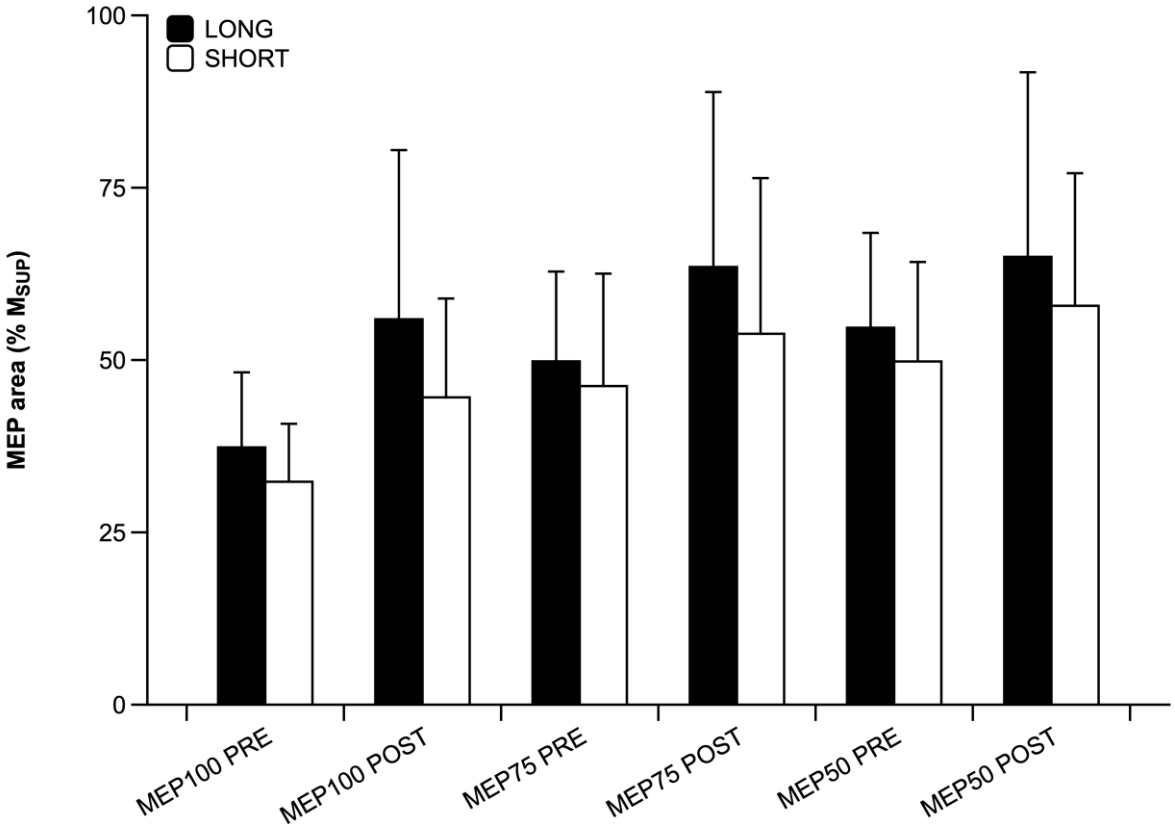
Supplementary File 3 Spearman rank-order correlation coefficients (ρ) for the relationship between race distance or race time and the percentage PRE-POST change (Δ) in neuromuscular parameters and leukocyte concentration and POST CK and CRP concentrations.

	Distance	Time
KE MVC	ρ = -0.556 *	ρ = -0.505 *
KE TwPot	ρ = -0.521 *	ρ = -0.442 *
KE $Db10 \cdot Db100^{-1}$	ρ = -0.163	ρ = -0.104
KE VA_{FNES}	ρ = -0.116	ρ = -0.147
KE VA_{TMS}	ρ = -0.034	ρ = 0.001
VL M_{MAX} amplitude	ρ = 0.090	ρ = 0.105
VL M_{SUP} area	ρ = 0.206	ρ = 0.256
VL RMS/M_{MAX}	ρ = -0.442 *	ρ = -0.367
PF MVC	ρ = -0.120	ρ = -0.122
PF TwPot	ρ = 0.143	ρ = 0.076
PF $Db10 \cdot Db100^{-1}$	ρ = 0.248	ρ = 0.142
PF VA_{TNES}	ρ = 0.034	ρ = -0.026
SOL M_{MAX} amplitude	ρ = -0.326	ρ = -0.345
GM M_{MAX} amplitude	ρ = -0.229	ρ = -0.108
SOL RMS/M_{MAX}	ρ = 0.126	ρ = 0.147
GM RMS/M_{MAX}	ρ = -0.051	ρ = -0.154
CRP (POST)	ρ = 0.762 *	ρ = 0.740 *
CK (POST)	ρ = 0.810 *	ρ = 0.809 *
Leukocytes	ρ = -0.391	ρ = -0.470 *

Abbreviations: CK, creatine kinase; CRP, C-reactive protein; $Db10 \cdot Db100^{-1}$, ratio of potentiated 10-Hz doublet to potentiated 100-Hz doublet; GM, gastrocnemius medialis; KE, knee extensors; M_{MAX}, maximal M wave elicited in relaxed muscle; M_{SUP}, maximal M wave elicited during a voluntary contraction at 50% MVC; MVC, maximal voluntary contraction; PF, plantar flexors; POST, post-race assessment; RMS, root mean square; SOL, soleus; TwPot, potentiated twitch; VA_{FNES}, voluntary activation of the knee extensors assessed by femoral nerve electrical stimulation; VA_{TMS}, voluntary activation of the knee extensors assessed by transcranial magnetic stimulation; VA_{TNES}, voluntary activation of the plantar flexors assessed by tibial nerve electrical stimulation; VL, vastus lateralis.
* $P < 0.05$ after correction for multiple comparisons using the Benjamini and Hochberg procedure.



Supplementary File 4 Relationship between race distance and the PRE-POST percentage change for (A) KE MVC, ρ = -0.556, $P < 0.001$ and (B) KE TwPot, ρ = -0.521, $P = 0.003$. KE, knee extensors; MVC, maximal voluntary contraction; PF, plantar flexors; PRE, pre-race assessment; POST, post-race assessment; TwPot, potentiated twitch.



Supplementary File 5 MEP area elicited by TMS during contractions at 100, 75, and 50% MVC at PRE and POST LONG and SHORT trail-running races. Black bars indicate LONG and white bars indicate SHORT. Values are presented as mean ± SD. There was a main contraction intensity effect such that MEP area at 100% MVC was smaller than at 75 or 50% MVC (both $P < 0.001$). There was a main time effect and time × contraction intensity interaction showing that MEP area for all contraction intensities increased PRE-POST (all $P < 0.001$). LONG, trail-running race > 100 km; MEP, motor-evoked potential; M_{SUP}, M-wave area elicited during voluntary contractions at 50% MVC; MVC, maximal voluntary contraction; PRE, pre-race assessment; POST, post-race assessment; SHORT, trail-running race < 60 km; TMS, transcranial magnetic stimulation.