Sex differences in neuromuscular fatigue and changes in cost of running after mountain trail races of various distances

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ABSTRACT

Introduction: Females have been shown to experience less neuromuscular fatigue than males in knee extensors (KE) and less peripheral fatigue in plantar flexors (PF) following ultra-trail running, but it is unknown if these differences exist for shorter trail running races and whether this may impact running economy. The purpose of this study was to characterize sex differences in fatigability over a range of running distances and to examine possible differences in the post-race alteration of the cost of running (Cr).

Methods: Eighteen pairs of males and females were matched by performance after completing different races ranging from 40 to 171 km, divided into SHORT vs LONG races (< 60 and > 100 km, respectively). NM function and Cr were tested before and after each race. NM function was evaluated on both KE and PF with voluntary and evoked contractions using electrical nerve (KE and PF) and transcranial magnetic (KE) stimulation. Oxygen uptake, respiratory exchange ratio and ventilation were measured on a treadmill and used to calculate Cr.

Results: Compared to males, females displayed a smaller decrease in maximal strength in KE (−36% *vs* −27%, respectively, $p < 0.01$), independent of race distance. In SHORT only, females displayed less peripheral fatigue in PF compared to males (Δ peak twitch: −10% *vs* −24%, respectively, $p < 0.05$). Cr increased similarly in males and females.

Conclusion: Females experience less neuromuscular fatigue than men following both ‘classic’ and ‘extreme’ prolonged running exercises but this does not impact the degradation of the energy cost of running.

Keywords

Energy cost, fatigability, females, males, trail running

INTRODUCTION

In recent years, participation in trail and ultra-trail (defined by the International Trail Running Association as trail races with a distance > 80 km) running has increased considerably, with reports of numerous events having to limit registration numbers due to over-demand. However, participation in trail and ultra-trail events remains considerably more popular in males than females. For instance, at the UTMB® 2019, one of the most popular ultra-trail events in the world, only 257 out of 2543 starters, i.e. 10%, were females. Despite the low participation rates for females, ultra-trail running is one of the rare disciplines in which females have outperformed males in some events (1).

The increasing enthusiasm for trail running has led scientists and researchers to take a particular interest in the physiological and neuromuscular consequences of prolonged trail running events. Neuromuscular (NM) fatigue, also known as performance fatigability, can be defined as the progressive change that occurs in the central nervous system and/or muscles due to exercise, resulting in a force output that is less than anticipated for a given voluntary contraction or stimulation (2). It has been reported from isometric studies in isolated muscle groups that females are less fatigable than males, particularly at submaximal intensities (3). While part of the sex difference in fatigability has been attributed to greater strength and consequent higher blood flow occlusion in males, previous studies have demonstrated that the sex difference in fatigability during submaximal isometric contractions persists when males and females were matched for strength (4, 5). Furthermore, Ansdell, et al. (6) assessed sex differences in fatigability relative to the critical power, and showed that females are less fatigable during intermittent isometric contractions above critical torque, and experience less muscle deoxygenation at intensities both above and below critical torque. Thus, it was suggested that the lower fatigability was mediated by lower muscle deoxygenation, likely due, at least in part, to a higher proportional area of type-I fibres (7), greater muscle capillarisation (7) and greater

vasodilatory capacity in females compared with males (8). While the studies mentioned above [see Hunter (3) for a review] bring interesting insights to the mechanisms responsible for sex differences in fatigability after single-joint tasks, whether these findings are transferable to whole-body exercise such as running is unclear.

It is well-known that trail and ultra-trail running induces alterations to both central and peripheral components of NM function (9-12). The reduction in maximal strength increases with exercise duration until a duration corresponding to ~100 miles (13, 14). Based on the lower level of fatigability in females during isometric tasks as well as the out-performance of males by females in recent ultra-trail events, one may be tempted to believe that females experience less NM fatigue during prolonged exercises. Surprisingly, this question remains understudied. After two hours of running on a treadmill at gas exchange threshold, Glace, et al. (15) reported a significant decrease in isokinetic maximal voluntary knee-flexor and knee-extensor (KE) force in males only when measured at low angular velocities (60°/s). On the contrary, Boccia, et al. (16) reported a similar decrease between sexes in maximal KE force after a half-marathon performed in race conditions by amateur runners. Over a much longer distance, our group (12) assessed NM fatigue in females and males matched by relative level of performance after a 110-km ultra-trail-running race. We observed that females showed i) less peripheral fatigue in the plantar flexors (PF) and ii) a lower decrease in maximal force loss in KE compared to their male counterparts. A recent review compiled physiological outcomes that can confer females an advantage over longer distances (17) such as greater distribution of type I fiber and better substrate efficiency (higher rate of lipid oxidation and lower carbohydrate utilization), however, a systematic comparison between males and females over various running distances has yet to be performed. In addition, whether or not this difference is due to NM fatigue resistance *vs* the competition intention of the participants (i.e. the effort put into the race) is not known. Indeed, this latter factor may influence the race-induced fatigue but has never been considered when

comparing males and females. Sex differences such as greater sporting motivation and competitiveness in males could be expected (18), although the physiological impact of these psychological characteristics are still unknown.

In addition to NM fatigue, prolonged running may induce alterations in energy cost of running (Cr) (19). Interestingly, after a 2-hour treadmill run, Glace, et al. (15) reported significant maximal knee strength loss (see above) and an increase in Cr in males only. Additional motor unit recruitment to compensate for muscle fatigue could partly explain greater oxygen uptake ($\dot{V}O_2$) demand (19, 20). As such, it is possible that attenuated muscle fatigue in females could reduce the compensatory increase in motor unit recruitment and thus $\dot{V}O_2$ demand. However, it should be noted that conflicting evidence exists surrounding the effects of muscle fatigue on changes in $\dot{V}O_2$, with some studies reporting no interaction between these variables (21-24). In addition to Glace, et al. (15) only two other studies have to the best of our knowledge examined sex difference on Cr change with fatigue and observed similar changes in males and females: one following a 1-h run at marathon pace (25) and another one following a 5-km run at 80-85% of maximal oxygen uptake ($\dot{V}O_{2max}$) (26). However, these experiments were conducted on a treadmill, i.e. not in ecologically valid conditions. Furthermore, since an effect of distance on sex differences in fatigability may exist, a comparison of the change in Cr between sexes for various distances is warranted.

The aim of this study was to further characterize sex differences in NM fatigue and Cr changes by examining various running distances from 40 to 171 km. It was hypothesized that i) females would exhibit less NM fatigue, particularly its peripheral component, and be better able to preserve their Cr than their male counterparts and ii) sex differences would increase with race distance.

METHODS

Participants

Seventy-five experienced trail runners were included in the study after medical examination (Table 1). Specific qualifying race criteria needed to be met to register for the races of the study. To participate in the study, applicants have to meet inclusion criteria: men and women of over 18 years old, free from muscular, bone or joint injuries and free from neurologic disease. Participants were excluded if i) they were taking neuroactive substances that can alter corticospinal excitability, ii) they have contraindication to experimental procedures including transcranial magnetic stimulation and iii) they were currently participating in a structured exercise program. Four participants dropped out of the study, seventeen runners did not complete the race and three runners were excluded from analysis because they did not complete the race in a competitive manner, due to completing the race by accompanying another race participant (Figure 1). Thus, 56 participants participated in the post test session of this study. A subgroup of 36 finishers was further separated into two groups of 18 males and 18 females matched by relative performance to the first male and the first female of their specific race (4, 5, 4, 1 and 4 pairs came from the MCC, OCC, CCC, TDS and UTMB races, respectively), respectively (158% ± 11% *vs* 158% ± 9%, respectively; $p = 0.897$). All pairs of male and female participants completed the same race. The male and female winner of each race had an ITRA (International Trail Running Association) performance index that was, on average, 95% of the world best ITRA performance index in their respective race category (more details are showed in supplemental Table S1). This shows that, on average, the relative level of the female winner is equivalent to the level of the male winner for each race despite a lower rate of participation in females. The study was performed according to the Declaration of Helsinki and was approved by the ethics committee (Comité de Protection des Personnes Ouest VI) and was registered at ClinicalTrials.gov (#NCT04025138). All participants gave their written informed consent

before their participation. The present study was part of a larger study investigating the effect of trail and ultra-trail racing on different physiological and biomechanical responses in males and females.

Design

Participants performed three testing sessions in total, comprising one familiarization session. One month before the race, participants visited the lab for a familiarization session. The other two testing sessions were performed before (PRE) and after (POST) a trail running race to assess NM function, Cr, blood parameters and participants' sensations in an isolated room close to the finish line (Figure 2). The PRE session was completed at least 24 h and less than 128 h before the race, while the POST session was completed as soon as possible after the race. The delay between the end of the race and KE NM evaluation was 36 ± 14 min, 44 ± 13 min for PF and 80 ± 19 min for Cr. The testing sessions were performed in a laboratory installed in a building located around 500m from the finishing line at an altitude on 1035m. After finishing their race, runners were offered food and drink and were allowed to lie down if necessary, for medical reasons.

Familiarization session

The familiarization session comprised a medical examination, familiarization with the NM testing protocol and a maximal running test to determine $\dot{V}O_{2max}$. The NM familiarization consisted of sustained submaximal and maximal isometric contractions (MVC) on both KE and PF muscles. Participants were then familiarized with peripheral electrical nerve stimulation on both femoral and tibial nerves. During KE contractions, participants were also familiarized with transcranial magnetic stimulations (TMS). The maximal running test consisted of incremental

running to exhaustion on a treadmill set with a 12% slope. Participants started at 5 to 6 km/h depending on their fitness and the speed increased by 0.5 km/h every minute until exhaustion.

Trail running race

Participants completed various races across five days at the Ultra-Trail du Mont-Blanc® (the race characteristics are detailed in Table 1). In addition to sex, participants were further subdivided into two groups by distance of race completed: SHORT (< 60 km) *vs* LONG (> 100 km). The event took place at the end of August 2019 under summer temperatures, ranging from 11°C (at night) to 31°C in Chamonix throughout the duration of the event, with a weather very similar between days (data derived from https://www.timeanddate.com/weather). All races were mountain trail or ultra-trail races, mostly composed of trail sections with a range of technical difficulties and gradients.

Neuromuscular testing

Neuromuscular function was assessed on both KE and PF muscles. The protocol was the same between PRE and POST sessions, apart from the inclusion of a standardized warm-up of ten submaximal isometric contractions in the PRE session only. During the POST session, the order of KE and PF NM function tests depended on the availability of testing stations in order to minimize the delay before assessment.

The NM testing protocol (Figure 2) consisted of one MVC without stimulation, followed by two MVCs with paired-pulse (100 Hz) peripheral nerve stimulation (PNS) delivered at peak torque, and on relaxed muscle separated by 3 s (100- and 10-Hz paired pulse and single pulse ; Db100, Db10, and Pt, respectively). During MVCs, participants were instructed to contract as strongly as possible for ~4 s. A 30-s resting period separated the first two MVCs. Electrical

PNS were delivered on the femoral nerve and on the tibial nerve for KE and PF, respectively. Further details on torque recordings and PNS are provided below.

For KE, participants performed two series of 3 contractions with TMS delivered at the target torque level (100%, 75%, and 50% MVC). PNS was also delivered after the TMS stimulation during the 50% contraction. Real-time visual feedback of the torque level was given to the participants so that they could maintain the desired level of contraction. Contractions were separated by 5 s and series by ~30 s at PRE and ~10 s at POST.

Torque and electromyography (EMG) recordings

Knee-extensor torque was measured during MVC and evoked contractions with an isometric knee dynamometer (ARS dynamometry, SP2, Ltd., Ljubljana, Slovenia). Participants were seated on the chair in an upright position with the hip and the right knee at 90° of flexion. The right leg was attached to the chair by a noncompliant strap just proximal to the malleoli of the ankle joint. Hips were securely strapped to maintain the position during contractions.

Plantarflexor torque was measured by an instrumented pedal (CS1060 300 Nm; FGP Sensors Les Clayes Sous Bois, France). Participants were seated in a custom-built chair with hip, knee, and ankle angles of 90°. The chest was strapped to the chair, and heel and forefoot were securely attached to the pedal with noncompliant straps to avoid displacement of the foot during MVC.

EMG activity of KE (vastus lateralis, VL) and PF (gastrocnemius medialis and soleus, GM and SOL, respectively) was recorded using pairs of self-adhesive surface electrodes (Meditrace 100; Covidien, Mansfield, MA) with a 10-mm recording diameter. The electrodes were placed in bipolar configuration and spaced by a 30-mm interelectrode distance. A reference electrode was placed on the right patella and the right medial malleolus for KE and PF, respectively. Prior to placing the electrodes, the skin was prepared to obtain low impedance (<5 kΩ) by shaving, gently abrading the skin and cleaning it with alcohol. The electrode placement was drawn on

the skin using a permanent marker to ensure the same placement between PRE and POST sessions. EMG data were recorded with PowerLab system (16/30 - ML880/P, ADInstruments, Bella Vista, Australia) with a sampling frequency of 2000 Hz. The EMG signal was amplified with octal bio-amplifier (Octal Bioamp, ML138, ADInstruments) with a bandwidth frequency ranging from 5 to 500 Hz (common mode rejection ratio = 85 dB, gain = 500), transmitted to the computer and analysed with LabChart 8 software (ADInstruments).

Electrical nerve stimulation

Single electrical stimuli of 1 ms duration and 400 V maximal output voltage were delivered via constant-current stimulator (DS7A or DS7R; Digitimer, Welwyn Garden City, Hertfordshire, UK) to both the right femoral and the tibial nerves. For the femoral nerve, stimulations were sent via a 30-mm-diameter surface cathode manually pressed into the femoral triangle (Meditrace 100) and a 10 × 5 cm self-adhesive stimulation electrode (Medicompex SA, Ecublens, Switzerland) located in the gluteal fold. Stimulations were delivered to the tibial nerve via a bipolar bar stimulating electrode with 30-mm anode-cathode spacing (Bipolar Felt Pad Stimulating Electrode part no. E.SB020/ 4 mm; Digitimer) placed in the popliteal fossa and parallel to the nerve.

For both KE and PF, the optimal intensity was determined by delivering single stimuli incrementally to the relaxed muscles until maximal M-wave (M_{max}) and the torque response plateaued. A stimulation intensity of 130% of the intensity that produced the maximal torque response and M-wave amplitude was used to ensure supramaximality of the twitch responses. For both KE and PF, the stimulation intensity was determined at the beginning of both the PRE and the POST sessions.

Transcranial magnetic stimulation

Single TMS pulses were manually delivered to obtain motor-evoked potentials (MEP) and superimposed twitches (SIT) during isometric KE contractions. TMS was delivered to the left motor cortex using a magnetic stimulator (Magstim 200^2; The Magstim Company Ltd., Whitland, UK) with a 110-mm concave double-cone coil (maximum output of 1.4 T) to induce a postero-anterior current. Participants wore a swim cap on which the optimal stimulation position was drawn to ensure a consistent coil position during the protocol. At the beginning of the protocol, the vertex was identified by drawing a line between the preauricular points and from nasion to inion. Six stimulation spots were drawn on the swim cap every centimetre from the vertex to 2 cm posterior along the nasal–inion line and 1 cm to the left of those 3 points. A single stimulation was delivered over each spot. The optimal coil position was determined during 20% MVC contractions and was chosen as the site which elicited the greatest SIT and MEP response. Optimal stimulus intensity was defined as the lowest stimulus intensity eliciting maximal MEP amplitude during short voluntary contractions at 20% MVC (27). Two stimulations were delivered at each intensity. The same TMS intensity and coil position was used in POST. During the protocol, participants were asked to recontract as quickly as possible to the pre-stimulus torque level after TMS delivery.

Energy cost of running

Following the neuromuscular assessments, participants were asked to run for two bouts of four minutes on a motorized treadmill (Pulsar 3p, h/p/cosmos, Munich, Germany) with a 1-min resting period in between: 4 min at 0% incline (FLAT) and 4 min at 15% uphill incline (UH). The speed was set at 90% and 70% of the average speed sustained during the last minute of the maximal test performed during the familiarization for FLAT and UH respectively. During this test, heart rate and breath-by-breath $\dot{V}O_2$ were measured using a portable system (Metamax 3B, Cortex Biophysik, Leipzig, Germany).

265

266 **Blood parameters**

267 Peripheral venous blood samples were taken from an antecubital vein of the participants at PRE
268 and POST sessions. Blood samples were collected in nonadditive collection tubes under sterile
269 conditions. Tubes were then centrifuged for ten minutes at 1000 g and 4°C. A Cobas C501
270 integrated system (Roche, Basel, Switzerland) was used for simultaneous assay of C-reactive
271 protein (CRP) and creatine phosphokinase (CPK) with reagents from the manufacturer.

272 **Race intensity**

273 The average intensity at which participants performed their race was calculated using the ratio
274 of the mean speed achieved during their race relative to the maximal speed reached on the
275 treadmill during the $\dot{V}O_{2max}$ test performed during the familiarization session. This parameter
276 was calculated for both males and females and for both SHORT and LONG and was expressed
277 in percentage of the speed reached during the $\dot{V}O_{2max}$ test.

278

279 **Participants' sensations and competitive intention**

280 PRE and POST race, participants were asked to report their fatigue, perceived pain (for both
281 KE and PF) and level of gastrointestinal discomfort on a 10-cm visual analog scale. Each scale
282 was anchored with the verbal descriptors "not at all" and "extremely". Furthermore, at the end
283 of the race, participants were asked their competitive intention going into the race by rating
284 from 0 to 10, with 0 being performing the race as fast as possible (i.e. maximum effort) and 10
285 being for fun (i.e. minimal effort). The exact question provided to the participants was the
286 following: *How would you rate your state of mind between 0 and 10, between pleasure mode*
287 *and competition mode? 0: Competition mode (I tried to do the best time possible). 10: Fun*
288 *mode (my only goal was to finish the race).*

289

290 **Data analysis**

291 *Voluntary and evoked torque.* The maximal torque values were determined as the highest peak
292 torque recorded from the MVC contractions (out of 3 MVCs for PF and out of 5 MVCs for
293 KE). The ratio of the amplitude of the superimposed doublet to the resting doublet was then
294 calculated to obtain the percentage of voluntary activation (VA) as follows:

295 $$\mathrm{VA_{PNS}} = (1 - \left[\frac{\text{superimposed Db100}}{\text{resting Db100}}\right]) \times 100 \qquad [1]$$

296 The VA and amplitudes of Db100, Db10 and Pt were measured on the trial where the torque
297 value was the highest when the superimposed doublet was delivered. The ratio of Db10 to
298 Db100 (Db10:Db100) was calculated to evaluate the presence of low-frequency fatigue (28).

299 *EMG.* M-wave peak-to-peak amplitude (Mmax) was analysed from the single-pulses elicited
300 when the muscle was at rest. EMG root mean square (RMS) was calculated over a 500-ms
301 period after the torque had reached a plateau and before the delivery of PNS during the best
302 MVC trial. The RMS was then normalized to Mmax (RMS/Mmax).

303 *TMS.* VL peak–to-peak MEP amplitude (MEP_{AMP}) and MEP area (MEP_{AREA}) were obtained at
304 each contraction level (MEP100, MEP75 and MEP50 corresponding to 100%, 75% and 50%
305 MVC contraction, respectively) and used as an index of corticospinal excitability. MEP
306 amplitude and area were then normalized to the amplitude and area, respectively, of the M-
307 wave obtained during the 50% MVC contraction. TMS voluntary activation (VA_{TMS}) was
308 measured by the twitch interpolation technique (29). The estimated resting twitch was
309 determined as the y-intercept of a linear regression of SIT amplitudes elicited by optimal TMS
310 intensity and absolute voluntary force during the two series of the three contractions at 50%,
311 75% and 100% MVC. Estimated resting twitch regression was considered not linear for two
312 participants only ($r < 0.9$) who were then discarded from analyses. In all other participants, the

313 regression was linear (r > 0.9) for at least one series at both PRE and POST sessions (30).
314 VA_{TMS} was then calculated with the following equation:

315 $$VA_{TMS} = (1 - \left[\frac{SIT_{MVC}}{\text{estimated resting twitch}}\right]) \times 100 \quad [2]$$

316 The duration of the silent period, i.e. as index of corticospinal inhibition, was visually
317 determined and defined as the duration from the TMS stimulus to the return of continuous
318 voluntary EMG (31). Both MEP (area and amplitude) and silent period were averaged from the
319 two series of contractions.

320 *Energy cost of running.* Oxygen uptake, carbon dioxide production and ventilation were
321 measured during the Cr test during both level and uphill running (Cr_{FLAT} and Cr_{UH},
322 respectively). Cr was calculated from oxygen consumption using the energy equivalent of
323 oxygen taking into account the respiratory exchange ratio (32).

324 *Participants' sensations.* For general fatigue, perceived pain (for both KE and PF) and level of
325 gastrointestinal discomfort, the analysis was made on the PRE to POST change (measured in
326 millimetres on the 100 mm scale).

327

328 **Statistical analysis**

329 Statistics were performed the same way on two different groups: on pairs (i.e. performance
330 matched) and on all participants (i.e. irrespective of performance; see Figure 1 for more details).
331 Statistical analyses were performed using Statistica software (Statsoft Inc., Tulsa, OK).
332 Normality distribution was verified with a Shapiro-Wilk test and variance homogeneity using
333 Levene's test. A mixed-model ANOVA for time (PRE-POST) with sex (Males-Females) and
334 distance (SHORT-LONG) as between-participant factors were used to assess PRE to POST
335 alterations. A mixed-model ANOVA for time (PRE-POST) and voluntary contraction intensity

(100%, 75% and 50% MVC) with sex (Males-Females) and distance (SHORT-LONG) as between-participants factors were used to evaluate changes in MEP (amplitude and area) and silent period. Because there were too few participants on LONG for Cr_{UH} (only two pairs), a two-way ANOVA (time × sex) was performed for Cr. Effect size is presented for significant findings as partial eta squared (η^2_p). In the event of a significant time × sex × distance interaction, a time × sex mixed-model ANOVA was performed on both SHORT and LONG. Paired t-test was used to compare i) the relative performance of paired males and females and ii) the relative race intensity of paired males and females in both SHORT and LONG. An independent sample t-test was performed to compare the relative intensity of all males and females. When normality or homogeneity conditions were not met (for subject sensation parameters) a Mann-Whitney *U* test was performed on PRE to POST changes for males versus females in both SHORT and LONG. A Mann-Whitney *U* test was also used to compare the competitive intention of males and females in SHORT and in LONG. The POST concentrations of blood parameters (CRP and CPK) were assessed by independent-sample *t* test or Mann-Whitney *U* test (i.e. when assumption of normality or heterogeneity were not met) to compare males versus females in SHORT and LONG. The level of significance was set at $p<0.05$.

RESULTS

Pair comparisons

Maximal voluntary contraction, voluntary activation and EMG RMS

There was no time × sex × distance interaction for MVC, VA_{PNS}, VA_{TMS} or EMG RMS for either KE or PF ($p \geq 0.18$; Table 2). A significant time × sex interaction was found for KE MVC ($p < 0.01$, $F = 11.7$, $\eta^2_p = 0.28$). KE MVC change was −36% ± 17% *vs* −27% ± 15% for males and females, respectively (Figure 3A). The time × sex interaction did not reach the statistical

significance ($p = 0.051$, $F = 4.2$, $\eta^2_p = 0.14$) for PF MVC (−34% ± 11% *vs* −30% ± 15% for males and females, respectively; Figure 3B). VA_{PNS} decreased significantly for both KE and PF ($p<0.001$, $F=25$, $\eta^2_p=0.45$ and $p < 0.001$, $F = 23.2$, $\eta^2_p = 0.47$ for KE and PF, respectively; Figure 3E-F) but no significant time × sex interaction for either muscle group was found. Similarly, the ANOVA displayed a significant decrease in VA_{TMS} ($p < 0.001$, $F = 20.1$, $\eta^2_p = 0.44$) but no significant time × sex interaction ($p = 0.170$, $F = 2.0$, $\eta^2_p = 0.07$; Figure 3G). As reported in Table 2, RMS/Mmax decreased for VL ($p < 0.001$, $F = 16.3$, $\eta^2_p = 0.35$), GM ($p < 0.001$, $F = 14.9$, $\eta^2_p = 0.36$) and SOL ($p = 0.028$, $F = 5.4$, $\eta^2_p = 0.17$), independently of sex.

Resting twitch responses

No time × sex interaction was found for KE Pt ($p = 0.202$, $F = 1.7$, $\eta^2_p = 0.05$; Figure 3C) and KE Db100 (−15% ± 9% *vs* −17% ± 9% for males and females, respectively; $p = 0.123$, $F = 2.5$, $\eta^2_p = 0.07$). There was a significant time × sex × distance interaction in PF Pt ($p = 0.006$, $F = 8.7$, $\eta^2_p = 0.25$, Figure 3D). Then, a significant time × sex interaction was observed in PF Pt for SHORT ($p = 0.019$, $F = 7.0$, $\eta^2_p = 0.33$) but no such interaction was observed in LONG ($p = 0.122$, $F = 2.8$, $\eta^2_p = 0.19$). Change in PF Pt on SHORT was −24% ± 14% *vs* −10% ± 9% for males and females, respectively. A significant triple time × sex × distance interaction was also found for PF Db100 ($p < 0.05$, $F = 4.7$, $\eta^2_p = 0.15$) followed by a significant time × sex interaction in SHORT ($p = 0.005$, $F = 10.8$, $\eta^2_p = 0.44$) but not in LONG ($p = 0.836$, $F = 0.1$, $\eta^2_p < 0.01$). In SHORT, the PRE-POST change was −19% ± 10% for males and −4% ± 9% for females. The analyses on the Db10:Db100 revealed the presence of low-frequency fatigue on both KE and PF, independent of sex and distance ($p < 0.01$, $F = 8.5$, $\eta^2_p = 0.22$ and $p < 0.05$, $F = 7.1$, $\eta^2_p = 0.21$, respectively). The decrease in Db10:Db100 was −5% ± 19% *vs* −7% ± 13% in KE and −3% ± 9% *vs* −5% ± 6% in PF for males versus females, respectively.

MEP and Silent Period

A significant time × sex interaction was observed in MEP_{AREA} ($p = 0.022$, $F = 5.9$, $\eta^2_p = 0.17$). The *post hoc* revealed that MEP_{AREA} increased in males ($p = 0.002$) and did not change in females ($p = 0.478$ and $p = 0.967$, respectively). However, the analyses did not reveal any time × sex interaction in MEP_{AMP} ($p = 0.239$, $F = 1.4$, $\eta^2_p = 0.05$) or silent period ($p = 0.340$, $F = 0.94$, $\eta^2_p = 0.03$). All values and statistics concerning MEP and silent period are presented in Table 3.

Energy cost of running

A significant increase in both Cr_{FLAT} ($p = 0.011$, $F = 7.7$, $\eta^2_p = 0.26$) and Cr_{UH} ($p = 0.023$, $F = 6.5$, $\eta^2_p = 0.32$) conditions (+6% ± 10% and +4% ± 7%, respectively) was observed (Figure 4A-B), but no sex differences were identified ($p = 0.208$, $F = 01.7$, $\eta^2_p = 0.07$ and $p = 0.704$, $F = 0.2$, $\eta^2_p = 0.01$ for Cr_{FLAT} and Cr_{UH}, respectively).

Blood parameters

The statistical analyses did not reveal any sex differences in either CRP ($p = 0.860$ and $p = 0.115$ for SHORT and LONG, respectively) or CPK ($p = 0.171$ and $p = 0.916$ for SHORT and LONG, respectively).

Race intensity

No significant differences were observed between males and females in both SHORT (60% ± 5% vs 61% ± 11% of the speed reached during the $\dot{V}O_{2max}$ test, respectively; $p = 0.798$) and LONG (44% ± 7% vs 47% ± 5% of the speed reached during the $\dot{V}O_{2max}$ test, respectively; $p = 0.131$).

Participants' sensations and competitive intention

Males reported more general fatigue compared to females in SHORT ($p = 0.027$) but not in LONG ($p = 0.353$, Figure 5A). No sex differences were observed in perceived KE pain, perceived PF pain or digestive system feeling for either LONG or SHORT (Figure 5A-B-C-D).

The competitive intention was significantly different between males and females in SHORT (3.6 ± 1.9 *vs* 6.2 ± 2.9, respectively; p = 0.042) but not in LONG (5.8 ± 1.6 *vs* 7.0 ± 2.6, respectively; p = 0.171, Figure 5E).

All participants

The race performance of all participants relative to the best male and the best female finishers was not different between sexes (157% ± 13% for males *vs* 159% ± 9% for females, p = 0.46). Analyses of all participants for NM parameters were not different than the pairs analyses already presented so the results are not presented here.

The significant time × sex interaction did not reach the level of significance for Cr_{FLAT} (p = 0.108, F = 2.7, η^2_p = 0.06) or for Cr_{UH} (p = 0.057, F = 3.9, η^2_p = 0.10), the deterioration being +7% ± 12% vs +3% ± 8% in FLAT and +10% ± 7% vs +2% ± 6% in UH for males versus females, respectively.

DISCUSSION

The purpose of this study was to investigate whether distance has an effect on the magnitude of sex differences in neuromuscular fatigue and energy cost of running following trail running races. Our results showed that females are less fatigable as evidenced by a lower decrease in KE maximal strength, independent of the distance. Females also demonstrated less peripheral fatigue of the PF muscles compared to males on short distances, this result being possibly due to sex differences in competitive intentions. Furthermore, a sex difference was displayed in the fatigue-induced change in corticospinal excitability, with an increase in MEP_{AREA} only in males. The neuromuscular fatigue sex differences did not statistically translate into energy cost of running sex differences.

Effect of trail race on neuromuscular fatigue

The strength losses found in the present study in SHORT (−23% and −25% for KE and PF, respectively) and LONG (−36% and −33% for KE and PF, respectively) fit with previous studies which assessed NM fatigue following running races of distances shorter (9, 33) and longer than 100 km (10, 12, 34, 35). However, most of these studies did not include females or did not compare sexes. For both males and females and for both KE and PF, torque reduction was accompanied by central (i.e. decrease in VA) and peripheral (i.e. decrease in Pt, Db100 and Db10:Db100) alterations, as previously reported following trail-running races (9-12, 33-36).

Sex differences in neuromuscular fatigue

Maximal Voluntary Contraction

Maximal torque decreases in LONG KE for both males and females are comparable with Temesi, et al. (12) (110 km; 10 males and 10 females), i.e. −38% *vs* −40% in males, −29% *vs* −33% in females, respectively. However, the decrease of maximal strength in PF in LONG was higher in the present study compared to Temesi, et al. (12) (−39% *vs* −26% in males, −37% *vs* −31% in females, respectively). The decrease in KE MVC was greater in males than in females, in accordance with Temesi, et al. (12). Despite the interaction not reaching the level of significance (p=0.051), the results observed in PF seem consistent with the results in KE. Contrary to our hypothesis, the sex differences observed in MVC decrease were independent of distance. Indeed, sex differences in NM fatigue did not increase with distance, and the results for peripheral fatigue in fact showed the opposite (i.e. a sex difference was only shown in SHORT; see below). In addition to the results from our previous study on this topic (12) and the known physiological differences between males and females which could give females a greater advantage over longer distances (e.g. muscle fiber type, muscle capillarisation and

vasodilatory capacity, lower carbohydrate metabolism), it must be acknowledged that this hypothesis was based on anecdotal evidence from observing females beat males in ultra-endurance races, i.e. no direct scientific evidence existed.

Peripheral fatigue

Despite a greater strength loss in males in KE, no sex differences were observed in peripheral parameters (Pt, Db100, Mmax and Db10:Db100) in this muscle group. This result is in agreement with the study of Temesi, et al. (12). The present data showed greater PF peripheral fatigue (greater decrease in Pt and Db100) in males compared to females but unexpectedly it was found in SHORT distance only (−24% *vs* −10% of PF Pt decrease after SHORT in males and females, respectively). Since Temesi, et al. (12) reported a sex-difference for PF Pt after 110 km, we would have expected such observations also in runners who performed races longer than 100 km. The sex difference observed in peripheral fatigue in SHORT cannot be explained by low-frequency fatigue since no sex differences were observed in Db10:Db100 ratio. Based on data collected in rat soleus (37), Temesi, et al. (12) speculated that the maintenance of work output driven by a large amount of eccentric component during a mountain ultramarathon could induce a smaller decrease in evoked responses in females because of more compliant Achilles tendon properties (38). Yet, it seemed that a similar amount of muscle damage were observed in males and females in both SHORT and LONG since no sex differences were observed in CRP and CPK, although these markers are only indirect indices of muscle damage. Alternatively, sex differences in fatigability have previously been associated with contractile mechanisms (3). Glycogen depletion is also an important contributor to impairments in contractile function (Pt) after endurance exercise (39, 40). Males have been found to use ~25% more muscle glycogen than females during moderate-intensity exercise (41) which is likely the intensity at which ultra-marathons are performed. Thus, a greater level of glycogen depletion

in males could also be a plausible explanation for the smaller twitch amplitude reduction in females than males.

Yet, one must be cautious before attributing an attenuated force decrease to better fatigue resistance. Field studies performed during competitions are relevant for that type of experiment since (i) asking subjects to exercise over 10 to 20 h in a laboratory is extremely challenging (although doable (42)) and (ii) competitions minimize motivation issues, i.e. it is assumed that participants complete the race as fast a time as possible. However, this may not be the case and for the first time, the present study presented data on the competitive intention of the participants. The objective of the questionnaire was to better understand the competitive intention of the participants during the race. It is a relevant, yet under-investigated, question since this type of event brings together runners from a wide range of backgrounds and experience, and with different motivations for the race (e.g. ranking, best personal performance, finishing the race within the time limits, enjoyment). Despite males and females being matched by relative performance, the present results showed that males were more competitively oriented and reported more general fatigue compared to females on SHORT but not on LONG. It has been suggested that during ultra-endurance exercise, runners have a security reserve set by the brain to prevent excessive fatigue levels (14). This security reserve described in the Flush model is highly influenced by motivation. Thus, it could be speculated that with greater motivation for the competition, males could have stretched the limit of their security reserve to a greater extent, involving greater decreases of force capacities, explaining the greater peripheral fatigue in SHORT. Interestingly, although difficult to explain, males and females performed their race with similar competitive intention in LONG. This could partly explain the lower sex differences in terms of peripheral fatigue in LONG compared to SHORT. However, this suggestion should be balanced against the fact that performing exercise at a submaximal effort for a given distance would result in a longer duration of exercise, and it is unclear what

effect this trade-off would have on muscle function post-race. Furthermore, it should be noted that despite the lower competitive intention during the short races in females, the present data showed that the intensity of exercise relative to the speed at $\dot{V}O_{2max}$ was similar between sexes. Taken together, these findings suggest i) that for a given relative intensity of prolonged exercise, females exhibit attenuated impairments in neuromuscular function, and ii) females might be capable of performing prolonged exercise at a greater relative intensity than males.

Central fatigue and corticospinal excitability and inhibition

No sex differences were observed in voluntary activation on KE and PF muscles. These results are in accordance with Temesi, et al. (12) Despite the decrease in VA in KE being approximately twice as large among males compared to females (−18% ± 19% *vs* −9% ± 11% in VA_{TMS} and −22% ± 20% *vs* −11% ± 17% in VA_{PNS} for males and females, respectively), there was no statistically significant difference, likely due to the large variability, the power of the statistical analysis and/or the delay to POST evaluation.

The present study showed a sex difference in the fatigue-induced change in MEP_{AREA}, with a post-exercise increase in MEP_{AREA} in males only. These results are in contrast to that of Keller, et al. (43) and Hunter, et al. (30) who reported similar results between MEP_{AMP} and MEP_{AREA} and did not show any effect of sex on the fatigue-induced change in MEP_{AREA} following isometric exercise, but the fatiguing task differed considerably to the present study. Speculatively, the greater increase in MEP_{AREA} in males could be related to the greater strength loss in the KE in males. For example, during sustained MVCs, MEPs have been shown to increase, with this increase thought to occur due to an increase in cortical output in order to compensate for impairments in neuromuscular function occurring downstream of the motor cortex (31, 44). Surprisingly, the sex differences observed in MEP_{AREA} were not found in MEP_{AMP}, and the lack of difference in MEP_{AMP} between males and females is consistent with

our previous study (12). While the reasons why MEP_{AREA} and MEP_{AMP} behaved differently are unclear, this could indicate a reduction in the firing frequency of the multiple descending volleys elicited by TMS, causing an elongation of the MEP and thus an increase in its area (45). Silent period was not differently altered in males and females, suggesting that corticospinal inhibition does not change with fatigue following ultramarathon races and that is true in either sexes (12).

Sex differences in energy cost of running

It was hypothesized that females would be better able to preserve their Cr than their male counterparts based on changes observed following a 2-hour treadmill run (15). Specifically, given that males have been shown to exhibit greater decrements in Pt following trail running (12), it was expected that males would be required to increase motor unit recruitment in order to compensate for greater impairments in contractile function relative to females, with a consequent increase in oxygen demand and Cr. Although we observed greater reductions in Pt in males in the PF following SHORT, no sex differences were observed in Cr_{FLAT} or Cr_{UH}. First, although the present results on NM alterations are consistent with existing literature (12) and seem to go in same direction across muscles (i.e. with females appearing less fatigued), differences are probably not strong enough to differently alter Cr between males and females. Second, as mentioned in the introduction, contrary to popular hypothesis, NM fatigue is possibly not as strongly related to oxygen uptake kinetics (21-24) and therefore energy cost of running. However, a type II error cannot be ruled out given that out of the 18 matched pairs who finished the race, only 13 were able to perform the Cr POST evaluations (7 pairs in SHORT and 6 in LONG). Furthermore, when running the analysis on all subjects (i.e. on 17 females and 26 males), the deterioration in Cr_{UH} in males was approximately 5 time larger than in females despite the difference not reaching statistical significance ($p = 0.057$). These results are not

readily comparable with the existing literature since the few studies that have assessed fatigue-induced sex differences in Cr (15, 25, 26) were performed over shorter durations on a treadmill without gradient. Nevertheless, the present findings are intriguing, and future research should further investigate potential sex differences in Cr following ultra-marathons.

Limitations

In the present study, there was an imbalance between the number of male versus female race finishers, impairing our ability to match pairs of males and females based on performance and decreasing our statistical power. It was difficult to recruit females in the study even though the percentage of females of the present sample (34%) was much larger than the rate of participation at these type of events (e.g. 10% on UTMB). The sample size was estimated to take into account an anticipated ~30% of dropout rate (calculated using data on the previous 3 years), however, it is difficult to predict how many runners would be able to perform the POST sessions after such a demanding effort. Another limitation is that females were in different phases of their menstrual cycle, as assessed using a medical questionnaire prior to the race. Conflicting findings exist in the literature about the effects of menstrual cycle on fatigability (46-49). It is worth mentioning that studies showing effects of the menstrual cycle on fatigability were conducted on fatiguing tasks using local, single-joint exercise (50) while no effects have been found when considering whole body exercise (51, 52) as in the present study. The delay to POST evaluation is another limitation of this study despite measurements were done as soon as possible after the race. It should be noted that the time to post assessment was similar between males and females for both KE (37 ± 15 min vs 34 ± 13 min, respectively) and PF (44 ± 14 min vs 45 ± 12 min, respectively) NM assessment and for Cr evaluation (81 ± 19 min vs 77 ± 20 min, respectively). Although most of the metabolic perturbations occurring during the race likely recovered by the post assessment, such perturbations were likely to be minimal given the

low intensities at which trail runs are performed. Indeed, slower recovery is associated with more prolonged exercises, as shown for instance by Kruger, et al. (53) in cycling, and the low frequency fatigue induced by muscle damage during trail running most likely even further delayed recovery. Although the delay before Cr assessment was similar between males and females, this delay could still have affected the results by changing their Cr as well as their substrate use since participants had some time to eat and digest after the race. Furthermore, given that carbohydrate intake was not controlled during and after the race, it is possible that differences in carbohydrate intake, or simply the 'mouth rinsing' effect, could have impacted the degree of neuromuscular impairment (54, 55). Finally, the potential limitations behind our matching of participants based on performance level relative to the winner of the race warrants discussion. In the races of the UTMB, there is a considerable sex difference in participation, such that substantially more males than females compete. Consequently, matching performance relative to the winner might be associated with limitations if the relative standard of the male race is greater than the female races. However, the International Trail Running associated (ITRA) performance index of both male and female winners of each race was over 95% of the world's best as the UTMB is one of the most renowned races in the world. Thus, despite the differences in participation, we are confident that our experimental approach is reliable to address sex differences in fatigability and Cr amongst males and females of a similar participation level. Moreover, our analysis examining the average speed at which the race was completed relative to the speed at $\dot{V}O_{2max}$ revealed that these participants were also not different in terms of the relative intensity of exercise throughout the races. Finally, important limitations surrounding the use of the competitive intention scale should be acknowledged. The goal of the scale was to understand the level of effort put into the race and any potential sex differences. While the results interestingly revealed a lower competitive intention in females, which warrants further investigation and has potential implications for sex differences in

neuromuscular function, it is important to note that the competition intention scale has not undergone a validation process. Thus, the results surrounding this scale should be interpreted with caution. Future studies, performed in collaboration with a psychologist, should reconsider this question.

CONCLUSION

The present study showed lower decrements in force in the KE and lower decrements in contractile function following SHORT in the PF in females compared to males following prolonged running exercises. This data is consistent with our previous study performed on a 110-km trail running race (12). The present study performed on distances ranging from 40 to 171 km allowed the conclusion that, contrary to our hypothesis, sex differences in fatigability do not increase with race distance. This study also brings a novel element on the competition intention of the participants. Although it likely does not explain all physiological outcomes, the effort extended during self-paced race events is an important consideration for future research when comparing males and females after such efforts.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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SUPPLEMENTAL DIGITAL CONTENT

Supplemental Table S1

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790

791

792 **FIGURE CAPTION**

793

794 **Figure 1.** Inclusion table. Cr, cost of running; NM, neuromuscular function. M, males. F,
795 females. * Not tested in POST because of inability to perform the test due to extreme fatigue or
796 specific injury. ** Excluded from analysis because not meeting the criteria (see detail in the
797 text).

798

799 **Figure 2.** Description of a testing session to assess the neuromuscular function (panel A) and
800 energy cost of running (Panel B). PNS, Peripheral Nerve Stimulation; MVC, Maximal
801 Voluntary Contraction; TMS, Transcranial Magnetic Stimulation.

802

803 **Figure 3.** PRE to POST change in MVC (Panel A for KE and Panel B for PF), in Pt (Panel C
804 for KE and Panel D for PF), in VA_{PNS} (Panel E for KE and Panel F for PF) and VA_{TMS}.
805 Continuous lines represent the group mean values and the dashed lines represent individual
806 participants. $^{\dagger\dagger}p<0.01$: Significant time × sex interaction. $^{\$\$}p<0.01$: Significant time × sex ×
807 distance interaction (see results section for details).

808

809 **Figure 4.** PRE to POST change in Cr on both FLAT (Cr_{FLAT}, panel A) and UH (Cr_{UH}, panel
810 B) conditions. Continuous lines represent the group mean values and the dashed lines
811 represent individual participants.

812

813 **Figure 5.** Subject sensation (Panels A, B, C and D) and competitive intention (Panel E) of the
814 participants. $^{\dagger}p<0.05$: Significantly different between males and females.

815

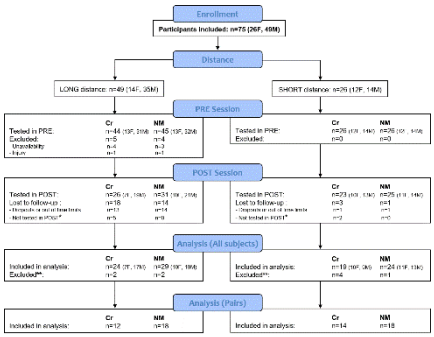

Enrollment
Participants included: n=75 (26F, 49M)
Distance
LONG distance: n=49 (14F, 35M)
SHORT distance: n=26 (12F, 14M)
PRE Session
Cr
NM
Tested in PRE:
n=44 (19F, 25M)
n=45 (19F, 26M)
Excluded:
n=5
n=4
- Unavailability:
n=4
n=3
- Injury:
n=1
n=1
Tested in PRE:
Excluded:
Cr
n=26 (12F, 14M)
n=0
NM
n=26 (12F, 14M)
n=0
POST Session
Cr
NM
Tested in POST:
n=26 (14F, 12M)
n=28 (16F, 12M)
Lost to follow-up :
n=18
n=14
- Responds to social media only:
n=13
n=14
- Not known anymore*:
n=5
n=0
Tested in POST:
Lost to follow-up:
- Unknown out of time limits
- Not tested in PRE*:
Cr
n=23 (10F, 13M)
n=3
n=2
NM
n=25 (11F, 14M)
n=1
n=1
n=0
Analysis (All subjects)
Cr
NM
Included in analysis:
n=24 (7F, 17M)
n=29 (10F, 19M)
Excluded**:
n=2
n=2
Included in analysis:
Excluded**:
Cr
n=19 (10F, 9M)
n=4
NM
n=24 (11F, 13M)
n=1
Analysis (Psfrs)
Cr
NM
Included in analysis:
n=12
n=18
Included in analysis:
Cr
n=14
NM
n=18

Figure 1

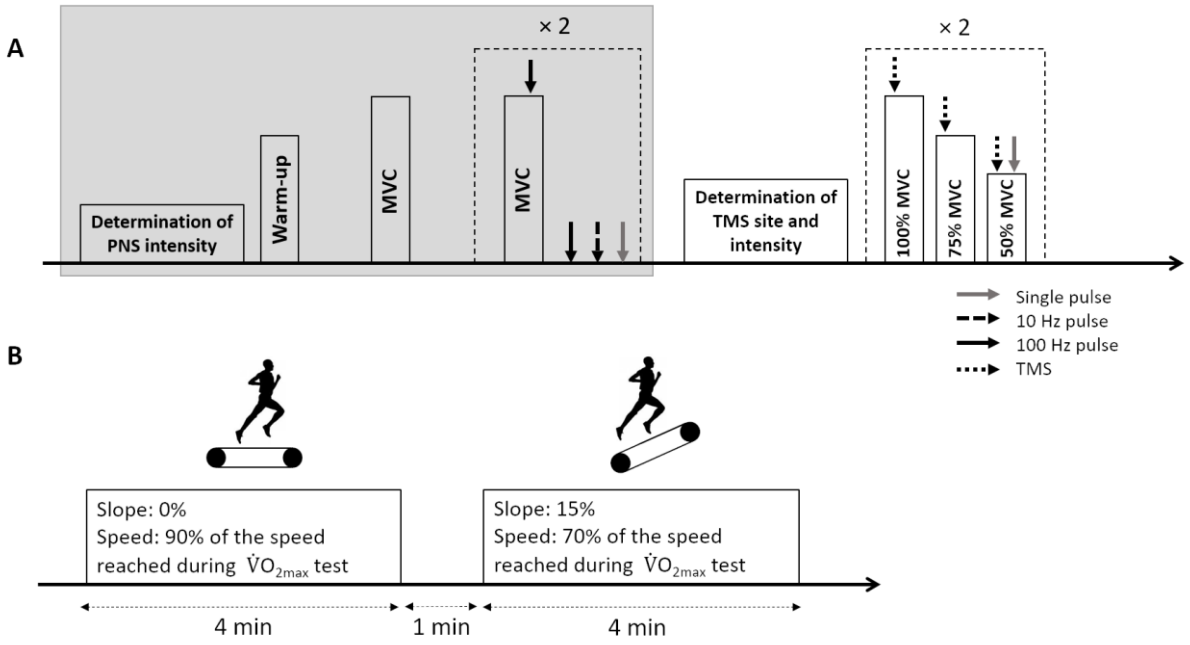

A
× 2
× 2
Determination of PNS intensity
Warm-up
MVC
MVC
Determination of TMS site and intensity
100% MVC
75% MVC
50% MVC
Single pulse
10 Hz pulse
100 Hz pulse
TMS
B
Slope: 0%
Speed: 90% of the speed reached during $\dot{V}O_{2max}$ test
Slope: 15%
Speed: 70% of the speed reached during $\dot{V}O_{2max}$ test
4 min
1 min
4 min

Figure 2

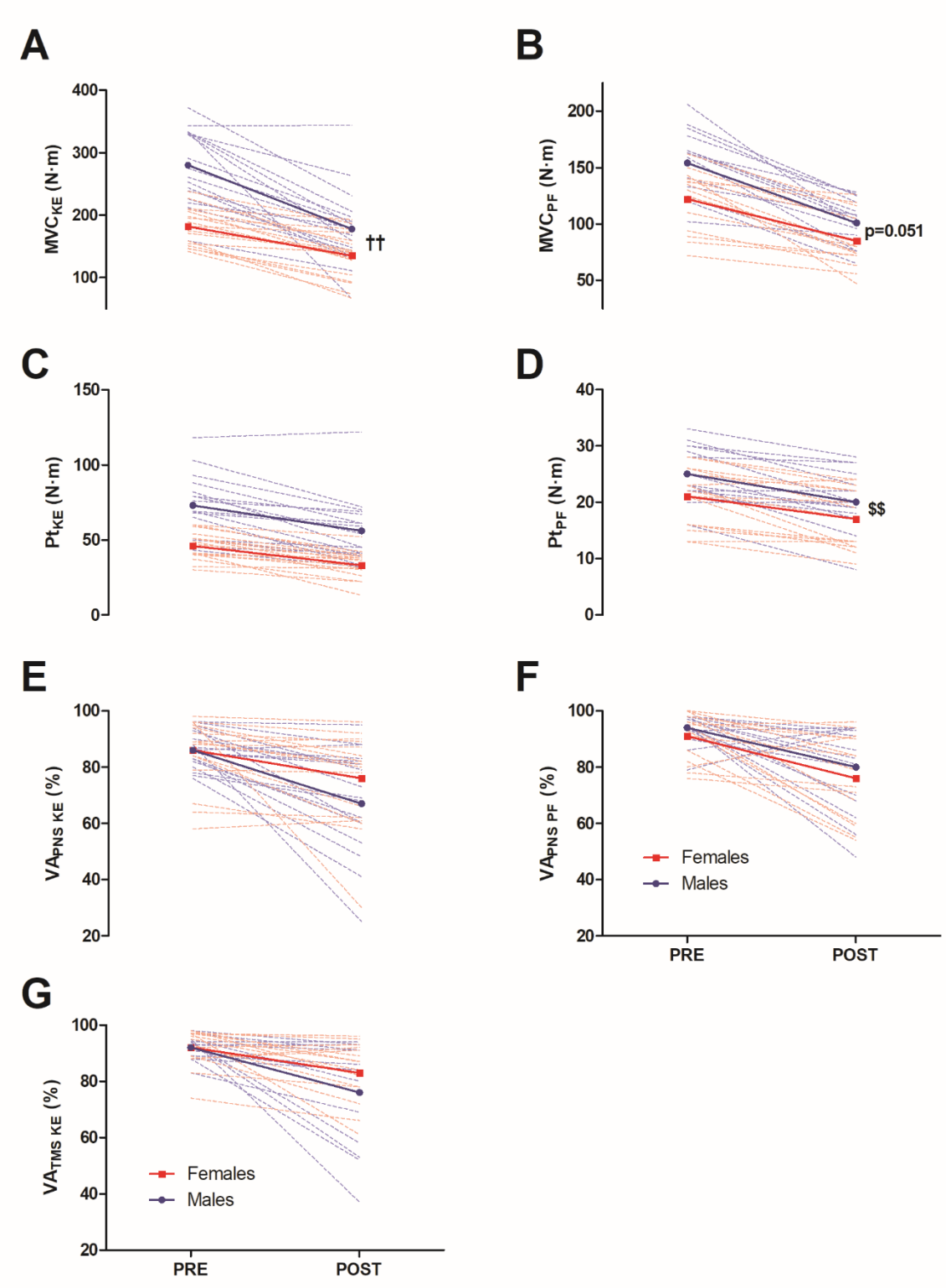
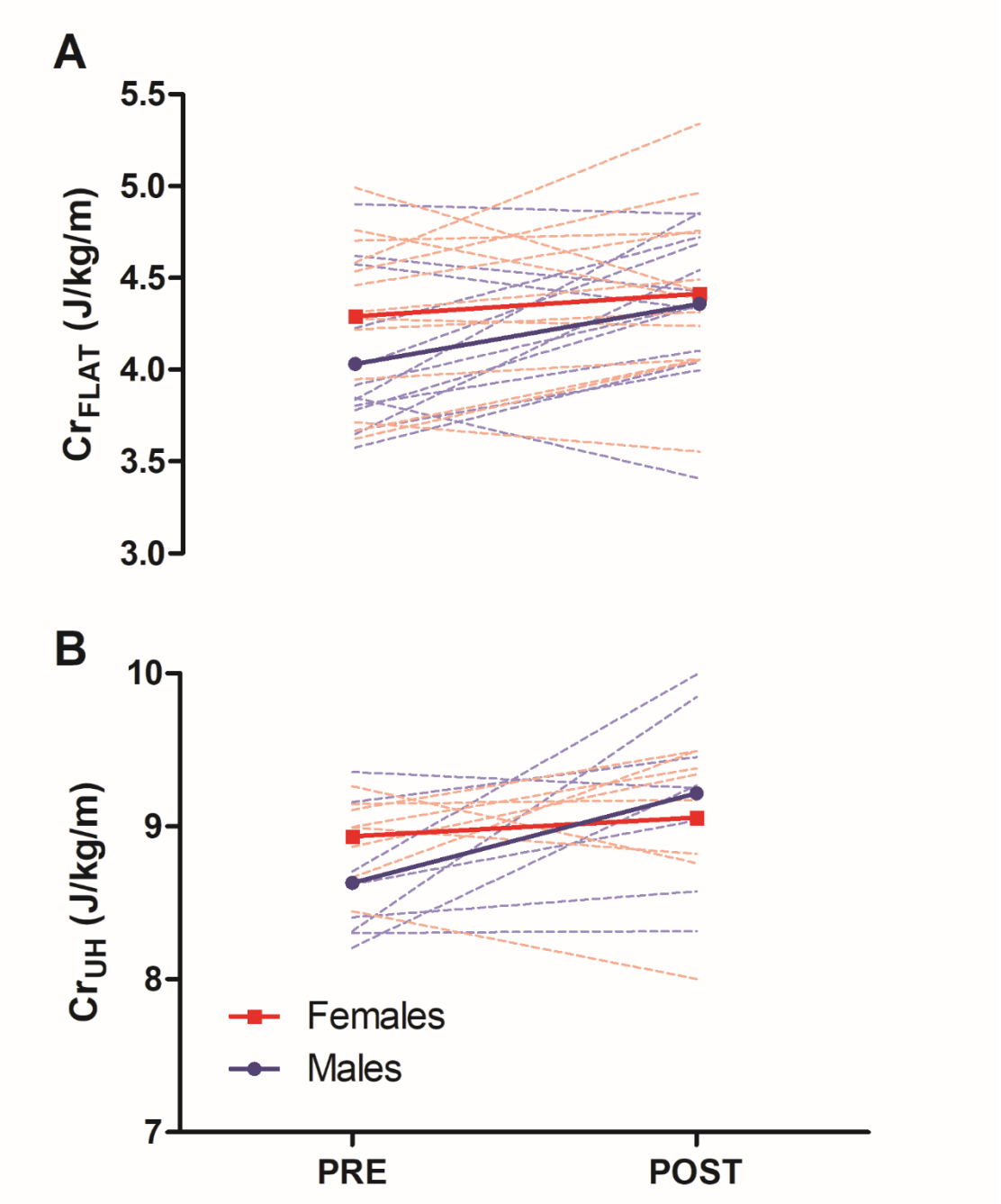
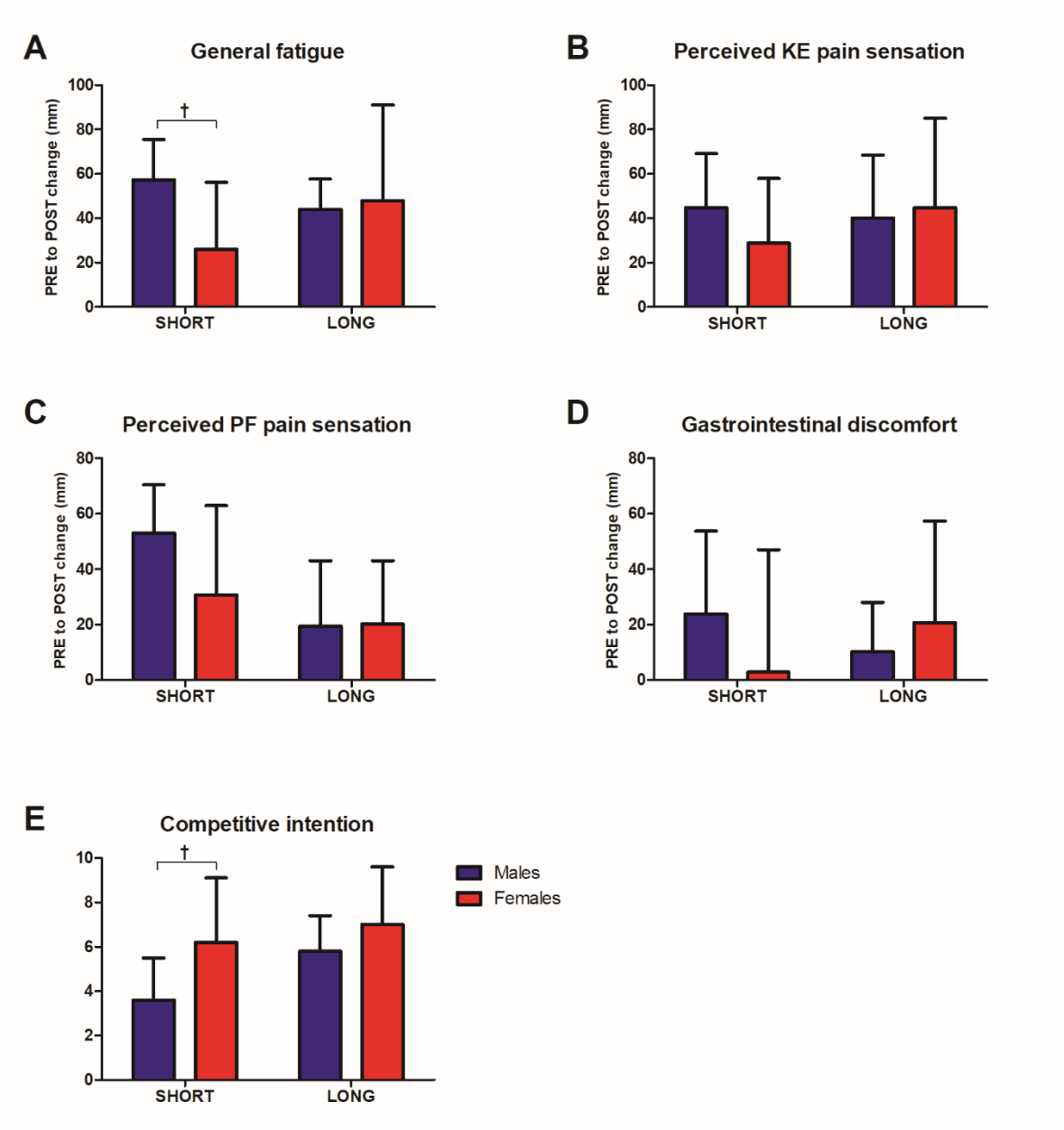

A
MVC$_{KE}$ (N·m)
400
300
200
100
††
B
MVC$_{PF}$ (N·m)
200
150
100
50
p=0.051
C
Pt$_{KE}$ (N·m)
150
100
50
0
D
Pt$_{PF}$ (N·m)
40
30
20
10
0
$$
E
VA$_{PNS\ KE}$ (%)
100
80
60
40
20
F
VA$_{PNS\ PF}$ (%)
100
80
60
40
20
Females
Males
PRE
POST
G
VA$_{TMS\ KE}$ (%)
100
80
60
40
20
Females
Males
PRE
POST

Figure 3



823

824 **Figure 4**



825

826 **Figure 5**

827 **Table 1.** Race details and participant characteristics

Race	Group	Distance (km)	D+ (m)	ITRA KM-effort	ITRA Category	Male Winning Time	Participants (n)	Age (yrs)	Height (cm)	Weight (kg)	VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)
MCC®	SHORT	40	2300	63	S	3:40:46	F: 7	39 ± 12	169 ± 6	62 ± 6	52.5 ± 4.0
							M: 7	34 ± 8	179 ± 6	73 ± 10	60.8 ± 14.7
OCC®		56	3500	91	M	5:19:24	F: 5	36 ± 8	168 ± 6	60 ± 5	54.5 ± 5.5
							M: 7	35 ± 7	180 ± 11	76 ± 15	64.7 ± 9.3
CCC®	LONG	101	6100	162	XL	10:28:49	F: 5	33 ± 6	163 ± 4	57 ± 8	57.9 ± 8.2
							M: 13	37 ± 10	180 ± 3	74 ± 6	61.5 ± 6.4
TDS®		145	9100	236	XXL	18:03:06	F: 2	47 ± 4	162 ± 10	54 ± 11	54.8 ± 5.7
							M: 4	43 ± 8	182 ± 5	75 ± 17	54.0 ± 7.6
UTMB®		171	10300	274	XXL	20:19:07	F: 7	35 ± 3	164 ± 7	57 ± 6	53.3 ± 4.3
							M: 18	39 ± 7	177 ± 6	72 ± 9	61.5 ± 7.0

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Table 2. EMG RMS and M-wave data

		Females		Males		
		PRE	**POST**	**PRE**	**POST**	
VL Mmax (mV)	SHORT	12.4 ± 1.1	12.2 ± 2.3	14.6 ± 6.7	14.7 ± 6.1	#
	LONG	8.5 ± 4.9	10.4 ± 6.1	12.5 ± 5.5	15.9 ± 4.6	
VL RMS (mV)	SHORT	0.58 ± 0.18	0.49 ± 0.22	0.60 ± 0.32	0.47 ± 0.18	*
	LONG	0.46 ± 0.29	0.31 ± 0.14	0.64 ± 0.44	0.48 ± 0.18	
VL RMS/Mmax (%)	SHORT	4.6 ± 1.2	3.9 ± 1.2	4.3 ± 1.4	3.6 ± 1.6	***
	LONG	5.5 ± 2.3	4.0 ± 3.0	5.0 ± 1.3	3.1 ± 1.0	
GM Mmax (mV)	SHORT	8.9 ± 3.6	9.5 ± 3.7	8.1 ± 2.9	8.1 ± 2.9	
	LONG	8.8 ± 4.4	8.5 ± 2.7	13.2 ± 6.9	11.5 ± 5.5	
GM RMS (mV)	SHORT	0.19 ± 0.10	0.16 ± 0.10	0.23 ± 0.15	0.15 ± 0.06	**
	LONG	0.19 ± 0.12	0.11 ± 0.05	0.23 ± 0.15	0.13 ± 0.08	
GM RMS/Mmax (%)	SHORT	2.2 ± 1.0	1.9 ± 1.0	3.0 ± 1.5	1.9 ± 0.7	***
	LONG	2.7 ± 1.8	1.5 ± 0.8	2.0 ± 1.0	1.5 ± 1.0	
SOL Mmax (mV)	SHORT	10.6 ± 2.7	9.4 ± 3.0	12.3 ± 3.3	11.8 ± 3.8	*
	LONG	10.8 ± 4.6	9.5 ± 3.7	8.4 ± 3.0	8.6 ± 3.0	
SOL RMS (mV)	SHORT	0.22 ± 0.09	0.21 ± 0.09	0.30 ± 0.16	0.23 ± 0.11	**
	LONG	0.31 ± 0.17	0.20 ± 0.06	0.30 ± 0.09	0.23 ± 0.06	
SOL RMS/Mmax (%)	SHORT	2.2 ± 1.0	2.4 ± 1.2	2.8 ± 2.1	2.2 ± 1.4	*
	LONG	2.8 ± 0.8	2.3 ± 0.6	3.8 ± 1.2	3.1 ± 1.9	

832 **Table 3.** MEP and SP data

		Females		Males		
		PRE	**POST**	**PRE**	**POST**	
MEP_{AMP} 100% MVC (%)	SHORT	33.2 ± 15.3	34.9 ± 12.1	31.5 ± 5.9	38.2 ± 8.9	**
	LONG	37.5 ± 13.3	46.1 ± 14.0	35.9 ± 14.5	47.3 ± 9.3	
MEP_{AMP} 75% MVC (%)	SHORT	43.5 ± 14.2	42.4 ± 14.1	43.0 ± 14.7	42.8 ± 14.7	
	LONG	47.3 ± 15.4	50.0 ± 13.8	44.2 ± 12.3	53.8 ± 9.5	
MEP_{AMP} 50% MVC (%)	SHORT	43.0 ± 16.8	44.4 ± 18.8	40.7 ± 11.6	43.9 ± 17.7	
	LONG	46.8 ± 15.1	51.1 ± 13.2	42.9 ± 13.2	53.4 ± 12.9	
MEP_{AREA} 100% MVC (%)	SHORT	33.4 ±14.2	37.4 ± 14.8	34.4 ± 6.4	47.2 ± 15.8	**, †
	LONG	42.5 ± 18.7	48.0 ± 18.8	40.2 ± 13.8	56.6 ± 13.9	
MEP_{AREA} 75% MVC (%)	SHORT	49.1 ± 17.3	48.3 ± 15.5	49.7 ± 17.1	58.2 ± 26.2	
	LONG	53.5 ± 19.5	55.6 ± 18.6	53.1 ± 15.2	65.0 ± 11.4	
MEP_{AREA} 50% MVC (%)	SHORT	55.0 ± 21.5	55.1 ± 20.0	54.2 ± 15.2	61.3 ± 21.5	
	LONG	59.2 ± 20.3	60.0 ± 18.8	56.7 ± 14.0	68.7 ± 15.6	
SP 100% MVC (ms)	SHORT	212 ± 86	232 ± 94	185 ± 77	191 ± 78	
	LONG	210 ± 58	188 ± 44	202 ± 91	184 ± 87	
SP 75% MVC (ms)	SHORT	209 ± 82	213 ± 79	179 ± 78	178 ± 78	
	LONG	198 ± 64	197 ± 47	201 ± 86	178 ± 81	
SP 50% MVC (ms)	SHORT	218 ± 76	216 ± 77	179 ± 74	185 ± 82	
	LONG	206 ± 62	199 ± 45	203 ± 79	175 ± 76	

833

834 **Table S1.** Best male and female levels and participation rate at the different races of the UTMB
835 event.
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	Male winner ITRA performance index relative to world best	Female winner ITRA performance index relative to world best	Percentage of female participation
MCC (40 km)	92%	84%	30%
OCC (55 km)	94%	100%	23%
CCC (101 km)	97%	97%	16%
TDS (145 km)	95%	96%	11%
UTMB (170 km)	98%	100%	10%
Average	**95%**	**95%**	/

837 The ITRA (International Trail Running Association) performance index is a tool for evaluating the
838 performance level of trail runners. It corresponds to the maximum speed of each trail runner on a scale
839 of 1000 points, corresponding to their performance against the theoretical world best for that distance.

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