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Original Article

Comparison of the effects of simulated and actual short trail running tests on neuromuscular function in master trail runners

SIRAPRAPA PANTHONG[1], NATEE THONGSIRI[2], DAROONWAN SUKSOM[3]
[1,3]Area of Exercise physiology, Faculty of Sports Science, Chulalongkorn University, Bangkok, THAILAND.
[2]Department of Mathematics, Faculty of Science, Chiang Mai University, Chiang Mai, THAILAND.
[3]Exercise Physiology in Special population Research Unit, Chulalongkorn University, Bangkok, THAILAND.

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Abstract:
Trail running is an increasingly popular endurance sport with races over variety of distances and terrains. Those prolonged running elicits decrease in muscle power output mediated by reductions in neuromuscular function. However, the evaluation of neuromuscular function is still limited in trail runners and difficult on the field setting. **Purpose** The present study aimed to compare the impact of simulated and field trail running races on neuromuscular function trail runners. **Methods** 26 trail runners (42 ± 6 years) with 4.2 ± 1.3 years of trail-running practice were studied. A simulated trail running test (STR) was conducted in a laboratory, consisting of 30 minutes of 15% grade uphill running at 75% of heart rate reserve (HRR) and 30 minutes of 15% grade downhill running at 40% HRR on a treadmill. The short distance trail running race (TRR) was run on mountain single tracks with technical rocky sections and a total distance of 18 km with a positive elevation of 1,080 m. **Results** After STR, quadriceps maximal voluntary contraction (MVC) and rectus femoris M-wave decreased significantly ($p<0.05$), indicating total and peripheral fatigue. However, no significant changes were observed in central fatigue as assessed by maximal voluntary activation. After TRR, the average time was 279 ± 45 minutes, and height and peak power of counter movement jump (CMJ) decreased significantly ($p<0.05$). The decreases in MVC observed in the laboratory were associated with the corresponding reductions in peak power of CMJ ($r = 0.480$, $p = 0.013$) obtained in the field setting. **Conclusion** The simulated trail running test produced total and peripheral fatigue but not central fatigue in trail runners. Such simulated trail running test could be used as an experimental tool to investigate neuromuscular fatigue in trail runners.
Key Words: **trail running, neuromuscular fatigue, maximal voluntary contraction, counter movement jump, fatigue sensation**.

Introduction

Trail running defines as a pedestrian and off-road race conducted in a natural environment (e.g. mountain) with minimal possible paved or asphalt road (20% of the total duration race) (Ehrstrom et.al., 2018b). In trail running races where the distance may vary from short (<42 km) to ultra-long (100 km) are involved extensive vertical displacement in both uphill and downhill sections (Ehrstrom et.al., 2010). Prolonged concentric and eccentric muscle actions during uphill and downhill sections are known to induce specific mechanical and metabolic alterations (Zimmermann et al., 2020). Severe alterations in neuromuscular function were reported with substantial failures in both central and peripheral neuromuscular function (Ehrstrom et.al., 2018a). Neuromuscular fatigue is an exercise-related decrease in the maximal voluntary force or power of a muscle or muscle group (Borji et al., 2019). This potentially involves processes at all levels of the motor pathway from the brain to skeletal muscle (Millet et.al., 2011). It is usually divided into central and peripheral components (Wan et al., 2017). Central fatigue originates in the central nervous system (CNS), which reduces the neural drive to the muscle. Peripheral fatigue is caused by changes at or distal to the neuromuscular junction. It is well known that trail running induces alterations to both central and peripheral components of neuromuscular function (Besson et al., 2021). Neuromuscular fatigue is a commonly experienced phenomenon that limits athletic performance and prolonged activity (Wan et al., 2017). The progressive change that occurs in the neuromuscular fatigue due to exercise, resulting in a force output that is less than anticipated for a given voluntary contraction or stimulation.

Recent studies investigating trail races reported aspects of neuromuscular fatigue mainly assessed by maximal voluntary contraction (MVC) torque and changes in twitch and activation parameters (Easthope et.al., 2010). Greater impairment of muscle contractile properties in longer trail running races, contributed to the correlation between knee extensors MVC loss and race distance (Temesi et al., 2021). The countermovement jump (CMJ) test has been also used to monitor neuromuscular fatigue in athletes (Armada-Cortés et al., 2022). Previous study showed that CMJ jump height were significantly lower in male ultra runners immediately after the 75 km Interlacs Trail (Balducci et al., 2017). However, the effects of trail running on neuromuscular function

Corresponding Author: DAROONWAN SUKSOM, E-mail: Daroonwan.S@chula.ac.th

and muscle fatigue are not fully understood, mainly because assessing such function in trail runners in the field is challenging. Therefore, it may be more effective to evaluate strategies and modalities that may influence neuromuscular fatigue in a laboratory setting. The simulated trail running test is valuable tool for athletes and can be conducted on treadmills, indoor that closely resemble the race conditions. A previous study reported that trail running performance using laboratory exercise testing (the graded exercise test) most accurately predicted field-based (a 31.1 km trail running performance) outcomes (Scheer et al., 2019). However, no one has investigated the induction of neuromuscular fatigue in the laboratory compared to the field test.

Accordingly, the present study was aimed to compare the impact of simulated and field trail running races on neuromuscular function. We hypothesized that simulated trail running test would evoke similar responses in neuromuscular fatigue same as the actual trail running race in master trail runners. In an attempt to tease out the influence of testing, a separate group of gender was also determined.

Material & methods

Participants

Forty-five amateur male and female trail runners, age 42.3 ± 6.3 yr, who had regular training experience in long distance running volunteered to participate in this study. Participants had a mean of 4.0 ± 1.3 yr of trail running experience. The sample size was calculated according to a previous study with a statistical power of 80% and a significance at $P<0.05$. All participants completed a medical history questionnaires and physical activity readiness questionnaire to determine eligibility. They had no chronic medical history such as cardiovascular disease or any other conditions. No participants were taking prescribed medications in the present study. Subjects gave their informed written consent to participate in this study, which was approved by the research ethics review committee of Chulalongkorn University and conducted according to the Declaration of Helsinki.

Study Design

Participants visited the laboratory and mountain race on three different occasions. During the first visit, participants were requested to abstain from alcohol consumption for 24 h and caffeine and tobacco for 12 h before the tests. No vigorous exercise was performed for at least 24 h before the testing. Participants completed a health history questionnaire and an exercise participation questionnaire. Each participant had their vital signs and body composition measured. Blood sampling was then performed at the same time of day (e.g., 7:30 h) for each participant. Two hours after having breakfast, the participants were asked to perform measurements of muscle function and aerobic fitness. During the second visit (1 day after), the neuromuscular function in both central fatigue, assessed by maximal voluntary activation and peripheral fatigue assessed by maximal voluntary contraction (MVC) were evaluated before and after a simulated trail running test. Heart rate were monitored during the running test. During the third visit (separated by 1 week), participants performed a short trail running race. Heart rate, blood pressure, CMJ, blood lactate concentration and time were recorded before and after the race.

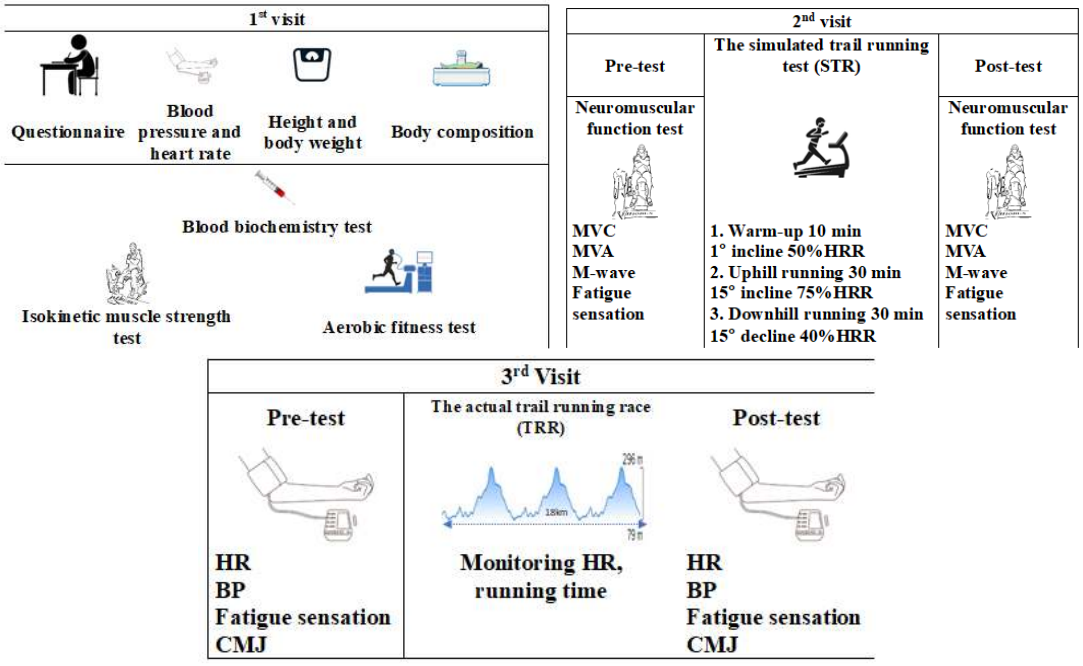

Figure 1. Schematic representation of the study design

The simulated trail running test (STR)

STR was conducted in a laboratory, consisting of 60 minutes of uphill and downhill running. Subjects performed running on a motorized treadmill (Pulsar® 3p, HP cosmos®, Germany) for warm-up 10 min at 50% heart rate reserve (HRR) in a level (1%) followed by uphill (15%) running for 30 min at 75 % HRR (Garnier et al., 2019) and then downhill (15%) running on a motorized treadmill (Gait Trainer™ 3, Biodex Medical Systems, Shirley, NY) for 30 min at 40 % HRR (Ehrstrom et al., 2018). HRR was calculated in accordance with Karvonen's formula (Garnier et al., 2020).

HRR = [(maximal heart rate − resting heart rate) × %intensity]+ resting heart rate

The short trail running race (TRR)

TRR was run on mountain single tracks with technical rocky sections and a total distance of 18 km located in Khao Chalak, Chon Buri in Thailand. The race was composed of a climbing segment 1,021 m (31.2%, 298 m ascent) followed by a downhill segment 1,018 m (-0.7%, 61 m descent). All subjects wore a cardio-GPS watch (H10; Polar, Kempele, Finland) during the TRR for continuous HR and speed monitoring. Although different refueling points were available during the TRR, subjects limited at 500 calories to carry light backpacks or drinking belts containing fluids or carbohydrates (e.g., drinks, gels, bars) (Ehrström et al., 2018).

Measurements

Measurements were conducted in the exercise physiology laboratory with the Faculty of Sports Science at Chulalongkorn University. Upon entering the exercise laboratory, each participant was familiarized with the equipment and given clear but brief instructions regarding the protocol. All data collection and analyses were performed by the same trained technician.

General characteristics. Height and body weight were measured using the electric bioimpedance device (ioi-353; Jawon Medical, Seoul, Korea). Heart rate and blood pressure were recorded after >5 min of rest with the participant in seated position using a semiautomated blood pressure device (CARESCAPE V100 monitor; GE Healthcare, Milwaukee, WI). Body composition (body fat percentage, total fat mass, total lean mass, and leg lean mass) was assessed using dual-energy x-ray absorptiometry (GE Healthcare, Madison, WI).

Blood biochemistry. After 12 h of overnight fasting, a venous blood sample was collected from the antecubital vein and then centrifuged for separation of erythrocyte and plasma. Complete blood count (via automated cell counter), fasting blood glucose (enzymatic assay using hexokinase reaction), lipids, and lipoproteins (enzymatic assays) were measured in the certified clinical laboratory within the Faculty of Allied Health Science at Chulalongkorn University.

Aerobic fitness. During the graded exercise test, oxygen consumption was measured continuously breath by breath using a cardiopulmonary gas exchange system (Vmax Encore 29 System, VIASYS Healthcare, Inc., Yorba Linda, CA). Subjects started running on a treadmill the slope was fixed at +10% at a velocity of 5 km·h-1, which increased by 1 km·h-1 every 2 min until exhaustion. Maximal oxygen consumption (VO2max), maximal heart rate, and ventilatory threshold were recorded. VO2max was defined as the highest 15-s average measured before the termination of the test. Attainment of VO2max was confirmed on the basis of the following criteria: a change in VO2 of no greater than 2 mL·kg−1 ·min−1 with increasing stages, respiratory exchange ratio greater than 1.1, and achievement of heart rate greater than 85% of their age-predicted maximal heart rate (Ehrstrom et al., 2018).

Isokinetic muscle strength. Isokinetic muscle strength of quadriceps (knee extension) and hamstrings (knee flexion) were measured using an isokinetic dynamometer (Biodex System 4; Biodex Medical Systems, Shirley, NY). During the tests, participants were securely strapped into an isokinetic dynamometer (Biodex System 3, Shirley, NY) with a knee joint angle of 90- (full leg extension, 0-) for the assessed dominant leg. The axis of the knee joint was carefully aligned with the rotational axis of the dynamometer, and all settings were kept constant throughout the experiment. Muscle strength was measured with five maximal efforts with flexion and extension movement at a velocity of $60°\cdot s^{-1}$. The subjects were provided strong verbal encouragement during exercise, yet they had no feedback regarding their performance during the protocol. Settings for the arm length and chair position were recorded and repeated during posttesting. Peak torque (newton) was recorded for both knee extension and flexion (Sundby and Gorelick, 2014).

Neuromuscular function. The neuromuscular function was tested using the method of electrical stimulation similarly on the right knee extensor (KE) muscle before and after STR test. During both the maximal voluntary contraction (MVC) and electrical stimulation, subjects were secured to a Biodex System 3 isokinetic dynamometer (Biodex Medical System, Shirley, NY, USA) by chest and hip strapping to avoid excessive lateral and frontal movements. The seating was adjusted for each subject with the right knee femoral epicondyle aligned with the axis of the rotation arm of the dynamometer. The right lower leg was attached to the lever arm just above the lateral malleolus. The knee and hip angles were positioned at 90 and 110 degrees, respectively. Subjects maintained their hands in the same position by holding the chest strapping of the dynamometer (Froyd et.al., 2018).

Maximal voluntary contraction (MVC). Subjects performed 4 s isometric MVC of the right knee extensors. They were instructed to reach maximum torque in 1 s and then to maintain this level for 3 s. They received strong verbal encouragement to maintain a maximal effort during all contractions. Torque was measured in the isokinetic dynamometer.

Electrical stimulation. Before each test, the optimal stimulation intensity was identified by delivering successive single electrical stimuli at increasing intensities on relaxed muscles on the femoral nerve. The stimulation intensity used during all tests was 130% of optimal intensity to ascertain full spatial recruitment (Ehrstrom et al., 2018). During the neuromuscular tests, transcutaneous electrical stimulations were applied to the femoral nerve via a self-adhesive electrode cathode (10 mm in diameter) pressed manually by a researcher into either the femoral triangle. The self-adhesive rectangular anode (50 mm × 90 mm) was located either in the gluteal fold. A constant current stimulator (Intelect® TranSport 2 Channel Electrotherapy Unit, Guildford Surrey, United Kingdom) delivered a square wave stimulus of 1 ms duration and 400 V maximal voltage and the interval of stimuli in the doublet were 100 and 10 ms for doublets at 10 Hz (Db10) and 100 Hz (Db100), respectively (Vercruyssen et al., 2017). After a specific KE isometric warm-up (i.e., 3-min submaximal contractions performed at increasing force levels), participants performed a neuromuscular evaluation for KE which first consisted of a 4-s MVC followed by two single potentiated twitches separated by 2 s on the relaxed muscles. This procedure was repeated a second time after 15 s of rest. Following a resting period of 30 s, the subjects performed a third 4-s MVC superimposed with Db100 and followed after 2 s by two potentiated doublets in the relaxed muscle, i.e., Db100 and Db10, delivered 2 s apart. After 15 s of rest, this procedure was repeated a second time. The amplitude of the potentiated Db10, Db100, and the amplitude of the potentiated twitch peak torque (TW) that followed the two doublets as well as the ratio of paired stimulation peak forces at 10 Hz over 100 Hz (Db10:100) were analyzed for KE. Throughout the testing sessions, subjects were strongly encouraged during their MVC. On the contrary, they were asked to be as relaxed as possible during the peripheral fatigue measurements. For each condition, neuromuscular evaluation was conducted twice. Values were then averaged from the two series. The variability in VA was determined to assess central fatigue for KE using a high-frequency doublet (100 Hz) superimposed on MVC. VA was calculated from the maximal force (Fmax) attained during the MVC, the force just before the superimposed doublet (Fbefore), the peak force following the superimposed doublet (Db100sup), and control Db100 on relaxed muscle (Ehrstrom et al., 2018) as follows:

$$VA = \left[1 - \frac{(Db100\ sup - Fbefore) \times \frac{Fbefore}{Fmax}}{controlDb100}\right] \times 100$$

EMG recordings. During the neuromuscular function test, surface EMG signals were continuously recorded from the vastus lateralis (VL) muscle with a pair of self-adhesive surface (10 mm diameter) electrodes connected to an EMG data collection system with the wireless apparatus of a Cometa miniwave infinity waterproof device (Cometa slr, Milan, Italy) and the signals were collected using customized software named EMG and Motion Tools, Inc. software version 7 in bipolar configuration with a 20-mm interelectrode distance. Data analysis and processing were performed using the raw EMG signal fully rectified offline and calculated as a high-pass filtered cut-off frequency of 20 Hz and a low-pass filtered cut-off frequency of 400 Hz. (Phothirook et al., 2022). EMG data quantified by using the root mean square (RMS). Maximal RMS EMG of VL muscle was set as the maximal 500-ms RMS value found over the 3-s MVC (i.e., 500 ms window width, 1-ms overlap). During evoked stimulation performed before the MVC, peak-to-peak amplitude (PPA) and peak to-peak duration (PPD) of the M-wave were determined for the VL muscle. Amplitude was defined as the sum of absolute values for maximum and minimum points of the biphasic (one positive and one negative deflection) M-wave. Duration was defined as the time from maximum to minimum points of the biphasic M-wave (Ehrstrom et al., 2010).

Countermovement Jump (CMJ) Performance. Three completed CMJs with a 15-second rest between each effort were assessed. The subjects performed a countermovement downward immediately followed by a complete extension of the lower limbs with hands on their hips, starting from a static position. All jump testing was performed on the power cage (FT700; Fitness Technology, Adelaide, Australia) using the linear position transducer (LPT) and the 400 series force plate. The height and peak power of countermovement jump were calculated via Ballistic Measurement System Software program (Balducci et al., 2017).

Fatigue sensation. The fatigue sensation was measured at pre and post STR and TRR tests on a 10-cm visual analog scale. The subjects were requested to report the severity of their general fatigue feeling (Millet et.al., 2011).

Statistical Analyses Descriptive data are expressed as means ± SD. Data were analyzed using SPSS (version 23; IBM, Armonk, NY). Before the parametric tests, the tests for normal distribution (Shapiro– Wilks test) and homogeneity of variance were confirmed. Student's paired t-tests was used to examine possible differences between pre- and post- STR and TRR tests. Independent t-tests was used to examine possible differences between groups (Male and Female). Pearson correlation analyses were used to assess associations of interest. Statistical significance was set at an α level of 95% ($P < 0.05$) for all statistical measures.

Results

All the participants completed 100% of laboratory-based and field trail running races. No adverse events were reported. The general physiological characteristics of trail runners at baseline were shown in Table 1. There were 26 trail runners, average age 42 ± 6 years (13 male, age 42 ± 6 years and 13 females, age 41 ± 6 years). The participants had body fat and lean mass of 26 ± 8%/44±10 kg measured on DEXA, a maximal oxygen consumption (VO2max) of 42.7 ± 8.1 ml/kg/min measured on a treadmill and knee extension/flexion

torque of 144 ± 41/62 ±21 Nm measured on a Biodex machine. Male trail runners had significantly higher in body composition, blood pressure, VO2max, muscular strength, hemoglobin, hematocrit and red blood cell than female trail runners (all p<.05). There is no significant difference between male and female trail runners in age, body mass index, heart rate, WBC, glucose, cholesterol, HDL, LDL and triglyceride.

Table 1. General physiological characteristics of trail runners.

Variables	Total (n=26)	Male (n=13)	Female (n=13)	t	*p*-value
Age (years)	42.3 ± 6.3	42.7 ± 6.9	41.8 ± 6.0	0.278	0.786
Height (cm)	165.1 ± 7.4	171.0 ± 5.7	159.2 ± 2.2†	6.931	0.000
Body weight (kg)	62.1 ± 11.7	69.7 ± 9.7	54.6 ± 8.3†	4.625	0.001
Body mass index (kg/m^2)	22.6 ± 3.3	23.8 ± 2.1	21.4 ± 3.9	2.068	0.061
Body fat (%)	26.3 ± 7.9	21.5 ± 5.6	31.1 ± 7.1†	-3.776	0.003
Fat mass (kg)	47.0 ± 9.8	55.3 ± 5.9	38.7 ± 4.0†	11.247	0.000
Lean mass (kg)	44.1 ± 9.6	52.4 ± 5.6	35.9 ± 3.7†	9.188	0.000
Leg lean mass (kg)	15.1 ± 4.2	18.0 ± 4.2	12.3 ± 1.6†	4.593	0.001
Heart rate (bpm)	62.0 ± 8.7	62.0 ± 6.7	62.1 ± 10.7	-0.019	0.985
Systolic BP (mmHg)	114.4 ± 10.0	117.8 ± 9.3	111.0 ± 9.9†	2.229	0.046
Diastolic BP (mmHg)	67.5 ± 9.5	71.1 ± 8.4	63.9 ± 9.5†	2.415	0.033
Mean BP	83.7 ± 9.0	87.2 ± 8.4	80.3 ± 8.7†	2.400	0.034
VO_2max (ml/kg/min)	42.7 ± 8.1	48.1 ± 5.7	37.3 ± 6.4†	5.054	0.000
Knee extension torque (Nm)	144.2 ± 41.5	172.7 ± 37.6	115.7 ± 20.9†	4.798	0.000
Knee flexion torque (Nm)	61.8 ± 21.9	75.8 ± 19.7	47.8 ± 13.8†	3.713	0.003
Hemoglobin (g/dL)	13.7 ± 1.6	14.9 ± 1.2	12.4 ± 0.8†	5.258	0.000
Hematocrit (%)	42.5 ± 4.7	45.9 ± 3.7	39.1 ± 2.6†	4.871	0.000
WBC (103/µL)	6.1 ± 1.4	6.0 ± 1.5	6.1 ± 1.3	-0.034	0.974
RBC ($10^6 \cdot \mu L^{-1}$)	4.8 ± 0.7	5.1 ± 0.5	4.4 ± 0.6†	2.572	0.024
Glucose (mg·dL^{-1})	86.5 ± 8.2	87.5 ± 9.0	85.6 ± 7.6	0.416	0.685
Cholesterol (mg·dL^{-1})	215.8 ± 42.4	212.7 ± 36.1	218.9 ± 49.2	-0.389	0.704
HDL (mg·dL^{-1})	67.8 ± 14.9	62.3 ± 15.3	73.3 ± 12.8	-1.670	0.121
LDL (mg·dL^{-1})	137.9 ± 49.33	140.2 ± 54.6	135.7 ± 45.6	0.251	0.806
Triglyceride (mg·dL^{-1})	69.5 ± 28.3	79.77 ± 34.2	59.3 ± 16.5	2.114	0.056

Data are means ± SD. †$P < 0.05$ Men vs female BP= blood pressure, VO2max= maximal oxygen consumption, HDL= high density lipoprotein, LDL= low density lipoprotein

As illustrated in Table 2, VA did not change significantly after STR in total, male and female groups. MVC, Db100, Db10:100 amd M-wave decreased after STR in total, male and female groups (all p<.05). There is no significant difference in all parameters of neuromuscular function that induced fatigue by STR between male and female athletes.

Table 2. Neuromuscular function at pre-and post- the simulated trail running test in trail runners

Variable	Total (n=26)			Male (n=13)			Female (n=13)		
	Pre	Post	%Change	Pre	Post	%Change	Pre	Post	%Change
VA (%)	112.1 ± 72.3	78.8 ± 43.2	-18.8 ± 57.0	133.7 ± 99.0	68.9 ± 56.7	14.7 ± 39.1	108.6 ± 33.9	107.9 ± 37.3†	0.2 ± 22.1
Db100 (Nm)	429.9 ± 145.3	378.2 ± 129.3*	-8.3 ± 26.1	451.2 ± 127.3	387.4 ± 123.9*	-13.1 ± 18.1	408.5 ± 163.8	368.9 ± 138.9*†	-3.5 ± 32.2
Db10 (Nm)	352.7 ± 145.8	275.0 ± 142.5*	-23.9 ± 24.9	368.5 ± 139.0	290.9 ± 161.3*	-23.6 ± 24.8	336.9 ± 156.2	259.0 ± 125.5*†	-24.3 ± 26.0
Db10:100 (%)	15.1 ± 31.8	29.0 ± 30.6*	-3.4 ± 17.2	18.4 ±27.9	25.4 ± 37.0	1.6 ± 5.6	11.7 ± 36.1	32.6 ± 23.5	-8.5 ± 23.0
M-wave (µV)	324.3 ± 106.6	286.6 ± 102.0*	-11.2 ± 12.0	357.5 ± 110.3	306.0 ± 98.6*	-12.1 ± 13.5	290.1 ± 110.2	266.8 ± 105.5*†	-0.2 ± 22.1
MVC (Nm)	151.7 ± 63.2	122.9 ± 50.9*	-11.2 ±22.9	188.4 ± 64.2	144.4 ± 57.2*	-22.1 ± 18.6	108.6 ± 33.9	107.9 ± 37.3*	-0.2 ± 22.1†

Data are means ±SD. *$P < 0.05$ Pre vs Post-test †$P < 0.05$ Men vs female
VA= voluntary activation, VA= voluntary activation, Db100=doublets at 100 Hz, Db10=doublets at 10 Hz , MVC= maximal voluntary contractions

As illustrated in Table 3, all participant were performed short trail running in the mountain with sunny conditions at similar temperatures (31.4 ± 2.2 °C), low wind speeds (16.00 ± 2.5 km·h-1), dry conditions and good visibility. The total, male and female subjects had average race time as 279.9 ± 45.5 min, 270.8 ± 34.2 min and 289.1 ± 54.5 min respectively, which corresponds to an average running speed of 13.8 ± 5.2 kg/min, 14.1 ± 3.7 kg/min and 13.5 ± 6.5 kg/min, respectively.

Height and peak power of CMJ decreased significantly after TRR in total, male and female groups (all p<.05) and the decrease of those in female trail runners had significantly higher than male trail runners. Heart rate, systolic BP and diastolic BP increased significantly after TRR in total, male and female groups (all p<.05).

Table 3. Trail running performance at pre-and post- the actual trail running race in trail runners

Variable	Total (n=22)			Male (n=11)			Female (n=11)		
	Pre	Post	%Change	Pre	Post	%Change	Pre	Post	%Change
Time (min)	-	279.9 ± 45.5	-	-	270.8 ± 34.2	-	-	289.1 ± 54.5	-
Average pace (kg/min)	-	13.8 ± 5.2	-	-	14.1 ± 3.7	-	-	13.5 ± 6.5	-
Heart rate (bpm)	70.7 ± 10.7	100.9 ± 8.1*	35.4 ± 48.4	68.7 ± 8.5	104.3 ± 6.7*	45.4 ± 30.0	72.7 ± 12.6	98.0 ± 11.0*	25.0 ± 61.3
Systolic BP (mmHg)	122.3 ± 15.1	108.2 ± 11.6*	-16.2 ± 26.8	127.4 ± 9.8	113.3 ± 13.9*	-13.5 ± 12.5	117.2 ± 18.0	104.2 ± 8.8*	18.8 ± 36.4
Diastolic BP (mmHg)	78.1 ± 11.6	76.9 ± 7.8	-9.3 ± 33.1	83.1 ± 9.3	79.8 ± 7.4	-7.9 ± 16.4	73.2 ± 11.8	74.6 ± 8.1	-13.3 ± 49.0
Height CMJ (cm)	0.4 ± 0.1	0.4 ± 0.1*	9.0 ± 10.3	0.5 ± 0.1	0.4 ± 0.1*	11.0 ± 10.2	0.4 ± 0.1	0.3 ± 0.1*†	10.2 ± 8.8
Peak power CMJ (W)	3119.0 ± 969.5	2690.4 ± 992.9*	-11.8 ± 28.2	3810.3 ± 792.4	3542.5 ± 512.4*	-2.0 ± 31.7	2427.7 ± 543.1	1838.4 ± 567.2*†	-21.7 ± 20.9

Data are means ±SD. *$P < 0.05$ Pre vs Post-test †$P < 0.05$ Men vs female
CMJ= countermovement jump, BP= blood pressure

As show in Fig. 2, fatigue sensation after STR of total, male and female groups were 2.7 ± 2.3, 2.3 ± 2.1 and 2.5 ± 2.6 scores, respectively. Fatigue sensation after TRR of total, male and female groups were 6.8 ± 1.9, 6.8 ± 1.6 and 6.7 ± 2.2 scores, respectively. Fatigue sensation scores after TRR were significantly higher in total, male and female groups (all p<.05).

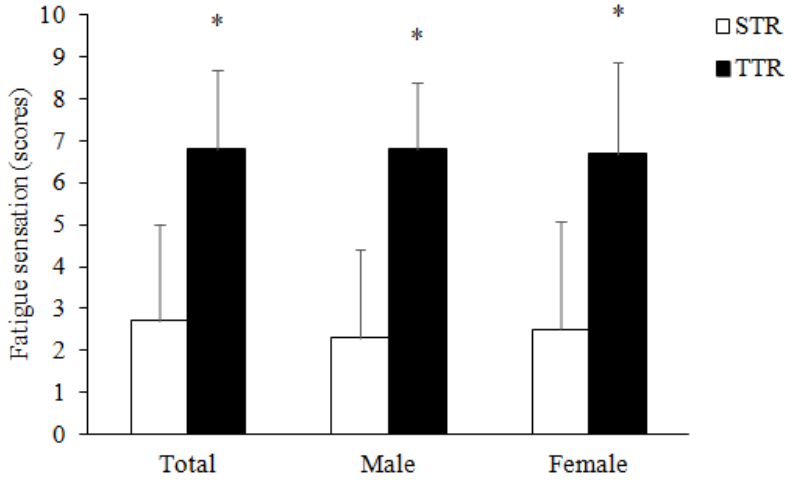

Figure 2. Fatigue sensation scores at pre-and post- the simulated trail running test (STR) and the actual trail running race (TRR) in trail runners. Data are means ± SD. *P < 0.05 Pre vs Post-test

Fig. 3 shows associations of key neuromuscular functions measures after STR and TRR. The decrease of height and peak power of CMJ were positively associated with the decrease of MVC (r = 0.480, P = 0.013 and r = 0.559, P = 0.006). The decrease of height of CMJ was positively associated with the decrease of VO2max (r = 0.515, P = 0.007).

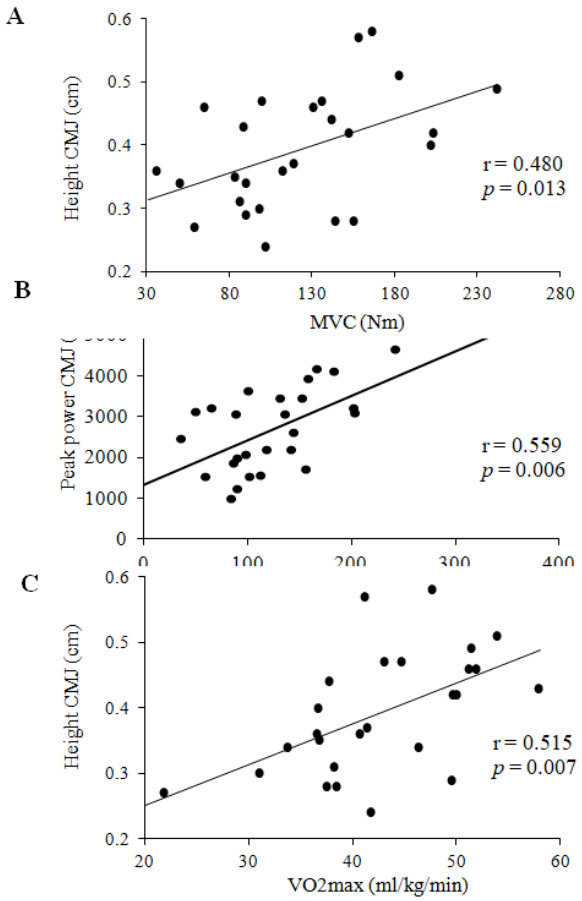

Figure 3. Correlations of A. Height of countermovement jump (CMJ) and maximal voluntary contraction (MVC) B. Peak power of CMJ and MVC and C. Height of CMJ and maximal oxygen consumption (VO2max).

Discussion

The major findings of the present study are as follows. After the simulated trail running test (STR), quadriceps maximal voluntary contraction (MVC) and rectus femoris M-wave decreased significantly ($p<0.05$) while there was no change in maximal voluntary activation (MVA). Height and peak power of countermovement Jump (CMJ) decreased after the actual trail running race (TRR). Fatigue sensation increased after both STR and TRR (all $p<0.05$) but it was significantly lower after STR than TRR ($p<0.05$). Male master trail runners had significantly higher in body composition, cardiorespiratory fitness and muscle strength than female master trail runners but female had less change in MVC after STR than male. The decreases in MVC after STR was associated with the reductions in peak power CMJ ($r = 0.480$, $p = 0.013$) after TRR.

Peak twitch torque is commonly used to evaluate peripheral fatigue. It has been reported that MVC values of master athletes were significantly lower than the young group's values (Ehrstrom et.al., 2010). During uphill sections, predominantly concentric muscle contractions induce less mechanical stress and thereby less potential muscle damage. In contrast, a marked decline in maximal voluntary contraction (MVC) torque for leg extensor muscles has been reported after treadmill and outdoor downhill running exercises. In the present study, quadriceps MVC torque was declined by 11% after the simulated trail running test. It is generally accepted that the structural muscle damage leading to MVC loss is generated by the eccentric muscle contractions occurring in running (Millet et al. 2003). Cellular mechanisms underpinning peripheral fatigue may be attributed to longer muscle lengths (i.e., overstretched sarcomeres) during eccentric muscle actions over braking phases, leading to myofibrillar damage such as disrupted weaker sarcomeres and/or excitation–contraction coupling failure (Ehrstrom et.al., 2017).

In this study, quadriceps maximal voluntary activation (VA) was not found to be significantly changes after a 30-min treadmill up-hill and 30-min treadmill down-hill running. This results indicated that the simulated trail running test contributed total and peripheral components to neuromuscular fatigue but not central fatigue. Inconsistent with previous study in which 55% of VO2max of downhill running 40 min has been reported to induce central neuromuscular fatigue by deficit in KE voluntary activation (Ehrstrom et.al., 2018). This contrast

may be due to difference in pattern, intensity and duration of exercise. Our simulated trail running test might be not have high intensity and/or prolong duration enough that could stimulate metabolites and damage to muscle spindles and failed to impact supra-spinal level or inhibitory reflexes (Martin et al., 2005). The etiology of fatigue depends on the type of exercise, particularly intensity and duration. Other critical task variables include muscle groups involved, activation patterns and types of muscle contraction (Millet et al. 2011). Thus, we suggest that that the amount of exercise bouts should be taken prolong enough when using as an induced fatigue test in order to evaluate neuromuscular performance. Especially, downhill exercise should be longer duration (>30-min) and greater slope (>15%grade) but should not change in constant speeds (10–14 km·h− 1) for reducing injury.

In the current study, the actual short trail running race was composed of a climbing segment 1,021 m, a downhill segment 1,018 m and total distance of 18 km. Vercruyssen et al. (2017) recently reported a ~4.5% to 6.5% decrease in quadriceps voluntary activation (central component) associated with a significant reduction in the low- to high-frequency doublet ratio (i.e., peripheral component) after various 18.6-km trail running sessions. Unfortunately, in this study we did not measure central and peripheral neuromuscular functions on field. However, we found that countermovement Jump (CMJ), a practical athlete monitoring tool used to examine neuromuscular status (Gathercole et al. 2015) was reduced after the 18 km trail running race. CMJ was one of the most common jump tests used because of its validity, reliability, and specificity (Marco-Contreras et al., 2021). The reduction of CMJ shown in this study reflects potential alterations in neurotransmission and/or excitation–contraction coupling mechanisms (Hunter, 2018. This potentially indicates decreased Ca2+ release from the sarcoplasmic reticulum and a related decrease in the amount of Ca2+ binding to myofilaments possibly limiting the amount of muscle force produced (Keeton and Binder-Macleod, 2006). Moreover, decreases CMJ performance (by 11%) obtained in the field were associated with the corresponding reductions in MVC (by 11%) observed in the laboratory setting, suggests that neuromuscular fatigue indued by STR achieved similar to induce by TRR. In this study, the simulated trail running test was created to mimic trail race as it comprised both uphill and downhill running. However, the mimic terrain was limited in laboratory test. We speculated that although the STR does not allow for the successful identification of actual trail situation, but it still have benefit for low cost and weather controlled to induce neuromuscular fatigue when compared with the field test.

Shear wave elastography revealed sex differences in resting skeletal muscle mechanical properties measured in a population of experienced trail runners (Fouré et al., 2021). In the present study, male master trail runners had significantly higher in knee extension and flexion torque than female. This can indirectly reflect a sex difference in muscle volume. It is reinforced by a larger body fat and a lesser lean mass in females in comparison to males. Additionally, the results showed that male trail runners had significantly higher in VO2max than female. This difference may be due to men typically have greater maximal aerobic capacity, due to larger stroke volumes and greater cardiac output during peak exercise, and increased hemoglobin (Navalta et al., 2018). It appears that neuromuscular function decreased after STR and TRR in both men and women. Our results also showed that female had less declined in MVC after STR than male but male had less declined in CMJ after TRR than female. Males have been shown to generally exhibit greater performance fatigability for various muscle groups compared with females for isometric fatiguing tasks, although such sex differences are less evident for dynamic fatiguing exercise (concentric and eccentric contractions) (Hunter, 2016).

There are a number of limitations to the present study that should be emphasized. Firstly, the present study uses a cross-sectional design. Therefore, a cause and effect relationship cannot be established. Secondly, the number of participants studied was relatively small and the results should be interpreted with caution. Thirdly, the neuromuscular fatigue index did not measure the same test in STR and TRR. Because it was limited to move the isokinetic machine to the field test, and either neuromuscular function test should be performed immediately after STR or TRR.

Conclusions

The results of this study indicate that the simulated trail running test induced total and peripheral fatigue, as indicated by decreased quadriceps maximal voluntary contraction (MVC) and rectus femoris M-wave, but not central fatigue, no change in maximal voluntary activation (MVA), in trail runners. In addition, the MVC measured after the simulated trail running was significantly associated with the counter movement jump obtained after the actual trail running test. Taken together, these results suggest that both the simulated trail running test and the actual trail running test have their own merits and contribute to an athlete's preparation for the sport. A simulated trail running test in the laboratory is a sufficient induction to produce neuromuscular fatigue that correlates with the actual trail running field test. This is a first step in creating a useful experimental tool for simulating short distance trail running and investigating neuromuscular fatigue in this population.

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Conflicts of interests

The authors have no conflict of interest to declare.

References

Armada-Cortés, E., Benítez-Muñoz, J. A., San Juan, A. F., & Sánchez-Sánchez, J. (2022). Evaluation of Neuromuscular Fatigue in a Repeat Sprint Ability, Countermovement Jump and Hamstring Test in Elite Female Soccer Players. International journal of environmental research and public health, 19(22), 15069.

Balducci, P., Clémençon, M., Trama, R., Blache, Y., & Hautier, C. (2017). Performance Factors in a Mountain Ultramarathon. International journal of sports medicine, 38(11), 819–826.

Besson, T., Parent, A., Brownstein, C. G., Espeit, L., Lapole, T., Martin, V., Royer, N., Rimaud, D., Sabater Pastor, F., Singh, B., Varesco, G., Rossi, J., Temesi, J., & Millet, G. Y. (2021). Sex Differences in Neuromuscular Fatigue and Changes in Cost of Running after Mountain Trail Races of Various Distances. Medicine and science in sports and exercise, 53(11), 2374–2387.

Borji, R., Zghal, F., Zarrouk, N., Martin, V., Sahli, S., & Rebai, H. (2019). Neuromuscular fatigue and recovery profiles in individuals with intellectual disability. Journal of Sport and Health Science, 8(3), 242-248.

Easthope, C. S., Hausswirth, C., Louis, J., Lepers, R., Vercruyssen, F., & Brisswalter, J. (2010). Effects of a trail running competition on muscular performance and efficiency in well-trained young and master athletes**.** European journal of applied physiology, 110, 1107-1116.

Ehrström, S., Gruet, M., Giandolini, M., Chapuis, S., Morin, J. B., & Vercruyssen, F. (2018a). Acute and delayed neuromuscular alterations induced by downhill running in trained trail runners: beneficial effects of high-pressure compression garments. Frontiers in physiology, 9, 1627.

Ehrström, S., Tartaruga, M. P., Easthope, C. S., Brisswalter, J., Morin, J. B., & Vercruyssen, F. (2018b). Short Trail Running Race: Beyond the Classic Model for Endurance Running Performance. Medicine and science in sports and exercise, 50(3), 580–588.

Fouré, A., Besson, T., Stauffer, E., Skinner, S. C., Bouvier, J., Féasson, L., Connes, P., Hautier, C. A., & Millet, G. Y. (2022). Sex-related differences and effects of short and long trail running races on resting muscle-tendon mechanical properties. Scandinavian journal of medicine & science in sports, 32(10), 1477–1492.

Froyd, C., Beltrami, F. G., & Noakes, T. D. (2018). Neuromuscular Fatigue at Task Failure and During Immediate Recovery after Isometric Knee Extension Trials. Sports (Basel, Switzerland), 6(4), 156.

Garnier, Y., Lepers, R., Assadi, H., & Paizis, C. (2020). Cardiorespiratory Changes During Prolonged Downhill Versus Uphill Treadmill Exercise. International journal of sports medicine, 41(2), 69–74.

Gathercole, R., Sporer, B., Stellingwerff, T., & Sleivert, G. (2015). Alternative countermovement-jump analysis to quantify acute neuromuscular fatigue. International journal of sports physiology and performance, 10(1), 84–92.

Hunter, S. K. (2016). The relevance of sex differences in performance fatigability. Medicine and science in sports and exercise, 48(11), 2247.

Hunter, S. K. (2018). Performance fatigability: mechanisms and task specificity. Cold spring harbor perspectives in medicine, 8(7), a029728.

Keeton, R. B., & Binder-Macleod, S. A. (2006). Low-frequency fatigue. Physical therapy, 86(8), 1146-1150.

Marco-Contreras, L. A., Bachero-Mena, B., Rodríguez-Rosell, D., & González-Badillo, J. J. (2021). Load Index and Vertical Jump to Monitor Neuromuscular Fatigue in an Elite 800-m Athlete. International journal of sports physiology and performance, 16(9), 1354–1358.

Martin, V., Millet, G. Y., Lattier, G., & Perrod, L. (2005). Why does knee extensor muscles torque decrease after eccentric-type exercise?. The Journal of sports medicine and physical fitness, 45(2), 143–151.

Millet, G. Y., Martin, V., Lattier, G., & Ballay, Y. (2003). Mechanisms contributing to knee extensor strength loss after prolonged running exercise. Journal of applied physiology (Bethesda, Md. : 1985), 94(1), 193–198.

Millet, G. Y., Tomazin, K., Verges, S., Vincent, C., Bonnefoy, R., Boisson, R. C., ... & Martin, V. (2011). Neuromuscular consequences of an extreme mountain ultra-marathon. PloS one, 6(2), e17059.

Navalta JW, Montes J, Tanner EA, Bodell NG, Young JC. Sex and Age Differences in Trail Half Marathon Running. Int J Exerc Sci. 2018 May 1;11(6):281-289.

Phothirook P, Amatachaya S, Peungsuwan P. Muscle Activity and Co-Activation of Gait Cycle during Walking in Water and on Land in People with Spastic Cerebral Palsy. International Journal of Environmental Research and Public Health. 2023; 20(3):1854.

Scheer, V., Janssen, T. I., Vieluf, S., & Heitkamp, H. C. (2018). Predicting Trail Running Performance With Laboratory Exercise Tests and Field Based Results. International journal of sports physiology and performance, 1–13. Advance online publication.

Sundby, O. H., & Gorelick, M. L. (2014). Relationship between functional hamstring: quadriceps ratios and running economy in highly trained and recreational female runners. Journal of strength and conditioning research, 28(8), 2214–2227.

Temesi, J., Besson, T., Parent, A., Singh, B., Martin, V., Brownstein, C. G., Espeit, L., Royer, N., Rimaud, D., Lapole, T., Féasson, L., & Millet, G. Y. (2021). Effect of race distance on performance fatigability in male trail and ultra-trail runners. *Scandinavian journal of medicine & science in sports*, *31*(9), 1809–1821.

Vercruyssen, F., Gruet, M., Colson, S. S., Ehrstrom, S., & Brisswalter, J. (2017). Compression Garments,

Muscle Contractile Function, and Economy in Trail Runners. International journal of sports physiology and performance, 12(1), 62–68.

Wan, J. J., Qin, Z., Wang, P. Y., Sun, Y., & Liu, X. (2017). Muscle fatigue: general understanding and treatment. *Experimental & molecular medicine*, *49*(10), e384.

Zimmermann, P., Müller, N., Schöffl, V., Ehrlich, B., Moser, O., & Schöffl, I. (2022). The Energetic Costs of Uphill Locomotion in Trail Running: Physiological Consequences Due to Uphill Locomotion Pattern-A Feasibility Study. Life (Basel, Switzerland), 12(12), 2070.