Are Females More Resistant to Extreme Neuromuscular Fatigue?

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BASIC SCIENCES

ABSTRACT

TEMESI, J., P. J. ARNAL, T. RUPP, L. FÉASSON, R. CARTIER, L. GERGELÉ, S. VERGES, V. MARTIN, and G. Y. MILLET. Are Females More Resistant to Extreme Neuromuscular Fatigue? *Med. Sci. Sports Exerc.*, Vol. 47, No. 7, pp. 1372–1382, 2015. **Purpose**: Despite interest in the possibility of females outperforming males in ultraendurance 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 ultraendurance 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 in females (−38% vs −29%, $P = 0.006$) although the reduction in plantar flexor torque was similar between sexes (−26% vs −31%). Evoked mechanical plantar flexor responses decreased more in males than in females (−23% vs −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. **Conclusions**: Females exhibited less peripheral fatigue in the plantar flexors than males did 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. **Key Words:** CENTRAL AND PERIPHERAL FATIGUE, KNEE EXTENSORS, PLANTAR FLEXORS, SEX DIFFERENCES, ULTRAENDURANCE RUNNING

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 Hunter [14]). Nevertheless, population-wide sex differences in physical activity levels and a bias toward 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

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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 after intermittent sustained isometric MVC 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 owing to differences in limiting factors (e.g., capacity to develop force, loss of activation, muscle metabolism) (for review, see Hunter [14]). For example, Glace et al. (9) attributed knee extensor MVC loss after 2 h of cycling to central and peripheral mechanisms in males but only central mechanisms in females. Studies using 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 the initial maximal power output (5). The diversity of protocols (e.g., intermittent vs continuous), exercises (e.g., isometric contractions vs dynamic whole-body exercise), and muscles investigated (e.g., elbow flexors vs 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 ultraendurance 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 3 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/ultramarathon 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 do 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 because of known physiological sex differences such as greater maximal oxygen consumption (38) and higher hemoglobin concentrations (46) in males.

Previous ultraendurance 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 in females at low angular velocities, whereas strength loss was unaffected at high angular velocities in both sexes. It remains to be determined whether similar neuromuscular sex differences occur in an ultraendurance race setting considering the myriad of other demands and factors influencing ultraendurance 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 in weaker individuals (19). The only studies to investigate supraspinal fatigue and corticospinal excitability and inhibitory sex differences (15,18,19) used isometric elbow flexion and observed no difference between males and females. It remains to be determined whether there is also a lack of sex difference with whole-body locomotor exercise, especially over a much longer exercise duration, that is, 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 hypothesized (i) that 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 ultraendurance 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 before 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 No. 1208048, Comité de Protection des Personnes Sud-Est 1, France). All subjects were experienced ultraendurance trail runners having participated in at least two trail-running races within the preceding 2-yr period.

Experimental Design

Each subject completed one familiarization session and two experimental sessions. During the familiarization session, conducted 6–8 wk before 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 3 d before the

TABLE 1. Subject characteristics and PRE maximal and evoked torques.

	Females	Males	
Percentage of winning time by sex	175 ± 22	174 ± 28	
Finishing time (hh:mm:ss)	21:53:32 ± 2:43:04	18:22:02 ± 2:59:51	*
Age (yr)	44 ± 7	41 ± 10	
Height (cm)	164 ± 4	179 ± 6	***
Mass (kg)	57 ± 6	74 ± 5	***
Body fat (%)	25 ± 3	11 ± 3	***
Lean mass (kg)	43 ± 4	66 ± 3	***
$\dot{V}O_{2max}$ (mL·min^{-1}·kg^{-1})	50 ± 3	59 ± 6	**
Time to POST KE evaluation (mm:ss)	58:41 ± 12:24	55:42 ± 18:47	
Time to POST PF evaluation (hh:mm:ss)	1:19:56 ± 32:38	1:06:52 ± 14:38	
PRE MVC KE (N·m)	115 ± 27	193 ± 31	***
PRE TwPot KE (N·m)	37 ± 6	51 ± 11	**
PRE Db10 KE (N·m)	61 ± 12	83 ± 19	**
PRE Db100 KE (N·m)	59 ± 9	87 ±14	***
PRE MVC PF (N·m)	115 ± 18	175 ± 26	***
PRE TwPot PF (N·m)	25 ± 4	31 ± 5	**
PRE Db10 PF (N·m)	39 ± 5	49 ± 7	**
PRE Db100 PF (N·m)	38 ± 4	49 ± 7	***

Body fat was calculated according to Durnin and Womersley (7). Values are presented as mean ± SD.
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; $\dot{V}O_{2max}$, maximal oxygen consumption.
*Significant sex difference: $P < 0.05$.
**Significant sex difference: $P < 0.01$.
***Significant sex difference: $P < 0.001$.

North Face® Ultra-Trail du Mont-Blanc® 2012 and the second ~1 h (POST) after completing the UTRR (Table 1). Because of 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 a total positive elevation change of 5862 m (see Supplemental Digital Content 1 in Temesi et al. [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 composed 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 MVC with and without tibial nerve electrical stimulation (TNES; see Neuromuscular Testing Protocol section). During knee extension with TMS, subjects also practiced returning to the prestimulus 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, 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 counterbalanced.

Knee extensors. Neuromuscular measures (Fig. 1) were assessed PRE and POST with real-time visual feedback. Maximal torque was determined from three 5-s MVC 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 suboptimal stimulus intensity (41); see below for further details). Contractions were separated by 15 s and series were separated by 30 s.

Plantar flexors. Maximal torque was determined from three 5-s MVC 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 MVC of the dorsiflexors separated by 30 s were performed to assess tibialis anterior coactivation.

Torque and EMG Recordings

Knee extensor force was measured during voluntary and evoked contractions by a calibrated force transducer (Meiri F2732 200 daN; Celians, Montauban, France) with amplifier attached by a noncompliant 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

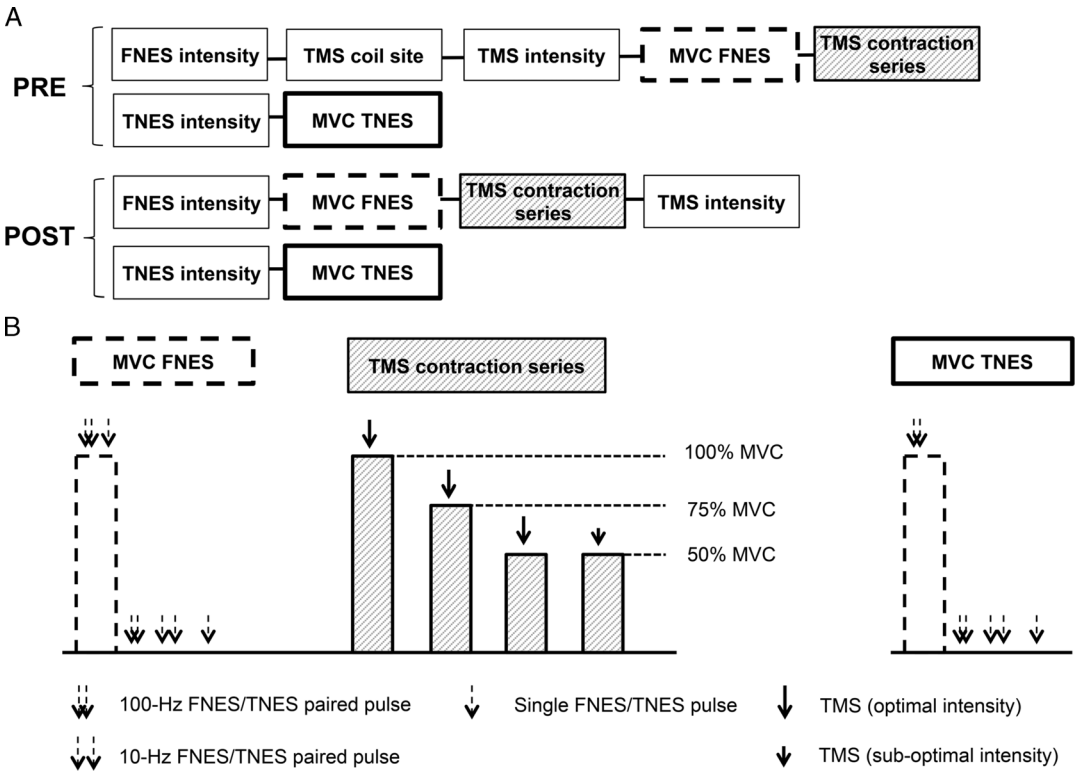

FIGURE 1—A, Neuromuscular testing order PRE and POST for FNES, TMS, and TNES. The order of testing POST was determined by equipment availability. B, Neuromuscular testing protocol for FNES and TNES MVC and TMS contraction series. See text for further details.

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 N·m; 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. Noncompliant straps secured the chest and hips as well as the heel and forefoot to limit heel lift and avoid lateral and frontal displacement, respectively, during the MVC.

EMG activity 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, MA) 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 converted from analog to digital at a sampling rate of 2000 Hz by PowerLab system (16/30-ML880/P; ADInstruments, Bella Vista, Australia) and octal bioamplifier (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 the 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 × 90 mm rectangular anode (Durastick Plus; DJO Global, Vista, CA) 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 × 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. A stimulus intensity of 130% of the intensity to produce Mmax and maximal twitch responses was used 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.

TMS

Single TMS pulses were manually delivered to elicit motor-evoked potentials (MEP) and superimposed twitches (SIT) during voluntary isometric knee extension. The left motor cortex was stimulated by a magnetic stimulator (Magstim 200[2]; The Magstim Company Ltd., Whitland, UK) with a 110-mm concave double-cone coil (maximum output of 1.4 T) to induce a posteroanterior current. Subjects wore a 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 was recorded, and the identical coil position was used 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 suboptimal stimulus intensity of 60% optimal intensity (i.e., corresponding to the rising part of the stimulus–response curve) was also used because 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 suboptimal stimulus intensities, respectively. There were no sex differences in selected TMS intensities. Identical TMS intensities were used PRE and POST. Immediately after POST, TMS intensity was redetermined 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% vs 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 recontract to the prestimulus torque level immediately after TMS delivery.

Blood Parameters

Venous blood samples were taken from an antecubital vein of subjects PRE and POST (just before 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, Chicago, IL) 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 on 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 MVC. EMG root mean square (RMS) was calculated as the mean from three MVC 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 ($Db10 \cdot Db100^{-1}$) (43). Voluntary activation was assessed by twitch interpolation from responses evoked by both FNES (VA_{FNES}) and TNES (VA_{TNES}). The superimposed and potentiated doublet amplitudes elicited by 100-Hz paired pulses during and after MVC with both muscle groups permitted VA to be calculated as: $[1 - (100\text{-Hz superimposed doublet amplitude} \cdot Db100^{-1})] \times 100$.

TMS. Peak-to-peak amplitude of MEP (as an index of corticospinal excitability) were measured and normalized to maximal M-wave amplitude during MVC measured at the same time point. Voluntary activation (VA_{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_{TMS} in all subjects (15). VA_{TMS} was assessed with the following equation: $[1 - (SIT \cdot (\text{estimated resting twitch})^{-1})] \times 100$ (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 prestimulus torque immediately after TMS delivery.

Statistics

Statistical analyses were performed with Statistica (version 8; Tulsa, OK). Shapiro–Wilk and Levene tests were used to verify data normality and homogeneity of variances. Independent-samples *t*-tests were used to evaluate sex differences PRE for all parameters and sex differences POST for blood parameters. Repeated-measures ANOVA 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 MEP and CSP. Repeated-measures ANOVA for time (PRE–POST) with sex as a

between-subject factor were used 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 ± SD in tables and figures.

RESULTS

Race performance and sex differences before the UTRR. Subjects completed the 110-km UTRR in a mean time of 20:07:47 ± 3:19:13 (range: 13:49:31 to 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, had greater lean mass, and had 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 in 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 $Db10 \cdot Db100^{-1}$ ($P = 0.056$) to be lower in males.

Maximal voluntary torque changes. There were significant decreases in knee extensor and plantar flexor MVC after the 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* analyses indicated that the PRE–POST Db10 decrease was greater in males (−20%) than in 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 $Db10 \cdot Db100^{-1}$, whereas plantar flexor $Db10 \cdot Db100^{-1}$ decreased PRE–POST (PRE = 1.01 ± 0.02 and 1.01 ± 0.06 and POST = 0.96 ± 0.05 and 0.96 ± 0.06 for males and females, respectively; $P < 0.001$). No PRE–POST–sex interactions were identified for $Db10 \cdot Db100^{-1}$ in either muscle group. There were also no PRE–POST changes or sex interactions for Mmax for any muscle (Tables 2 and 3).

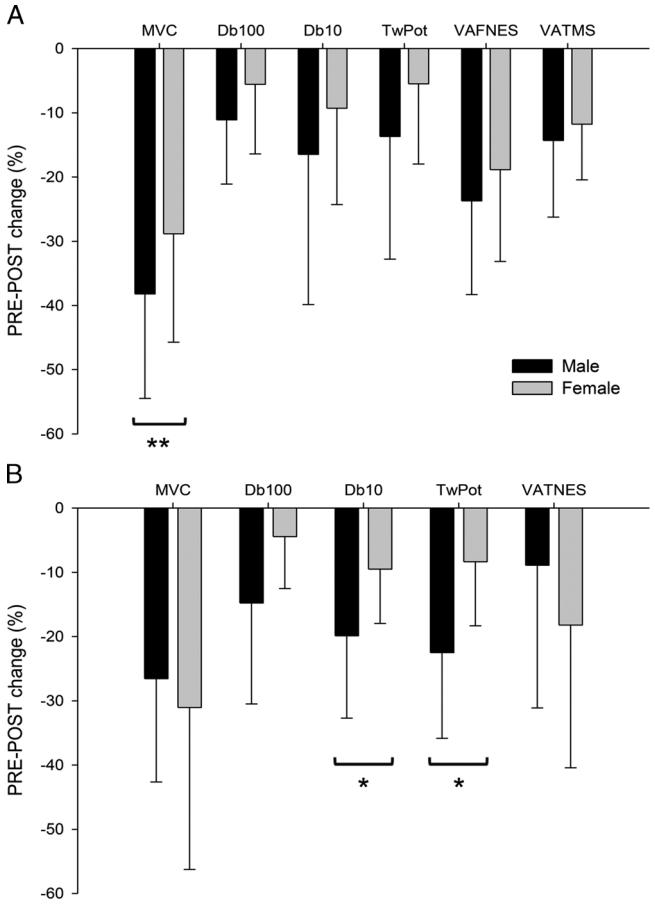

FIGURE 2—Changes in MVC and evoked torques (Db100, Db10, TwPot) and voluntary activation (VA_{FNES}, VA_{TMS}, VA_{TNES}) by sex from PRE to POST for (A) the knee extensors and (B) the plantar flexors. Values are presented as mean ± SD. Significant PRE–POST–sex interaction: *$P < 0.05$, **$P < 0.01$.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

Voluntary activation and EMG RMS. There were PRE–POST decreases in VA_{TMS} (by 14% and 12% for males and females, respectively, $P < 0.001$; Fig. 2A), VA_{FNES} (by 24% and 19% for males and females, respectively, $P < 0.001$; Fig. 2A), and VA_{TNES} (by 9% and 18% for males and females, respectively, $P = 0.002$; Fig. 2B). No PRE–POST–sex interactions for either muscle group were identified. Vastus lateralis RMS and $RMS \cdot Mmax^{-1}$ and gastrocnemius lateralis $RMS \cdot Mmax^{-1}$ 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).

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

TABLE 2. Knee extensor EMG parameters.

		Females	Males	
VL Mmax (mV)	PRE	10.6 ± 2.8	15.1 ± 3.8	****
	POST	10.0 ± 2.5	14.4 ± 4.8	
VL RMS (mV)	PRE	0.48 ± 0.15	0.72 ± 0.40	**,****
	POST	0.30 ± 0.10	0.45 ± 0.17	
VL RMS·Mmax^{-1}	PRE	0.047 ± 0.012	0.046 ± 0.017	***
	POST	0.031 ± 0.009	0.032 ± 0.010	
MEP 100% MVC (%)	PRE	38.7 ± 11.6	34.2 ± 12.3	***
	POST	46.5 ± 11.9	44.0 ± 11.9	
MEP 75% MVC (%)	PRE	48.9 ± 12.7	47.9 ± 14.0	***
	POST	51.7 ± 14.2	51.5 ± 11.8	
MEP 50% MVC (%)	PRE	51.5 ± 13.3	48.5 ± 10.2	***
	POST	57.7 ± 17.5	49.6 ± 10.7	
MEP 50S (%)	PRE	32.1 ± 11.4	33.5 ± 14.0	
	POST	36.1 ± 20.8	29.3 ± 15.9	
CSP 100% MVC (ms)	PRE	258 ± 42	267 ± 54	
	POST	278 ± 46	256 ± 47	
CSP 75% MVC (ms)	PRE	247 ± 57	256 ± 65	
	POST	259 ± 25	242 ± 46	
CSP 50% MVC (ms)	PRE	246 ± 53	243 ± 52	
	POST	256 ± 34	235 ± 41	
CSP 50S (ms)	PRE	105 ± 25	98 ± 24	*
	POST	123 ± 28	103 ± 24	

Values are presented as mean ± SD.
50S, elicited at suboptimal TMS intensity at 50% MVC; VL, vastus lateralis.
*Significant difference PRE–POST: $P < 0.05$.
**Significant difference PRE–POST: $P < 0.01$.
***Significant difference PRE–POST: $P < 0.001$.
****Significant sex difference: $P < 0.05$.

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

Because 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 whether 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 in females and (ii) there were similar magnitudes of central fatigue in the knee extensors (whether assessed by VA_{TMS} or VA_{FNES}) 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 UTRR: Comparison of Fatigue in Males to Existing Literature

Previous male-only ultraendurance 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 ultraendurance running bouts also

TABLE 3. Plantar flexor and dorsiflexor EMG parameters.

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

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

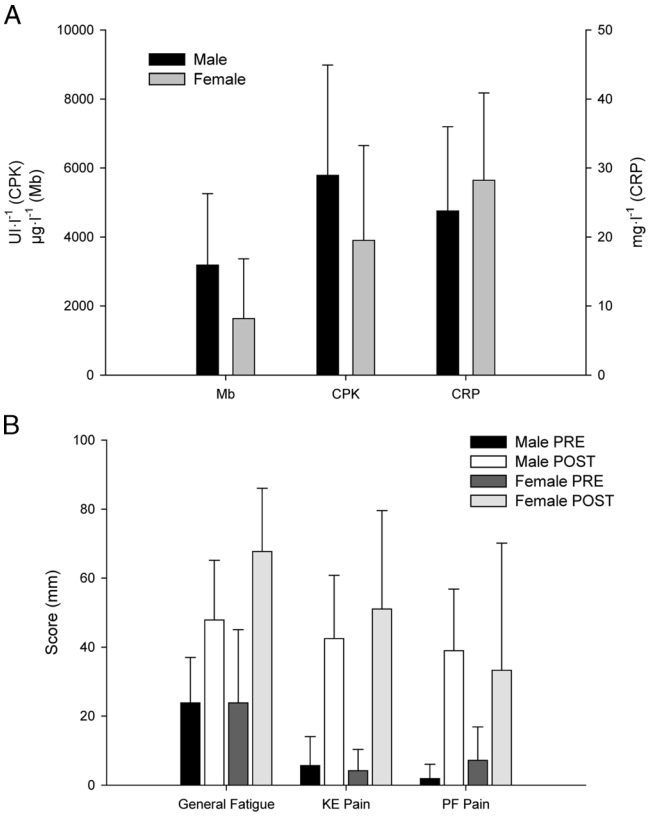

FIGURE 3—A, Concentrations of CRP, Mb, and CPK at POST by sex. B, General fatigue, knee extensor (KE), and plantar flexor (PF) pain and digestive discomfort PRE and POST by sex. Values are presented as mean ± SD. All parameters increased significantly from PRE to POST.

induced major central (i.e., decreased VA_{FNES} and VA_{TNES}) and peripheral (i.e., decreased evoked torques) fatigue (25,29,35). Finally, plantar flexor $Db10{\cdot}Db100^{-1}$ decreased, indicating low-frequency fatigue in ultralong running bouts with large elevation change as in UTRR (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 the 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 using 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 ($60°{\cdot}s^{-1}$) but not high ($300°{\cdot}s^{-1}$) 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 are 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, in the present study, males exhibited greater decreases in evoked plantar flexor torques than females after the UTRR. 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 $Db10{\cdot}Db100^{-1}$.

Our results suggest that there is a sex difference in the development of peripheral fatigue in the plantar flexors that either did 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 VA_{FNES} decrease was approximately twice as large in females (−9% vs −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, 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 intramuscular 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 the muscle and the skeletal frames. 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 (they were not 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 d 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) was 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 because greater recovery of knee extensor MVC was previously observed 1 h after 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.

CONCLUSIONS

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 after 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.

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