Are Females More Resistant to Extreme Neuromuscular Fatigue?

John Temesi¹,², Pierrick J. Arnal¹, Thomas Rupp³, Léonard Féasson¹,⁶, Régine Cartier⁷, Laurent Gergelé⁸, Samuel Verges⁴,⁵, Vincent Martin⁹, and Guillaume Y. Millet¹,²,⁵

¹Université de Lyon, Laboratoire de Physiologie de l’Exercice, Saint-Etienne, France; ²Human Performance Laboratory, Faculty of Kinesiology, University of Calgary, Calgary, Alberta, Canada; ³Université de Savoie, Laboratoire de Physiologie de l’Exercice, Chambéry, France; ⁴Université Grenoble Alpes, HP2 Laboratory, Grenoble, France; ⁵INSERM, U1042, Grenoble, France; ⁶Unité de Myologie, Centre Référent Maladies Neuromusculaires Rares Rhône-Alpes, Centre Hospitalier Universitaire de Saint-Etienne, Saint-Etienne, France; ⁷Hospices Civils de Lyon, Groupement Hospitalier Est, Centre de Biologie et de Pathologie Est, Département de Biochimie, Bron, France; ⁸Département d’Anesthésie-Réanimation, Service de Réanimation Polyvalente B, Hôpital Nord, CHU de Saint-Etienne, Université Jean-Monnet de Saint-Étienne, Université de Lyon, Saint-Priest-en-Jarez, France; ⁹Clermont Université, Université Blaise Pascal, EA 3533, Laboratoire des Adaptations Métaboliques à l’Exercice en Conditions Physiologiques et Pathologiques, BP 10448, Clermont Ferrand, France

Accepted for Publication: 2 October 2014
Are Females More Resistant to Extreme Neuromuscular Fatigue?

John Temesi1,2, Pierrick J. Arnal1, Thomas Rupp3, Léonard Féasson1,6, Régine Cartier7, Laurent Gergele8, Samuel Verges4,5, Vincent Martin9, and Guillaume Y. Millet1,2,5*

1Université de Lyon, Laboratoire de Physiologie de l’Exercice, Saint-Etienne, France; 2Human Performance Laboratory, Faculty of Kinesiology, University of Calgary, Calgary, Alberta, Canada; 3Université de Savoie, Laboratoire de Physiologie de l’Exercice, Chambéry, France; 4Université Grenoble Alpes, HP2 Laboratory, Grenoble, France; 5INSERM, U1042, Grenoble, France; 6Unité de Myologie, Centre Référent Maladies Neuromusculaires Rares Rhône-Alpes, Centre Hospitalier Universitaire de Saint-Etienne, Saint-Etienne, France; 7Hospices Civils de Lyon, Groupement Hospitalier Est, Centre de Biologie et de Pathologie Est, Département de Biochimie, Bron, France; 8Département d’Anesthésie-Réanimation, Service de Réanimation Polyvalente B, Hôpital Nord, CHU de Saint-Étienne, Université Jean-Monnet de Saint-Étienne, Université de Lyon, Saint-Priest-en-Jarez, France; 9Clermont Université, Université Blaise Pascal, EA 3533, Laboratoire des Adaptations Métaboliques à l’Exercice en Conditions Physiologiques et Pathologiques, BP 10448, Clermont Ferrand, France
* These authors contributed equally to this work.

Running title: Fatigue sex differences in ultramarathon

Disclosure of funding: This project received funding from the Institut Fédératif de Recherche en Sciences et Ingénierie de la Santé and John Temesi was supported by a doctoral research grant from the Rhône-Alpes Region.

Conflict of interest: The authors have no conflicts of interest to declare.

Corresponding Author:
John Temesi, Human Performance Laboratory, Faculty of Kinesiology, University of Calgary, 2500 University Drive NW, Calgary, Alberta, CANADA, T2N 1N4. Tel. +1 403 220 2452. Fax. +1 403 220 0448. E-mail: jtemesi@ucalgary.ca
ABSTRACT

Purpose: Despite interest in the possibility of females outperforming males in ultra-endurance sporting events, little is known about the sex differences in fatigue during prolonged locomotor exercise. This study investigated possible sex differences in central and peripheral fatigue in the knee extensors and plantar flexors resulting from a 110-km ultra-trail running race. Methods: Neuromuscular function of the knee extensors and plantar flexors was evaluated via transcranial magnetic stimulation (TMS) and electrical nerve stimulation before and after an ultra-trail running race in 20 experienced ultra-endurance trail runners (10 females and 10 males matched by percent of the winning time by sex) during maximal and submaximal voluntary contractions and in relaxed muscle. Results: Maximal voluntary knee extensor torque decreased more in males than females (-38% versus -29%, P = 0.006) although the reduction in plantar flexor torque was similar between sexes (-26% versus -31%). Evoked mechanical plantar flexor responses decreased more in males than females (-23% versus -8% for potentiated twitch amplitude, P = 0.010) indicating greater plantar flexor peripheral fatigue in males. Maximal voluntary activation assessed by TMS and electrical nerve stimulation decreased similarly in both sexes for both muscle groups. Indices of knee extensor peripheral fatigue and corticospinal excitability and inhibition changes were also similar for both sexes. Conclusion: Females exhibited less peripheral fatigue in the plantar flexors than males after a 110-km ultra-trail running race and males demonstrated a greater decrease in maximal force loss in the knee extensors. There were no differences in the magnitude of central fatigue for either muscle group or TMS-induced outcomes. The lower level of fatigue in the knee extensors and peripheral fatigue in the plantar flexors could partly explain the reports of better performance in females in extreme duration running races as race distance increases. Keywords: central and peripheral fatigue, knee extensors, plantar flexors, sex differences, ultra-endurance running
INTRODUCTION

It is recognized that females are less fatigable than males for sustained and intermittent isometric contractions at the same relative intensity in most muscle groups (e.g. dorsiflexors, elbow flexors, knee extensors) and intermittent maximal sprint cycling (14). Possible explanations for these sex differences include differences in central nervous system functioning, muscle mass, reproductive hormones and skeletal muscle metabolism and contractile properties (for a complete review see (14)). Nevertheless, population-wide sex differences in physical activity levels and a bias towards investigating and publishing studies of only male subjects in both human and animal studies limit our understanding of sex differences in physical performance and fatigue (27).

Neuromuscular fatigue is an exercise-related decrease in the maximal voluntary torque of a muscle or muscle group, whether or not a task can be maintained (4). Numerous studies have suggested that the proportion of fatigue attributable to peripheral (i.e. within the muscle) and central (i.e. proximal to the neuromuscular junction) mechanisms varies between males and females; however, results are contradictory. Most studies have investigated sex differences in single-joint protocols with several concluding that greater central fatigue occurs in males after intermittent (33) and sustained (24) isometric lower-limb maximal voluntary contractions (MVC). Conversely, Hunter et al. (15) observed similar declines in voluntary activation for males and females and greater reduction in estimated resting twitch amplitude in males following intermittent sustained isometric MVCs of the elbow flexors; thus, concluding that greater MVC torque loss in males was due to peripheral mechanisms. However, fatigue is task dependent and sex differences influencing how and where fatigue manifests in single-joint protocols may not be applicable to locomotor exercise due to differences in limiting factors (e.g. capacity to develop
force, loss of activation, muscle metabolism) (for review, see Ref. (14)). For example, Glace et al. (9) attributed knee extensor MVC loss after 2 h cycling to central and peripheral mechanisms in males but only central mechanisms in females. Studies employing repeated maximal sprint cycling bouts suggest that sex differences in locomotor exercise may be influenced by factors such as the amount of mechanical work performed and initial maximal power output (5). The diversity of protocols (e.g. intermittent versus continuous), exercises (e.g. isometric contractions versus dynamic whole-body exercise) and muscles investigated (e.g. elbow flexors versus knee extensors) may contribute to these variable results and for this reason, sex differences must specifically be examined in the conditions of interest.

The possibility that females may be capable of outperforming males in ultra-endurance sporting events has been discussed for many years. Ultramarathons are among the very rare sporting events where females can outperform males. For example, females have won or placed in the top three overall in major races such as the 100-mile Western States Endurance Run and the Badwater 135-mile ultramarathon. Two studies have compared males and females performance-matched at the marathon/ultra-marathon distances against performances in shorter and longer races (3,39) and concluded that while males are faster in shorter events, females are faster over longer distances. Bam et al. (3) further suggested that females have an advantage over longer distances because they are more resistant to fatigue than their male counterparts. Potential explanatory factors include anthropometric sex differences, the effects of reproductive hormones in females and sex differences in substrate utilization (6), tendon characteristics (21) and running biomechanics (8). Conversely, the capacity for females to maintain running speed better than males as race distance increases has not been established in ultra-trail running races (UTRR) (13). It is, however, unlikely that females will regularly outrun males due to known physiological
sex differences such as greater maximal oxygen consumption (38) and higher haemoglobin concentrations (46) in males.

Previous ultra-endurance fatigue studies have either investigated male-only populations (e.g. (25,29)) or pooled males and females (41). Only two studies (9,10) have investigated the effects of endurance locomotor exercise on sex differences in neuromuscular fatigue parameters. In the sole running study, Glace et al. (10) observed that a 2-h treadmill bout induced maximal strength loss of knee extensors and flexors in males but not females at low angular velocities while strength loss was unaffected at high angular velocities in both sexes. It remains to be determined if similar neuromuscular sex differences occur in an ultra-endurance race setting considering the myriad of other demands and factors influencing ultra-endurance performance such as cognitive stress, sleep deprivation, nutritional intake, other competitors and climatic conditions (28). Cognitive stress, for example, has been observed to reduce physical performance the greatest in females (47) and weaker individuals (19). The only studies to investigate supraspinal fatigue and corticospinal excitability and inhibitory sex differences (15,18,19) employed isometric elbow flexion and observed no difference between males and females. It remains to be determined if there is also a lack of sex difference with whole-body locomotor exercise, especially over a much longer exercise duration, i.e. when major central fatigue is expected.

The aim of this study was to investigate whether sex differences in neuromuscular fatigue in knee extensors and plantar flexors exist after completion of a 110-km UTRR. Results pertaining to the effect of this 110-km UTRR on supraspinal fatigue and corticospinal excitability and inhibition, independent of sex, have previously been published (41). We hypothesised that (i) a 110-km UTRR induces greater MVC loss and peripheral fatigue in males
than females and (ii) that central fatigue and changes in corticospinal excitability and inhibition are similar for males and females.

MATERIALS AND METHODS

Subjects

Twenty healthy experienced ultra-endurance trail runners (10 females and 10 males) matched by relative performance (i.e. percent of winning time of the same sex) completed all aspects of this study. Subject characteristics are presented in Table 1. Subjects 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 local ethics committee (protocol #1208048, Comité de Protection des Personnes Sud-Est 1, France). All subjects were experienced ultra-endurance trail runners having participated in at least two trail-running races within the preceding two-year period.

Experimental design

Each subject completed one familiarization session and two experimental sessions. During the familiarization session, conducted 6-8 weeks prior to the UTRR, subjects completed a maximal incremental running test and were introduced to all experimental procedures. The first experimental session (PRE) occurred on one of the three days before the North Face® Ultra-Trail du Mont-Blanc® 2012 and the second ~1 h (POST) after completing the UTRR (Table 1). Due to exceptional inclement weather conditions, the 2012 edition of the North Face® Ultra-Trail du Mont Blanc® was shortened to a total distance of 110 km running/walking with total positive elevation change of 5862 m (see Supplemental Digital Content 1 in (41)). Under conditions of a mixture of rain, snow and clouds, the temperature reached a maximum of 12°C in Chamonix and decreased below 0°C at altitudes above 1800 m.
Familiarization session

The familiarization visit comprised a medical inclusion, maximal incremental running test to task failure (41) and familiarization to neuromuscular evaluations. The familiarization was comprised of maximal and submaximal voluntary contractions of the knee extensors with and without femoral nerve electrical stimulation (FNES) and transcranial magnetic stimulation (TMS) and plantar flexor MVCs with and without tibial nerve electrical stimulation (TNES) (see neuromuscular testing protocol section). During knee extension with TMS, subjects also practiced returning to the pre-stimulus torque as soon as possible after the stimulus to permit accurate measurement of the cortical silent period (CSP; see below). Trials were repeated until subjects were able to perform all tests consistently and as directed.

Neuromuscular testing protocol

The neuromuscular testing protocol consisted of knee extensor and plantar flexor components. The evaluations at POST were conducted as soon as possible after completion of the UTRR. As such, in order to optimize the use of the testing stations some subjects performed POST evaluations of the knee extensors before POST evaluations of the plantar flexors and other subjects performed POST evaluations in the opposite order. The testing order POST was not counter-balanced.

Knee extensors

Neuromuscular measures (Fig. 1) were assessed PRE and POST with real-time visual feedback. Maximal torque was determined from three 5-s MVCs separated by 30 s with FNES (100-Hz paired pulses and single pulses) delivered at peak torque and immediately after in the relaxed state (100- and 10-Hz paired pulses and single pulses). Then three series of four ~3-s contractions were performed with TMS delivered at the desired torque level (100, 75 and 50%
MVC at optimal stimulus intensity and 50% MVC at sub-optimal stimulus intensity (41); see below for further details). Contractions were separated by 15 s and series by 30 s.

Plantar flexors

Maximal torque was determined from three 5-s MVCs performed with real-time visual feedback and separated by 30 s (Fig. 1). TNES (100-Hz paired pulses) was delivered at peak torque and immediately after in the relaxed state (100- and 10-Hz paired pulses and single pulses). Then two ~3-s MVCs of the dorsiflexors separated by 30 s were performed to assess tibialis anterior coactivation.

Torque and electromyographic recordings

Knee extensor force was measured during voluntary and evoked contractions by a calibrated force transducer (Meiri F2732 200 daN, Célians, Montauban, France) with amplifier attached by a non-compliant strap to the right leg just proximal to the malleoli of the ankle joint. Subjects were seated upright in a custom-built chair with both right knee and hips at 90° of flexion and secured by chest and hips straps. The force transducer was fixed to the chair such that force was measured in direct line to the applied force. Torque was calculated as force measured by the force transducer multiplied by the length of the lever arm (i.e. distance from the tibial condyles to where the force transducer was attached to the leg).

Plantar flexor torque was assessed by an instrumented pedal (CS1060 300 Nm, FGP Sensors, Les Clayes Sous Bois, France). Subjects were seated upright in a custom-built chair with right ankle, knee and hip joints at 90° from complete extension. Non-compliant straps secured the chest and hips and also heel and forefoot to limit heel lift and avoid lateral and frontal displacement, respectively, during the MVC.
Electromyographic activity (EMG) of the right knee extensors (vastus lateralis), plantar flexors (gastrocnemius lateralis, soleus) and dorsiflexors (tibialis anterior) was recorded with a pair 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 the knee extensors and medial malleolus for the plantar flexors and dorsiflexors. 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 7 software (ADInstruments).

**Electrical nerve stimulation**

Single electrical stimuli of 1-ms duration were delivered via constant-current stimulator (DS7A, Digitimer, Welwyn Garden City, Hertfordshire, UK) to both the right femoral nerve and right tibial nerve. Stimuli to the femoral nerve were delivered via a 30-mm diameter surface cathode manually pressed into the femoral triangle (Meditrace 100) and 50 x 90 mm rectangular anode (Durastick Plus, DJO Global, Vista, USA) in the gluteal fold. Stimuli to the tibial nerve were delivered via a 30-mm diameter surface cathode pressed manually into the popliteal fossa (Meditrace 100) and 50 x 90 mm rectangular anode (Durastick Plus) over the patellar tendon. Single stimuli were delivered incrementally in relaxed muscle until maximal M-wave (Mmax) and twitch amplitudes plateaued. Stimulus intensity of 130% of the intensity to produce Mmax and maximal twitch responses was employed to ensure supramaximality. Stimulus intensity was determined at the start of each session. Supramaximal FNES intensity increased from PRE (57 ±
14 mA) to POST (65 ± 18 mA) and supramaximal TNES was unchanged between PRE (25 ± 11 mA) and POST (24 ± 10 mA). There were no differences between males and females for either FNES or TNES intensity.

**Transcranial magnetic stimulation**

Single TMS pulses were manually delivered to elicit motor-evoked potentials (MEP) and superimposed twitches (SITs) during voluntary isometric knee extension. The left motor cortex was stimulated by a magnetic stimulator (Magstim 200², The Magstim Company Ltd, Whitland, UK) with a 110-mm concave double-cone coil (maximum output of 1.4 T) to induce a postero-anterior current. Subjects wore a latex swim cap on which lines were drawn between the preauricular points and from nasion to inion to identify the vertex. Every centimeter was demarcated from the vertex to 2 cm posterior to the vertex along the nasal-inion line and also to 1 cm over the left motor cortex. The optimal coil position was drawn on the swim cap and recorded and the identical coil position was employed for POST. Optimal stimulus intensity was defined as the lowest stimulus intensity eliciting maximal MEP amplitude during brief voluntary contractions at 20% MVC (40). A sub-optimal stimulus intensity of 60% optimal intensity (i.e. corresponding to the rising part of the stimulus-response curve) was also employed as different fatigue responses have previously been observed at different TMS intensities (26). Mean stimulus intensities PRE were 68 ± 9% and 40 ± 6% maximal stimulator output for optimal and sub-optimal stimulus intensities, respectively. There were no sex differences in selected TMS intensities. Identical TMS intensities were utilized PRE and POST. Immediately after POST, TMS intensity was re-determined in subjects still physically capable of maintaining the target torque level (20% MVC POST) (n = 16). Optimal stimulus intensity in these subjects was similar PRE and POST (68 ± 9% versus 67 ± 7% maximal stimulator output, respectively). TMS was
always delivered once the subject had contracted to the appropriate torque level and the torque had stabilized during voluntary contractions. Subjects were also instructed to re-contract to the pre-stimulus torque level immediately after TMS delivery.

**Blood parameters**

Venous blood samples were taken from an antecubital vein of subjects PRE and POST (just prior to neuromuscular testing). The samples were collected in blood collection tubes without additives and centrifuged at 1000 g for 10 min at 4°C to separate serum from whole blood. An Architect Ci8200 (Abbott Diagnostics, Abbott Park, USA) integrated system was used for simultaneous assay of C-reactive protein (CRP) and creatine phosphokinase (CPK) with reagents from the manufacturer. Myoglobin (Mb) was measured by access immunoassay (Abbott Diagnostics).

**Subjective sensations**

Subjects were asked to report their general fatigue and pain in the knee extensors and plantar flexors on a 100-mm visual analog scale at PRE and immediately upon arrival at the testing site POST.

**Data analysis**

*EMG and femoral and tibial nerve electrical stimulation*

M-wave peak-to-peak amplitude was calculated in both relaxed (Mmax) and contracted muscles. Maximal torque was calculated as the mean peak torque from three MVCs. EMG root mean square (RMS) was calculated as the mean from three MVCs over a 200-ms period after the torque had reached a plateau and before the delivery of electrical nerve stimulation. Then RMS was normalized to Mmax. Coactivation during maximal plantar flexion was calculated as the ratio between tibialis anterior RMS during plantar flexor MVC and dorsiflexor MVC.
The amplitudes of the potentiated peak twitch (TwPot) and doublet (100-Hz paired pulse, Db100; 10-Hz paired pulse, Db10) torques were also determined. The presence of low-frequency fatigue POST was evaluated from the change in the ratio of Db10 to Db100 (Db10·Db100\(^{-1}\)) (43). Voluntary activation was assessed by twitch interpolation from responses evoked by both FNES (VA\(_{\text{FNES}}\)) and TNES (VA\(_{\text{TNES}}\)). The superimposed and potentiated doublet amplitudes elicited by 100-Hz paired pulses during and after MVCs with both muscle groups permitted VA to be calculated as: \([1 – (100\text{-Hz superimposed doublet amplitude} \times \text{Db100}^{-1})] \times 100\).

Transcranial magnetic stimulation

Peak-to-peak amplitude of MEPs (as an index of corticospinal excitability) were measured and normalized to maximal M-wave amplitude during MVCs measured at the same time point. Voluntary activation (VA\(_{\text{TMS}}\)) during maximal effort was assessed with 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 by optimal intensity TMS at 100, 75 and 50% MVC and voluntary torque (42). Estimated resting twitch regression was linear (\(r > 0.9\)) in all subjects for at least one series at both PRE and POST, thus permitting determination of VA\(_{\text{TMS}}\) in all subjects (15). VA\(_{\text{TMS}}\) was assessed with the equation: \([1 – (\text{SIT}\times(\text{estimated resting twitch})^{-1})] \times 100\) (42). The duration of the CSP (as an index of intracortical inhibition) was determined visually and defined as the duration from the stimulus to the return of continuous voluntary EMG (41). Subjects were excluded from CSP analyses if they did not recontract to the pre-stimulus torque immediately after TMS delivery.
Statistics

Statistical analyses were performed with Statistica (version 8, Tulsa, USA). Shapiro-Wilk and Levene’s tests was used to verify data normality and homogeneity of variances. Independent samples t-tests were employed to evaluate sex differences PRE for all parameters and sex differences POST for blood parameters. Repeated-measures ANOVAs for time (PRE-POST) and voluntary contraction intensity (100, 75 and 50% MVC) with sex as a between-subject factor were used to evaluate changes in MEPs and CSPs. Repeated-measures ANOVAs for time (PRE-POST) with sex as a between-subject factor were employed to compare the effects of the UTRR on all other variables. When the ANOVA revealed significant interactions, the Newman-Keuls post-hoc test was used to identify differences. Statistical significance was set at \( P < 0.05 \). All data are presented as mean ± standard deviation (SD) in tables and figures.

RESULTS

Race performance and sex differences before the ultra-trail

Subjects completed the 110-km UTRR in a mean time of 20:07:47 ± 3:19:13 (range: 13:49:31 - 25:49:23). Males finished the UTRR significantly faster than females (\( P = 0.013 \)) although performance was very similar between the two groups relative to the fastest runner of their sex (Table 1).

As expected, males were taller and heavier than females, had a lower percentage of body fat, greater lean mass and greater maximal oxygen consumption (Table 1). Similarly, PRE MVC was greater in males for both the knee extensors and plantar flexors (both \( P < 0.001 \), Table 1). Evoked responses in the relaxed muscle state were always greater in males than females for both knee extensor and plantar flexor muscle groups (all \( P < 0.01 \), Table 1). In the knee extensors, there was also a tendency for Db10·Db100\(^{-1} \) (\( P = 0.056 \)) to be lower in males.
Maximal voluntary torque changes

There were significant decreases in knee extensor and plantar flexor MVC post-race (Fig. 2; both $P < 0.001$). There was also a PRE-POST × Sex interaction for knee extensor MVC ($P = 0.006$) whereby the MVC decrease was greater in males (-38% for males and -29% for females). There was no such interaction in the plantar flexors (-26% for males and -31% for females; $P = 0.52$).

Evoked responses

Knee extensor peripheral potentiated twitch and doublet (100 and 10 Hz) amplitudes decreased significantly by 14, 11 and 16%, respectively, for males and 5, 6 and 9%, respectively, for females (Fig. 2A; all $P < 0.05$). Plantar flexor peripheral potentiated twitch and doublet (100 and 10 Hz) amplitudes also decreased significantly (Fig. 2B; all $P < 0.01$) and there were PRE-POST × Sex interactions for TwPot ($P = 0.010$) and Db10 ($P = 0.026$). Post-hoc analysis indicated that the PRE-POST Db10 decrease was greater in males (-20%) than females (-10%) and that TwPot decreased PRE-POST in males only (-23% for males and -8% for females). There was also a trend for greater Db100 loss in males (-15% for males and -4% for females) although this did not reach statistical significance ($P = 0.07$). There was no PRE-POST change in knee extensor Db10·Db100$^{-1}$ while plantar flexor Db10·Db100$^{-1}$ decreased PRE-POST (PRE: $1.01 \pm 0.02, 1.01 \pm 0.06$ and POST: $0.96 \pm 0.05, 0.96 \pm 0.06$ for males and females, respectively; $P < 0.001$). No PRE-POST × Sex interactions were identified for Db10·Db100$^{-1}$ in either muscle group. There were also no PRE-POST changes or sex interactions for Mmax for any muscle (Tables 2 and 3).
Voluntary activation and electromyographic root mean square

There were PRE-POST decreases in $VA_{TMS}$ (by 14 and 12% for males and females, respectively; Fig. 2A; $P < 0.001$), $VA_{FNES}$ (by 24 and 19% for males and females, respectively; Fig. 2A; $P < 0.001$) and $VA_{TNES}$ (by 9 and 18% for males and females, respectively; Fig. 2B; $P = 0.002$). No PRE-POST $\times$ Sex interactions for either muscle group were identified. Vastus lateralis RMS and RMS·$M_{max}^{-1}$ and gastrocnemius lateralis RMS·$M_{max}^{-1}$ decreased PRE-POST (all $P < 0.01$). There were no other PRE-POST changes and no sex interactions in RMS or tibialis anterior coactivation (Tables 2 and 3).

Motor-evoked potentials and cortical silent periods

Normalized MEP amplitude elicited at optimal stimulus intensity was greater after the UTRR while there was no change in MEP size at sub-optimal TMS intensity. There was no change in CSP duration at optimal TMS intensity while CSPs elicited by sub-optimal intensity were longer POST. There were no sex interactions in UTRR-induced MEP or CSP changes (Table 2).

Blood parameters

All blood parameters (CK, CRP and Mb) increased during the UTRR ($P < 0.001$) similarly for males and females (Fig. 3A).

Subjective sensations

There were similar increases in global fatigue and both knee extensor and plantar flexor pain PRE-POST ($P < 0.001$) for both males and females (Fig. 3B).

DISCUSSION

Since some females have previously outperformed the best males in ultramarathons and it has been suggested that females are more fatigue resistant than males (3), the aim of this study
was to determine if there were sex differences in the origins of fatigue after an UTRR. The main results are that (i) peripheral plantar flexor fatigue was greater in males than females and (ii) there were similar magnitudes of central fatigue in the knee extensors (whether assessed by $V_{ATMS}$ or $V_{AFNES}$) and plantar flexors for males and females and no sex differences in changes in corticospinal excitability or inhibition after a 110-km UTRR.

**Impact of this ultra-trail running race: comparison of fatigue in males to existing literature**

Previous male-only ultra-endurance treadmill and trail running studies of greater distance reported similar knee extensor and plantar flexor maximal torque loss to males in the present study (25,29,35). These ultra-endurance running bouts also induced major central (i.e. decreased $V_{AFNES}$ and $V_{ATNES}$) and peripheral (i.e. decreased evoked torques) fatigue (25,29,35). Finally, plantar flexor Db10·Db100⁻¹ decreased, indicating low-frequency fatigue in ultra-long running bouts with large elevation change as in UTRRs (29,35).

**Fatigue in males versus females**

*Central fatigue and TMS parameters*

There were no differences in the magnitude of central fatigue that developed in males and females in either the plantar flexors or knee extensors. Similarly, there was no sex difference in the magnitude of supraspinal fatigue of the knee extensors. The lack of central fatigue sex difference is in agreement with a recent cycling study (9) and a study employing an intermittent incremental submaximal isometric knee-extensor protocol to task failure (2), both in the knee extensors. Conversely, Martin and Rattey (24) concluded that the greater knee extensor MVC loss in males was due to greater central deficits; however, this study comprised a sustained maximal effort. The authors are not aware of any fatigue studies that have investigated sex differences in central or peripheral components of fatigue in the plantar flexor muscles. The
present study also agrees with all studies investigating supraspinal fatigue sex differences, all isometric elbow flexion protocols that observed a lack of difference between males and females (15,18,19). Similarly, we did not observe any differences in other TMS parameter changes between males and females. Therefore, current evidence suggests that changes in central and supraspinal fatigue and corticospinal excitability and inhibition with fatigue are not influenced by the sex of the subject during an UTRR.

**Maximal voluntary torque and evoked torques**

Males had a greater MVC decrease than females in the knee extensors. This result is consistent with some single-joint isometric knee extensor studies (1,36) and isokinetic knee extension and flexion at low (60°·s⁻¹) but not high (300°·s⁻¹) angular velocities after a 2-h steady-state running bout (10). Conversely, isometric knee extensor MVC loss was similar for males and females after a 2-h cycling bout immediately followed by a 3-km time trial (9). Previous single-joint studies have suggested that the greater absolute MVC in males (e.g. (15,16) could be a reason for fatigue-related sex differences although this is debatable in the present study given the specificity of trail running (28). Interestingly, when assessed by concentric contractions after a high-velocity dynamic exercise protocol, power loss during maximal concentric contractions of the knee extensors was similar for males and females (36), underlining the importance of testing specificity.

In the present study, there were large sex differences in mean evoked torque changes in the knee extensors (Fig. 2A); however, these changes were not statistically significant. Similar reductions for males and females in evoked knee extensor torques is in agreement with a sustained 100-s knee-extensor MVC (24) yet in contrast to a >2-h cycling bout (9), where only males demonstrated peripheral fatigue. In contrast, males exhibited greater decreases in evoked
plantar flexor torques than females after the UTRR in the present study. The increased peripheral component of fatigue in the plantar flexor muscles in males was not due to greater low-frequency fatigue demonstrated by the lack of sex difference in \( \text{Db10(Db100)} \).

Our results suggest that there is a sex difference in the development of peripheral fatigue in the plantar flexors that either does not exist or was unable to be measured in the knee extensors. Despite greater peripheral fatigue in males, the present study did not observe a sex difference in plantar flexor MVC loss after the UTRR and central fatigue was not significantly greater in females. This apparent inconsistency might be due to the large variability in relative female plantar flexor voluntary activation loss where mean \( \text{VA}_{\text{FNES}} \) decrease was approximately twice as large in females (-9 versus -18% for males and females, respectively) despite a lack of statistically significant difference.

Previous animal research has observed estrogen- and progesterone-induced effects on skeletal muscle properties. For example, there is evidence from mice that female sex hormones have a role in maintaining strong-binding myosin evoked force generation (30), maintaining active muscle stiffness and reducing passive muscle stiffness (31) and reducing exercise-induced muscle damage (e.g. (20)); however, there is limited evidence for such differences in humans. The likelihood of reproductive hormonal differences contributing to observed sex differences is low because of the differences observed between the plantar flexors and knee extensors. Any protective intra-muscular effect of estrogen and/or progesterone on skeletal muscle performance would be expected to be observed in the knee extensors in addition to the plantar flexors. There was also no difference in POST CPK or Mb concentrations suggesting a similar amount of muscle damage for both sexes and inflammation was comparable between sexes as illustrated by similar CRP responses.
Several studies have demonstrated sex differences in tendon properties of the Achilles (21) and patellar (12) tendons. Both of these studies observed greater tendon stiffness in males. Hicks et al. (12) also observed increased fascicle lengthening in males that was partially attributable to the increases in tendon stiffness. An important difference between the knee extensors and plantar flexors is the lengths of their associated tendons. The Achilles tendon is longer than the combined length of the patellar and quadriceps tendons (12,32,45). Furthermore, the patella is situated between the patellar and quadriceps tendon whereas the Achilles tendon is a single, uninterrupted connection between muscle and skeletal frame. The combination of greater Achilles tendon length and smaller plantar flexor muscle mass may have accentuated any practical influence of these changes in the plantar flexors when compared with the knee extensors. Lichtwark and Barclay (23) simulated the effect of a compliant tendon on cyclic contractions of rat soleus muscle and observed a better maintenance of work output in the second half of the protocol and increased mechanical efficiency with a more compliant tendon. Thus, it is possible that the maintenance of work output, especially after a long-duration exercise bout with large eccentric component, with a more compliant tendon may translate into a smaller reduction in evoked mechanical responses in females after the UTRR.

**Limitations**

This study was conducted in conjunction with an existing UTRR and >5% of all female finishers participated in this study. Despite the high rate of female participation, the sample population may not have been large enough to detect sex differences. By partnering with an existing UTRR, it was impossible to control for the menstrual cycle in female subjects, nor were they asked about their menstrual cycle. Hormonal changes have been observed to alter TMS parameters (37) although the random distribution of female subjects across their menstrual cycles
would likely have negated any possible influence. Furthermore, PRE and POST evaluations occurred within 3-4 days for all subjects, thus limiting cyclic hormonal effects by performing all testing at the same point in the cycle. In isometric contraction protocols, neither time to task failure (18,19) nor the decrease in MVC (15,19) were influenced by the timing of the menstrual cycle and supraspinal deficits with exercise were similar between males and females (15,18,19). Contractile characteristics and knee extensor and plantar flexor MVC (17,22,44) are also unaffected by menstrual cycle phase although this is disputed (e.g. (34)). Another limitation concerns the timing of assessments. Although POST testing was conducted as soon as possible, there was still a large delay to both knee extensor (57:12 ± 15:34) and plantar flexor (1:13:24 ± 25:31) assessments. While the delay to both POST evaluations was similar for males and females, this delay may still have contributed to the observed PRE-POST × Sex interactions since greater recovery of knee extensor MVC was previously observed 1 h post-exercise in females compared to males (11). Finally, additional validation of the method using high- and low-frequency doublets to identify the presence or absence of low-frequency fatigue is required.

Conclusion

Females had less objectively assessed fatigue as indicated by a smaller decrease in knee extensor MVC and less peripheral fatigue in the plantar flexors than males. There were no sex differences in the magnitude of central fatigue in knee extensors and plantar flexors and no sex differences in supraspinal, fatigue or changes in corticospinal excitability and intracortical inhibition in the knee extensors following a 110-km UTRR. The greater peripheral plantar flexor fatigue induced by the UTRR in males than females may potentially be explained by reduced tendon compliance in males. Further studies are required to confirm and further elucidate these results, particularly the sex differences observed between knee extensors and plantar flexors.
ACKNOWLEDGEMENTS

We gratefully thank all the ultra-trail competitors that participated in this study for the generous contribution of their time. We sincerely acknowledge the medical commission and organizers of the North Face® Ultra-Trail® du Mont-Blanc 2012, the financial support of the Institut Fédératif de Recherche en Sciences et Ingénierie de la Santé and also the assistance of Philippe Gimenez and Marlène Giandolini for conducting the maximal incremental running tests, Dr Roger Ouillon and Dr Pascal Edouard for conducting medical inclusions and Régis Bonnefoi and John Holash for technical support. John Temesi was supported by a doctoral research grant from the Rhône-Alpes Region. The results of this study do not constitute an endorsement by the American College of Sports Medicine.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.
REFERENCES


44. White MJ, Weekes C. No evidence for a change in the voluntary or electrically evoked contractile characteristics of the triceps surae during the human menstrual cycle. *J Physiol*. 1998;506(SUPPL):119P.


FIGURE CAPTIONS

FIGURE 1 (A) Neuromuscular testing order PRE and POST for FNES, TMS and TNES. The order of testing POST was determined by equipment availability. (B) Neuromuscular testing protocol for FNES and TNES MVCs and TMS contraction series. See text for further details.

FIGURE 2 Changes in MVC and evoked torques (Db100, Db10, TwPot) and voluntary activation (VA$_{FNES}$, VA$_{TMS}$, VA$_{TNES}$) by sex from PRE to POST for (A) the knee extensors and (B) the plantar flexors. Values are presented as mean ± SD. Significant PRE-POST × Sex interaction, * ($P < 0.05$); ** ($P < 0.01$). For all parameters, the PRE-POST decrease was statistically significant.

FIGURE 3 (A) Concentrations of CRP, Mb and CPK at POST by sex. (B) General fatigue, knee extensor (KE) and plantar flexor (PF) pain and digestive discomfort PRE and POST by sex. Values are presented as mean ± SD. All parameters increased significantly from PRE to POST.
Figure 1

(A) PRE

FNES intensity → TMS coil site → TMS intensity → MVC FNES

TNES intensity → MVC TNES

POST

FNES intensity → MVC FNES

TMS contraction series → TMS intensity

TMS intensity → MVC TNES

(B)

MVC FNES → TMS contraction series → MVC TNES

100-Hz FNES/TNES paired pulse → Single FNES/TNES pulse → TMS (optimal intensity)

10-Hz FNES/TNES paired pulse → TMS (sub-optimal intensity)
Figure 2

(A) 

(B)
<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of winning time by sex</td>
<td>175 ± 22</td>
<td>174 ± 28</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44 ± 7</td>
<td>41 ± 10</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164 ± 4</td>
<td>179 ± 6</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>57 ± 6</td>
<td>74 ± 5</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>25 ± 3</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>43 ± 4</td>
<td>66 ± 3</td>
</tr>
<tr>
<td>VO$_{2\text{max}}$ (ml·min$^{-1}$·kg$^{-1}$)</td>
<td>50 ± 3</td>
<td>59 ± 6</td>
</tr>
<tr>
<td>Time to POST KE evaluation (mm:ss)</td>
<td>58:41 ± 12:24</td>
<td>55:42 ± 18:47</td>
</tr>
<tr>
<td>Time to POST PF evaluation (hh:mm:ss)</td>
<td>1:19:56 ± 32:38</td>
<td>1:06:52 ± 14:38</td>
</tr>
<tr>
<td>PRE MVC KE (N·m)</td>
<td>115 ± 27</td>
<td>193 ± 31</td>
</tr>
<tr>
<td>PRE TwPot KE (N·m)</td>
<td>37 ± 6</td>
<td>51 ± 11</td>
</tr>
<tr>
<td>PRE Db10 KE (N·m)</td>
<td>61 ± 12</td>
<td>83 ± 19</td>
</tr>
<tr>
<td>PRE Db100 KE (N·m)</td>
<td>59 ± 9</td>
<td>87 ± 14</td>
</tr>
<tr>
<td>PRE MVC PF (N·m)</td>
<td>115 ± 18</td>
<td>175 ± 26</td>
</tr>
<tr>
<td>PRE TwPot PF (N·m)</td>
<td>25 ± 4</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>PRE Db10 PF (N·m)</td>
<td>39 ± 5</td>
<td>49 ± 7</td>
</tr>
<tr>
<td>PRE Db100 PF (N·m)</td>
<td>38 ± 4</td>
<td>49 ± 7</td>
</tr>
</tbody>
</table>

Body fat was calculated according to Durnin and Womersley (7). Db10, potentiated low-frequency (10-Hz) doublet; Db100, potentiated high-frequency (100-Hz) doublet; KE, knee extensors; MVC, maximal voluntary contraction; PF, plantar flexors; POST, post-ultratrail running race assessment; PRE, pre-ultratrail running race assessment; TwPot, potentiated...
twitch; VO$_{2\text{max}}$, maximal oxygen consumption. Values are presented as mean ± SD.

Significant sex difference, * ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$).
Table 2 Knee extensor EMG parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VL Mmax</strong> (mV)</td>
<td>PRE</td>
<td>10.6 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>POST</td>
<td>10.0 ± 2.5</td>
</tr>
<tr>
<td><strong>VL RMS</strong> (mV)</td>
<td>PRE</td>
<td>0.48 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>POST</td>
<td>0.30 ± 0.10</td>
</tr>
<tr>
<td><strong>VL RMS·Mmax</strong>⁻¹</td>
<td>PRE</td>
<td>0.047 ± 0.012</td>
</tr>
<tr>
<td></td>
<td>POST</td>
<td>0.031 ± 0.009</td>
</tr>
<tr>
<td><strong>MEP 100% MVC (%)</strong></td>
<td>PRE</td>
<td>38.7 ± 11.6</td>
</tr>
<tr>
<td></td>
<td>POST</td>
<td>46.5 ± 11.9</td>
</tr>
<tr>
<td><strong>MEP 75% MVC (%)</strong></td>
<td>PRE</td>
<td>48.9 ± 12.7</td>
</tr>
<tr>
<td></td>
<td>POST</td>
<td>51.7 ± 14.2</td>
</tr>
<tr>
<td><strong>MEP 50% MVC (%)</strong></td>
<td>PRE</td>
<td>51.5 ± 13.3</td>
</tr>
<tr>
<td></td>
<td>POST</td>
<td>57.7 ± 17.5</td>
</tr>
<tr>
<td><strong>MEP 50S (%)</strong></td>
<td>PRE</td>
<td>32.1 ± 11.4</td>
</tr>
<tr>
<td></td>
<td>POST</td>
<td>36.1 ± 20.8</td>
</tr>
<tr>
<td><strong>CSP 100% MVC</strong> (ms)</td>
<td>PRE</td>
<td>258 ± 42</td>
</tr>
<tr>
<td></td>
<td>POST</td>
<td>278 ± 46</td>
</tr>
<tr>
<td><strong>CSP 75% MVC</strong> (ms)</td>
<td>PRE</td>
<td>247 ± 57</td>
</tr>
<tr>
<td></td>
<td>POST</td>
<td>259 ± 25</td>
</tr>
<tr>
<td><strong>CSP 50% MVC</strong> (ms)</td>
<td>PRE</td>
<td>246 ± 53</td>
</tr>
<tr>
<td></td>
<td>POST</td>
<td>256 ± 34</td>
</tr>
<tr>
<td><strong>CSP 50S</strong> (ms)</td>
<td>PRE</td>
<td>105 ± 25</td>
</tr>
<tr>
<td></td>
<td>POST</td>
<td>123 ± 28</td>
</tr>
</tbody>
</table>
VL, vastus lateralis; S50, elicited at sub-optimal TMS intensity at 50% MVC. Values are presented as mean ± SD. Significant difference PRE-POST, * (P < 0.05); ** (P < 0.01); *** (P < 0.001). Significant sex difference, † (P < 0.05).
Table 3 Plantar flexor and dorsiflexor EMG parameters.

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOL Mmax (mV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>5.0 ± 1.8</td>
<td>7.4 ± 3.2</td>
</tr>
<tr>
<td>POST</td>
<td>4.7 ± 1.8</td>
<td>7.4 ± 2.2</td>
</tr>
<tr>
<td>GL Mmax (mV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>5.5 ± 2.5</td>
<td>6.2 ± 2.6</td>
</tr>
<tr>
<td>POST</td>
<td>6.1 ± 2.5</td>
<td>7.5 ± 3.6</td>
</tr>
<tr>
<td>SOL RMS (mV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>0.15 ± 0.05</td>
<td>0.24 ± 0.07</td>
</tr>
<tr>
<td>POST</td>
<td>0.11 ± 0.04</td>
<td>0.26 ± 0.14</td>
</tr>
<tr>
<td>GL RMS (mV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>0.17 ± 0.11</td>
<td>0.13 ± 0.07</td>
</tr>
<tr>
<td>POST</td>
<td>0.12 ± 0.10</td>
<td>0.12 ± 0.08</td>
</tr>
<tr>
<td>SOL RMS·Mmax⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>0.031 ± 0.012</td>
<td>0.037 ± 0.014</td>
</tr>
<tr>
<td>POST</td>
<td>0.025 ± 0.010</td>
<td>0.034 ± 0.011</td>
</tr>
<tr>
<td>GL RMS·Mmax⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>0.030 ± 0.010</td>
<td>0.024 ± 0.016</td>
</tr>
<tr>
<td>POST</td>
<td>0.020 ± 0.013</td>
<td>0.018 ± 0.011</td>
</tr>
<tr>
<td>TA RMS (mV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>0.026 ± 0.006</td>
<td>0.025 ± 0.007</td>
</tr>
<tr>
<td>POST</td>
<td>0.025 ± 0.010</td>
<td>0.027 ± 0.017</td>
</tr>
<tr>
<td>TA RMS·TA RMSmax⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>0.17 ± 0.08</td>
<td>0.16 ± 0.09</td>
</tr>
<tr>
<td>POST</td>
<td>0.13 ± 0.09</td>
<td>0.14 ± 0.06</td>
</tr>
</tbody>
</table>

GL, gastrocnemius lateralis; RMSmax, RMS during antagonist maximal voluntary contraction; SOL, soleus; TA, tibialis anterior. Values are presented as mean ± SD. Significant difference PRE-POST, ** (P < 0.01). Significant sex difference, ‡ (P < 0.01); ‡‡ (P < 0.001).