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CENTRAL AND PERIPHERAL CONTRIBUTIONS TO NEUROMUSCULAR FATIGUE INDUCED BY A 24-HOUR TREADMILL RUN

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ABSTRACT

This experiment investigated the fatigue induced by a 24-hour running exercise (24 30 TR) and particularly aimed at testing the hypothesis that the central component would be the main mechanism responsible for neuromuscular fatigue. Neuromuscular function evaluation was performed before, every 4 hours during and at the end of the 24TR on 12 experienced ultra-marathon runners. It consisted of a determination of the maximal voluntary contractions (MVC) of the knee extensors (KE) and plantar flexors (PF), the maximal voluntary activation (%VA) of KE and PF, the maximal compound muscle action potential amplitude (M_{max}) on soleus and vastus lateralis. Tetanic stimulations were also delivered to evaluate the presence of low frequency fatigue, and the KE maximal muscle force production ability. Strength loss occurred throughout the exercise, large changes being observed after 24TR in MVC for both the KE and PF muscles (-40.9 ± 17.0% and - 30.3 ± 12.5%, respectively; $P < 0.001$) together with marked reductions of % VA (-33.0 ± 21.8% and -14.8 ± 18.9%, respectively; $P < 0.001$). A reduction of M_{max} amplitude was observed only on soleus and no low frequency fatigue was observed for any muscle group. Finally, KE maximal force production ability was reduced to a moderate extent at the end of the 24TR (-10.2%; $P < 0.001$) but these alterations were highly variable (± 15.7%). These results suggest that central factors are mainly responsible for the large maximal muscle torque reduction after ultra-endurance running, especially on the KE muscles. Neural drive reduction may have contributed to the relative preservation of peripheral function and also affected the evolution of the running speed during 48 the 24TR.

Key words: activation level, M wave, low frequency fatigue, ultra-marathon

INTRODUCTION

Muscle fatigue is an exercise-related decrease in the maximal voluntary force or power of a muscle or muscle group (3) associated with an increase in the perceived effort necessary to exert the desired force (9). This decline potentially involves processes at all levels of the motor pathway from the brain to the skeletal muscle. The typical strategy used to study fatigue has been to determine whether the mechanism responsible for fatigue is located in the exercising muscle or in the nervous system. This approach has resulted in the differentiation between central, *i.e.* nervous, and peripheral, *i.e.* muscle, fatigue (*e.g.* 9).

The mechanisms underlying the decline in maximal force capacity depend on the characteristics of the task being performed. Critical task variables include the muscle activation pattern, the type of muscle group involved and, the type of muscle contraction (9). However, the intensity and duration of activity are probably among the most important factors. We previously established that low intensity, prolonged running exercise induces a significant amount of central fatigue (for review see 25). Running duration seems to determine the amount of central fatigue: average central activation deficits were found to be –8% and –28% after competitive running bouts of 3h and 8.5h, respectively (26-27).

Whether central activation deficit is linearly related to running duration remains unknown. In their review related to neuromuscular fatigue, Millet and Lepers (25) proposed a non linear relationship for strength loss – exercise duration: as running duration increases, force decrement would tend to plateau. This could represent the influence of a central protective mechanism, aimed at limiting muscle work during prolonged running, to prevent extensive homeostasis disturbance, muscle damage and biological harm (29). Ultra-endurance running, *i.e.* any distance greater than that of a marathon (*e.g.* 100 km, 24h), constitutes an interesting paradigm to investigate this possibility. Indeed, that kind of exercise is challenging for the homeostasis, energetic and muscular systems and may therefore be able to trigger central

77 protective mechanisms. Ohta *et al.* (33) investigated biochemical modifications during a 24-
78 hour run and from their clinical observations concluded that this type of exercise induces
79 some supraspinal fatigue. Therefore, we can reasonably hypothesize that ultra-endurance
80 running may induce a significant level of central fatigue. Reductions of voluntary activation
81 for exercise durations of ~8.5h (26) for field studies and 5h for systematic laboratory studies
82 (37), support this hypothesis. However, an extreme running duration is the model required to
83 definitely challenge the idea that the intrinsic force generating capacity of the muscle is not
84 dramatically impaired after such task, and that central mechanisms are mainly responsible for
85 neuromuscular fatigue.
86 The implication of a central mechanism should confine peripheral fatigue to a moderate level.
87 Current knowledge on the origins of peripheral fatigue after endurance exercise suggests that
88 such exercise could impair three main components: the action potential transmission along the
89 sarcolemma, the excitation-contraction coupling (E-C) *i.e.* the release and reuptake of calcium
90 (Ca^{2+}) within the muscle cell, and the actin-myosin interaction (25). None of these
91 mechanisms have been assessed for exercises of extreme duration such as a 24h run.
92 However, available evidence suggests that intrinsic muscle force is moderately reduced (-
93 10%) after a 30-km trail run (27). Also, studies on prolonged running (8, 27, 37) have failed
94 to detect low frequency fatigue (LFF), which has been linked to E-C coupling alteration and
95 muscle damage (16, 18). This was unexpected since many studies have provided indirect
96 evidence of muscle damage after prolonged running (10, 34). Finally, evidence for the
97 occurrence of action potential transmission alteration after prolonged running is rather scarce
98 (25). Therefore, we can reasonably suggest that extreme duration running exercise may
99 induce a moderate peripheral fatigue.
100 Whether neuromuscular fatigue similarly affects locomotor muscles from the lower limbs
101 during ultra-endurance running remains unclear. Factors such as muscle fiber composition,

running technique and running course profile (*i.e.* level *vs.* uphill and downhill) could differentially influence the magnitude of strength loss on knee extensors (KE) and plantar flexors (PF). In particular, the relative contributions of these muscle groups to power production during slow running may influence their fatigue responses. Winter (43) reported that the positive work done by the PF averaged three times that done by the KE during slow level running. This is consistent with the proposition of Novacheck (30), who reviewed the biomechanics of running and concluded that the relative contributions of the PF and KE to power generation changes such that relatively more power is generated proximally as speed increases. At slow running speeds, the PF would then produce relatively more power than the KE. Data from glycogen depletion studies also confirm that the plantar flexors are more active than knee extensors during level running (20). In light of the above mentioned findings, we suggest that level ultra-endurance running would place a greater stress on PF as compared to KE. As a consequence, greater force decrements could be expected to occur in the PF, since the 24-hour running exercise was performed on a treadmill with no slope (24TR).

Therefore, the purpose of this experiment was to test the hypothesis that central fatigue would be the principal explanation for neuromuscular fatigue during a 24-hour running bout, and that this would minimize the extent of peripheral fatigue. The secondary purpose was to verify the assumption that PF muscles fatigue more than KE during level ultra-endurance running.

MATERIAL AND METHODS

Subjects

Twelve healthy male subjects (age: 41.6 ± 7.7 y; height: 1.78 ± 0.05 m; mass: 74.8 ± 7.4 kg; body fat: 17.9 ± 4.6%; $\dot{V}O_2$max 52.0 ± 6.2 ml · kg^{-1} · min^{-1}) were enrolled in this study after medical examination. Fourteen subjects were initially recruited but only 12 were able to complete the 24TR. All the participants were experienced ultra-marathon runners and had already run a race longer than 24 hours or greater than 100 km. On average, they had 15.3 ± 7.1 years of training history in running and 7.1 ± 4.4 years of ultra-endurance experience. The subjects were asked to refrain from strenuous exercise during the week preceding the 24TR. Force production capacity was also assessed in a control group of 12 physically active subjects (age: 34.2 ± 9.6 y; height: 1.77 ± 0.03 m; mass: 73.3 ± 6.2 kg) who did not run but stayed awake over the 24 hour period. The experiment was conducted according to the Declaration of Helsinki. The participants were fully informed of the procedure and the risks involved and gave their written consent. They were also allowed to withdraw from the study at will. Approval for the project was obtained from the local ethics committee (Comité de Protection des Personnes Sud-Est 1, France) and registered on http://clinicaltrial.gov (# NCT 00428779).

Experimental design

The participants came in 3 to 4 weeks before the experiment for a medical examination, including determination of body mass, height and percentage of body fat (skinfold thickness measurements). The subjects performed a maximal test on a motorized treadmill (Gymrol S2500, HEF Tecmachine, Andrezieux-Boutheon, France), that aimed at determining anaerobic threshold, maximal oxygen uptake ($\dot{V}O_2$max) and the velocity associated with $\dot{V}O_2$max ($V_{\dot{V}O_2max;}$ see Millet al. (24) for exact protocol). During this first visit, the subjects

were also fully informed regarding the experimental procedures. Particular attention was paid to familiarizing them with the maximal voluntary contractions (MVC) and electrical stimulation of the KE and PF muscles. The subjects repeated trials of the procedures until they were able to produce consistent results.

During the 24TR session, neuromuscular function was evaluated before (PRE), every 4 hours during and at the end (POST) of the 24-hour treadmill run to describe the progress of fatigue throughout the protocol. Neuromuscular evaluation consisted in determining the isometric MVC of KE and PF to provide a global index of fatigue. Maximal voluntary activation levels for KE and PF, as well as maximal vastus lateralis (VL) and soleus (SOL) electromyographic (EMG) activities normalized to the M-wave amplitudes were evaluated to evidence central fatigue. Finally, a superimposed tetanus (for KE only), and single and multiple electrical stimulations were delivered to the relaxed muscle to determine the extent and origin of peripheral fatigue. The measurements were conducted first on KE and then on PF.

The control group only performed the KE MVC trials and did not sleep during the experiment.

Protocol

The running exercise started between 4:30 and 6 p.m. and ended 24 hours later. The protocol is described in figure 1. Test sessions (Figure 1A) were organized every 4 hours. The ultra-marathon runners exercised on a calibrated level motorized treadmill (Gymrol S2500, HEF Tecmachine, Andrezieux-Boutheon, France and ProForm 585 Perspective, Health & Fitness Inc., Logan, UT) in the laboratory at a freely chosen pace (slope = 0%). The subjects were instructed to choose their speed and ask the investigator to set it. The speed could be modified at any time during the 24TR as in a normal 24-hour race. To avoid any influence of hypoglycaemia and hyperthermia on the development of central fatigue (31-32), the runners

were cooled with fans and fed ad libitum with meals containing mainly carbohydrates, energy bars and drinks. The food and water intake during the 24TR was recorded to ensure there was no major problem of energy intake during the experiment. This was checked 'live' by an experienced investigator.

Insert Figure 1 here

Experimental setting

The neuromuscular function evaluation was based on the measurements summarized in Figure 1B.

Torque measurements

For both muscle groups, the isometric contractions performed during the experiment included maximal voluntary contractions (MVC) and electrically evoked contractions. During all the MVCs, the subjects were strongly encouraged. For the KE testing, the subjects were seated in the frame of a Cybex II (Ronkonkoma, NY) and Velcro straps were strapped across the chest and hips to avoid lateral and frontal displacements. Subjects were also instructed to grip the seat during the voluntary contractions to further stabilize the pelvis. The KE muscles mechanical response was recorded with a strain gauge (SBB 200 Kg, Tempo Technologies, Taipei, Taiwan) located at the level of the external malleolus. Torque values were obtained from force measured by the strain gauge multiplied by the lever arm, *i.e.* knee-malleolus distance. All measurements were taken from the subject's right leg, with the knee and hip flexed at 90 degrees from full extension. MVC of the PF muscles was evaluated with a dynamometric pedal (Captels, Saint Mathieu de Treviers, France). For the PF testing, subjects were seated on an inclined bench, attached to the dynamometric pedal (see (38) for details). The hip, knee and ankle angles were set at 90 degrees from full extension. Velcro straps were also used to limit heel lift, hip extension and trunk movement. The isometric contractions

performed during the experiment included maximal voluntary and electrically evoked contractions. During all the MVCs, the subjects were strongly encouraged.

Electrical stimulation

After femoral (for KE) and posterior tibial nerve (PF) detection with a ball probe cathode pressed into either the femoral triangle (KE) or the popliteal fossa (PF), electrical stimulation was applied percutaneously to the motor nerve via a self-adhesive electrode pressed manually (10-mm diameter, Ag-AgCl, Type 0601000402, Contrôle Graphique Medical, Brie-Comte-Robert, France). The anode, a 10 × 5 cm self-adhesive stimulation electrode (Medicompex SA, Ecublens, Switzerland), was located either in the gluteal fold (for KE) or on the patella (for PF). A constant current stimulator (Digitimer DS7A, Hertfordshire, United Kingdom) was used to deliver a square-wave stimulus of 1000 μs duration with maximal voltage of 400 V. The optimal stimulation intensity (range: 25 mA to 72 mA on KE and 31 mA to 66 mA on PF) was determined from maximal twitch torque measurement (see below).

Percutaneous muscular stimulations were also given via self-adhesive electrodes (Medicompex SA, Ecublens, Switzerland) connected to a high-voltage stimulator set to deliver submaximal stimulations at high (80 Hz) and low (20 Hz) frequencies. This method was preferred to nerve stimulation because it is less painful during tetanic stimulation and its validity for the evaluation of LFF has been established (22). The subjects were instructed to relax while still seated and strapped. The positive electrodes (5 cm × 5 cm) were placed on the motor points of the vastus medialis and vastus lateralis (for KE), and the medial and lateral gastrocnemius muscles (for PF). The negative electrodes (10 cm × 5 cm) were placed over the upper part of the thigh for KE and over the proximal aspect of the gastrocnemii for PF. The stimulating electrodes were removed between each test session but their exact positions were marked on the skin. Two 0.5 s train stimulations separated by a 30 s rest interval were applied

at 80 and 20 Hz (respectively 41 and 11 stimuli). The intensity of stimulation (range: 40 mA to 65 mA on KE and 15 mA to 38 mA on PF) was initially set to reach 30 % of the MVC torque value at baseline when stimulating at 80 Hz. The same intensity was used for all test sessions.

Electromyographic recordings

The EMG signals of the right VL and SOL were recorded using bipolar silver chloride surface electrodes of 10-mm diameter (Type 0601000402, Contrôle Graphique Medical, Brie-Comte-Robert, France) during the MVC and electrical stimulation. The recording electrodes were taped lengthwise on the skin over the muscle belly following SENIAM recommendations (15), with an interelectrode distance of 25 mm. The position of the electrodes was marked on the skin so that they could be fixed in the same place should electrode replacement be required during the experiment. The reference electrode was attached to the patella (for VL EMG) or malleolus (for SOL EMG). Low impedance ($Z < 5$ kΩ) at the skin-electrode surface was obtained by abrading the skin with thin sand paper and cleaning with alcohol. Electromyographic signals were amplified (EISA 16-4, Freiburg, Germany) with a bandwidth frequency ranging from 10 Hz to 1 kHz (common mode rejection ratio = 90 dB, gain = 1,000) and simultaneously digitized together with torque signals using an acquisition card (DAQCard-6062E, National Instruments, Austin, TX), and the Imago software developed under Labview (National Instrument, Austin, TX). The sampling frequency was 2000 Hz.

Experimental variables and data analysis

M-wave

The optimal intensity of stimulation was set by progressively increasing the stimulus intensity until the maximal isometric twitch torque was reached. Three stimuli at supramaximal intensity (1.2 times the maximum M-wave stimulus intensity; range: 25 mA to 72 mA on KE and 31 mA to 66 mA on PF) were then delivered and the mean value of the three recorded M-waves was taken as the M_{max} value. This procedure was conducted on KE and PF. The same intensity was used over the whole experiment for a given subject. For data analysis, M-wave peak-to-peak amplitude and duration were considered. The M_{max} value was further used to normalize the maximal voluntary Root Mean Square (RMS) EMG ($RMS \cdot M_{ampl}^{-1}$; see below) on VL and SOL.

Mechanical responses to nerve stimulation

The amplitude of the potentiated twitch peak torque (Pt) that followed the first MVC was determined for KE and PF (see figure 1, panel B). To determine the maximal muscle force production ability, *i.e.* the true maximal force that can be produced by KE (27), a 0.3 s stimulation train (100 Hz) was delivered to the femoral nerve during the second MVC trial. The intensity of stimulation was the same as that used for M_{max}. The maximal absolute torque (MVC + superimposed evoked torque) was considered as the maximal muscle force production ability of KE (Figure 2, panel A). Maximal muscle force production ability could not be measured for PF because the first two subjects tested suffered from muscle cramps after the application of the stimulation train, so that this measurement was removed from the protocol.

Insert Figure 2 here

Low-to-high frequency ratio

The variable measured was the ratio of the torques induced by tetani delivered at low (20 Hz) and high (80 Hz) frequencies for both KE and PF.

Maximal voluntary contractions and maximal activation level

The subjects were asked to perform two MVCs of each muscle group for ~3 s separated by about 30-s. During the voluntary contractions, electrical stimulations were superimposed to evaluate the level of activation. The twitch interpolation technique (23) consisted in superimposing a single stimulation (supramaximal intensity) on the isometric plateau. A second stimulation (control twitch) was delivered to the relaxed muscle 1.5 s after the end of the contraction (Figure 2, panel B). This provided the opportunity to obtain a potentiated mechanical response and so reduce the variability in activation level (%VA) values. The ratio of the amplitude of the superimposed twitch over the size of the control twitch was then calculated to obtain %VA as follows:

$$\%\mathrm{VA} = \left[\frac{1 - \text{superimposed twitch}}{\text{control twitch}}\right] \times 100$$

The RMS values of the VL and SOL EMG activity and average torque level were calculated during the MVC trials over a 0.2 s period after the torque had reached a plateau and before the superimposed stimulation was evoked. This RMS value was then normalized to the maximal peak-to-peak amplitude of the M-wave ($RMS \cdot M_{ampl}^{-1}$).

Blood samples

Peripheral venous blood samples were taken from an antecubital vein of participants before,

every 4 hours during and after completing the 24TR. Samples were drawn into nonadditive tubes under sterile conditions. Serum was separated from whole blood by centrifugation at 1.000 g for 10 min at room temperature. Plasma levels of myoglobin (Mb), creatine phospho-kinase (CK), sodium and potassium were measured using an auto-analyzer (ADIVA 1650, Bayer, PA).

Perceived exertion

Every 2 hours, running speed was set to 8 $km.h^{-1}$ during 4 minutes. At the end of this period, rating of perceived exertion (RPE) was measured with the Borg RPE scale (4).

Statistics

All descriptive statistics presented in the text are mean values ± SD. Normal distribution was checked using a Shapiro-Wilk test of normality. Each study variable was then compared between the different instances using a 1-way (time) analysis of variance (ANOVA) with repeated measures. A 2-way (time × group) analysis of variance with repeated measures was performed for the variable measured in the control and experimental groups, *i.e.* KE MVC. Newhman-Keuls *post-hoc* tests were applied to determine between-means differences if the analysis of variance revealed a significant main effect for any factor or interaction. Pearson's product-moment correlation coefficients were also calculated for the following variables pairs: PRE-POST KE %VA variation *vs.* PRE-POST PF %VA variation; POST [CK] values *vs.* POST [Mb] values; POST [CK] values *vs.* POST relative values (%PRE) for maximal absolute torque. For all statistical analyses, a P value of 0.05 was accepted as the level of significance. Data presented in figures 3 to 8 are normalized to corresponding baseline values and expressed as percentages (mean ± SE).

RESULTS

Running performance, perceived exertion and maximal voluntary contraction

The effective running time averaged 18 hours 39 minutes (± 41 min) for an average distance of 149.2 ± 15.7 km. Average running speed, computed over 4-hour periods, displayed a progressive decrease during the exercise ($P < 0.001$; Figure 3). Overall, the average running speed represented 39 ± 4 % of $\dot{V}O_{2\,max}$. Speed declined regularly from the start of the exercise to 16h, but the first significant decrease occurred 8h after the start ($P < 0.001$) and declined continuously until 16h. Thereafter, average running speed remained constant.

RPE displayed the opposite pattern, *i.e.* increased regularly until 16h as compared to baseline ($P < 0.001$) and then tended to plateau. The first significant increase was observed 2h after the start ($P < 0.01$).

Insert Figure 3 here

MVC declined regularly during the exercise on both PF and KE muscles ($P < 0.001$, Figure 4, panels B & C). On KE muscles, data varied as a function of time and group (time × group interaction, $P < 0.001$). In the experimental group, when compared to baseline values (KE: 230 ± 40 N.m; PF: 174 ± 45 N.m), the first significant decline occurred at 8h (KE: 176 ± 45 N.m; $P < 0.001$; PF: 144 ± 41 N.m ; $P < 0.01$); MVC was then further reduced at 20h (KE: 143 ± 56 N.m; PF: 113 ± 35 N.m; $P < 0.05$) and POST (KE: 136 ± 49 N.m; PF: 118 ± 25 N.m; $P < 0.01$) as compared to 8h. At the end of the 24TR, MVC reductions from baseline reached -30.3 ± 12.5% and -40.9 ± 17.0% for PF and KE, respectively ($P < 0.001$). Torque decrements were significantly higher for KE as compared to PF ($P < 0.05$). Two-way analysis of variance with repeated measures for KE MVC revealed that KE MVC did not vary significantly during the 24-hour period in the control group (Figure 4, panel A). Therefore, KE MVC was

significantly higher than in the experimental group from 8h to POST ($P < 0.01$ at 8h; $P < 0.001$ from 12h to POST).

Insert Figure 4 here

Activation level

Variables related to nervous activation displayed a progressive decline throughout the exercise. On KE muscles, %VA declined regularly during the exercise ($P < 0.001$, Figure 5, panel A). When compared to baseline values (88 ± 9%), the first significant decline was observed at 8h (73 ± 15%; $P < 0.01$). At POST, %VA values (59 ± 20%) were further reduced as compared to 8h ($P < 0.001$) and the final decrement reached – 33.0 ± 21.8%. EMG data further confirmed the progressive reduction of nervous activation: VL $RMS \cdot M_{ampl}^{-1}$ during the MVC was reduced at 8h ($P < 0.001$) and then further declined at POST (-46.1 ± 16.4%, $P < 0.05$).

The development of central alterations was less pronounced in the PF muscle group. Although %VA declined progressively from baseline (97 ± 4%) during the exercise ($P < 0.001$), these alterations only became significantly different at 16h (84 ± 14%) and were maintained depreciated until POST (83 ± 20%; $P < 0.01$, Figure 5, panel B). After the 24TR, the PF %VA decrement was about half (-14.8 ± 18.9%) as compared to the %VA decrement measured on the KE muscles. Nevertheless, %VA variations between PRE and POST were significantly correlated between KE and PF ($R = 0.51$; $P < 0.001$). EMG data were less clear. Although a tendency to a gradual decline during exercise was observed, analysis of variance did not reveal any statistical difference.

Insert Figure 5 here

Single twitch

Tables 1 and 2 display the results of mechanical and EMG responses to a single electrical stimulation of the femoral and tibial motor nerves. Potentiated Pt decreased continuously until 16h for KE and 12h for PF. The values then stabilized during the second part of the event (12h-16h to POST). Final twitch peak torque reductions were similar for KE and PF (– 25% *vs.* – 23%, respectively). The VL M-wave showed slight but non-significant changes in amplitude at 12h. Conversely, the SOL M-wave amplitude began to decrease at 4h ($P < 0.01$) and remained reduced until the end of the 24TR ($P < 0.001$). The peak-to-peak duration of the VL and SOL M-waves did not change over the 24TR.

Trains of stimuli

The low-to-high frequency ratio remained unchanged over the 24TR for both KE and PF (range: 68% to 72%; Figure 6). The decrease in maximal absolute torque from baseline (242 ± 33 N.m) is shown in Figure 7. The alteration was significant at 8h (231 ± 33 N.m; $P < 0.01$), further decreased until 16h (216 ± 32 N.m; $P < 0.001$) and stayed at a similar level until the end of the 24TR (216 ± 43 N.m ; $P < 0.001$). The final decrement averaged -10.2 ± 15.7% ($P < 0.001$). A broad range of inter-individual responses was observed for this variable (Figure 8, panel B).

Insert Figures 6 & 7 here

Blood analysis

Plasma potassium and sodium concentration remained stable throughout the 24TR. Conversely, there was considerable variation in [CK] responses between subjects, ranging POST-24TR from 812 to 42,711 IU $\cdot$ l^{-1} (Figure 8, panel A) with an average value of 13319 IU $\cdot$ l^{-1}. The [Mb] response was similarly broad, ranging POST-24TR from 129 to 7014 µg $\cdot$ l^{-}

^{1} with an average value of 2,035 µg · l^{-1}. These two indexes of muscle damage were correlated at POST ($R = 0.90$; $P < 0.001$). Interestingly, POST-24TR [CK] values were slightly but significantly correlated with the POST-24TR relative values for maximal absolute torque ($R = -0.65$; $P < 0.05$; Figure 8, panel B).

Insert Figure 8 here

DISCUSSION

The main purpose was to test the hypothesis that central fatigue would be the principal explanation for neuromuscular fatigue during a 24-hour running bout, and that this would minimize the extent of peripheral fatigue. The results confirmed this hypothesis since large central activation deficits were observed, especially on the KE muscles. As expected, the extent of peripheral fatigue was moderate since no low-frequency fatigue was observed on any muscle group, the decline of KE maximal muscle force production ability was confined to a low level, and M-wave alterations were only observed on PF muscles. The present experiment also describes for the first time the development of central and peripheral fatigue appearance on a simulated ultra-marathon, showing that the muscle alterations were limited to the first part (12-16 hours) of the event. The secondary purpose of this experiment was to verify the assumption that PF muscles would fatigue more than KE during level ultra-endurance running. Although some M-wave alterations were observed only on PF, MVC was altered to a larger extent on the KE as compared to the PF muscles, therefore rejecting the initial hypothesis. Overall, this study shows that the etiology and amplitude, but not the evolution of the decrease in maximal strength capacity of the locomotor muscles after ultra-endurance running are dependent on the muscle group under consideration but that fatigue is mainly due to central alterations.

Torque impairment

The torque decrements reported in the current study are in accordance with the literature. Despite the flat terrain, strength loss is larger than those reported for shorter running exercises. Millet et al. (26) reported a 28% reduction of KE MVC after a running bout of 8.5 hours. In their literature review, Millet and Lepers (25) also referred to a reduction of 34% after a running exercise lasting 18.4 hours (unpublished data). Here, the KE MVC decrement averaged ~ 41% over the 24TR. This value agrees with the non linear relationship for strength loss – exercise duration proposed by Millet and Lepers (25) for running exercises of sufficient duration that the anaerobic metabolism does not play a significant role: as running duration increases, force loss first dramatically increases for exercise durations of 2 to 5 hours, and then tends to plateau for extreme exercise durations. The shape of this relationship could reflect the involvement of protective mechanisms brought into play to avoid extensive muscle damage, homeostasis disturbances and thus biological harm (29).

There is less information available on the evolution of PF MVC after endurance running, especially for running durations above 3 hours. MVC reductions after 1h30-2h30 flat running exercises have been reported between 11% and 18% (35, 38 , 40). The PF MVC impairment (~ 30%) of the present study seems to agree with these findings but is in contradiction with the results of Avela et al. (1) who observed a drop of 30% after a marathon run completed in ~3 hours. However, Avela et al. measured MVC while the blood flow was occluded with a pressure cuff, to avoid any recovery of metabolic fatigue during the measurements. This was not the case in the other studies mentioned above and in the present one. This procedure may have affected the amplitude of MVC reduction after the exercise.

One could also suggest that circadian rhythms and sleep deprivation would have influenced maximal strength (6, 12). Indeed, the 4^{th} to 12^{th} hours period of exercise corresponded to night-time and the torque decrement in the first half of the run tended to be larger than in the

second half (Figure 4). This may have resulted from the combined influence of fatigue and circadian rhythms. Although MVC did not vary significantly in the control group, it tended to decrease from 4h to 12h and to increase in the second part of the experiment (*i.e.* during day time) with a return to baseline values at POST. Therefore, it can reasonably be suggested that MVC kinetics observed in the runners were influenced mainly by fatigue, and only slightly by circadian rhythms.

Several studies have shown that the PF muscles are more active than KE during slow level running (20, 30, 43), leading to the hypothesis that larger force decrements would occur in PF than in KE. Since the decrease in PF MVC was ~30% *vs.* about 41% in KE, it can be suggested that KE was less resistant to fatigue than PF, maybe due to a lower percentage of type I fibers. These results are in agreement with those of Petersen et al. (35), who reported MVC decrements after a marathon of 17 and 22% for PF and KE, respectively. The relative contribution and fatigue of PF and KE muscles probably also depend on the runner's level of performance and training background or the runner's technique.

Central fatigue

The large central fatigue observed in the current study agrees well with previous data observed on the KE muscles after an ultra-marathon (26). The occurrence of central fatigue on the PF muscles after ultra-endurance running is an original finding. Although the amplitude of central drive reduction was lower for the PF muscles, there was a significant correlation between %VA changes for KE and PF. This result could reflect the existence of a common central mechanism aimed at reducing neural drive to the working muscles to limit the level of exhaustion (29). This safety mechanism may nevertheless be activated by peripheral feedback from muscle afferents directly at the supraspinal level (42) or at the spinal level. From a functional point of view, this mechanism may have contributed to preserve peripheral

function and may also have affected the evolution of the running speed and RPE during the 24TR. Of note is the fact that hyponatremia, which would have a large central effect on performance as athletes become progressively more disorientated, was not implicated in central fatigue.

Peripheral fatigue

Sarcolemmal propagation

The M-wave amplitude of VL did not change significantly, although the broad range of inter-individual responses could have concealed a latent tendency. Similar results were found after an ultra-endurance trail event (26). Conversely, a decrease in the M-wave amplitude of VL was found after shorter running exercises such as a 30 km run (27) or a 5 h treadmill run (37). Both the accumulation of intracellular Na^+ and the loss of muscle K^+ to the extracellular compartments from contracting muscles depend on the intensity of the work performed (7, 28), especially at low-to-moderate intensities (13). Contrary to measurements obtained after a 100 km run (34), no significant change in plasma [K^+] and [Na^+] was observed during the 24TR in the present study. Therefore, we suggest that in the current study, the exercise intensity was too low to cause marked ionic imbalance that would disturb muscle membrane excitability on the VL muscle. Although M-wave amplitude was unchanged on the VL muscle, a significant reduction was observed on the SOL muscles. These findings are consistent with those of Behm and St-Pierre (2), who showed that PF were more susceptible to M-wave amplitude reductions than KE muscles.

Lack of low-frequency fatigue

In line with previous studies on prolonged running, although not as extreme as in the present experiment (8, 27, 37), LFF was not observed for KE during the 24TR. Another original

finding of the present study is that no LFF was detected for the PF muscles, as for the KE muscles. It is then suggested that minimal exercise intensity is necessary to induce mechanical and metabolic disturbances that may promote the development of LFF (16-17). The low speed of the current exercise (9.0 to 7.2 $km.h^{-1}$) may have been insufficient to reach this threshold.

Intrinsic force and muscle damage

Since there was no change in the VL M-wave, no LFF and a moderate loss of maximal force production ability (~10%), average peripheral fatigue of the KE muscles appears very limited, with nevertheless a large inter-individual variability. Similar results were reported after a 30 km trail run (27). One limit of the present study is the fact that superimposed tetanus was performed only once at each instance due to the pain. As a result, two subjects reached 108% and 112% of initial values at the end of the 24TR (see Figure 8, panel B), especially because their baseline values were low. One may argue that the stimulation intensity was insufficient to be genuinely supramaximal throughout the experiment, *i.e.* notwithstanding potential changes in impedance over time, electrode-nerve position during contraction relative to rest, and axonal refractoriness. However, on the KE muscles, VL M-wave amplitude was not modified during the 24TR whereas twitch peak torque declined, suggesting that stimulation stayed maximal. To explain the increase in superimposed tetanus between PRE and POST in these two subjects, we rather suggest that the contraction of the antagonist muscles during superimposed tetanic stimulation may have influenced the results at baseline, despite the familiarization session.

The correlation reported in figure 9 suggests that the loss of maximal force production ability was partly influenced by the structural status of the fibers, as indirectly evidenced by [CK] activities. Nevertheless, as CK activities provide a gross indication of skeletal muscle injury, they do not inform on any relative degree of muscle damage or impairment of muscle function (11). In particular, the very long exercise duration in the present study may have accentuated

515 the accumulation of CK and Mb in the blood. Other factors, such as reduction in the force
516 produced by the active cross-bridges or modifications of the sensitivity of myofilaments to
517 Ca^{2+} (36) may also account for the reduction in maximal force production ability.
518 Because the nerve stimulation trains generated cramps on PF, it was unfortunately not
519 possible to evaluate the maximal force production ability of this muscle group. However,
520 twitch peak torque was depressed to the same extent on the PF and KE muscles. Although the
521 reduction of M-wave amplitude may partly explain the peripheral alterations, it can be
522 supposed that there would have been a loss of PF maximal force production ability since no
523 LFF occurred.
524
525 **Implications for ultra-endurance running performance**
526 The current results do not allow predicting directly the extent to which the fatigue
527 mechanisms identified during maximal isometric contractions would affect submaximal
528 muscle function during ultra-endurance running. It may nevertheless be speculated that the
529 relative level of muscle activation required for a constant running speed was progressively
530 increased, due to the reduction of maximal neural drive. In addition, peripheral fatigue implies
531 that higher muscle activation is required for a given mechanical power produced. As a
532 consequence, there was an increase of the subjective effort required for a given task (*i.e.*
533 running at 8 $km.h^{-1}$). Together with nociceptive feedback, this may eventually lead the subject
534 to reduce the speed, so that their relative level of activation during running would stay below
535 a maximal tolerated activation level. The progressive mismatch between perceived effort and
536 motor output, *i.e.* running speed, strongly suggests that central processes do impair some
537 aspects of ultra-endurance running performance (41).
538
539

A few limitations of our study must be noted. First, the stimulation intensity was 1.2 times optimal intensity rather than 1.5 times which further ensures supramaximality. We chose this intensity because it was better tolerated by the subjects during repetitive testing over the 24h-running exercise. A higher intensity may also increase the activation of the antagonist muscles for PF. This intensity (120%) was used in several studies investigating neuromuscular function alteration with fatigue (*e.g.* 14, 19, 21, 39). Another limitation is the possibility that axonal hyperpolarization may have affected our measurements (5) especially during the maximal evoked torque (MVC + superimposed tetanic train). This variable also needs to be interpreted with caution because antagonist co-activation may affect force development in this situation. This measurement should be interpreted as a relative indicator of muscle contractile status, rather than an absolute measure of maximal intrinsic force. In addition, axonal hyperpolarization could have preferentially depressed the high frequency response during tetanic muscle stimulation at submaximal intensity. Therefore, the absence of modification of the low-to-high frequency ratio could have resulted from the combined effects of LFF, which preferentially depresses low frequency response, and hyperpolarization, which preferentially depresses high frequency response. This limit is nevertheless present in all studies that have compared stimulations at low- and high-frequency to investigate the type of fatigue. Finally, the muscles were tested in the same order (KE then PF) which may have induced a small recovery for PF.

CONCLUSION

The purpose of this experiment was to investigate the development of fatigue over an extreme duration exercise, a unique occasion to study human physiology as it is stretched towards breaking point. More particularly, we aimed at testing the hypothesis that central fatigue would be the principal explanation for neuromuscular fatigue during a 24-hour running bout,

and that this would minimize the extent of peripheral fatigue. The results confirmed this hypothesis since large central activation deficits were observed, especially on the knee extensor muscles, as well as limited peripheral alterations. In addition to their relatively low amplitude, the muscle alterations were limited to the first part of the run. The disproportionate increase in the perceived effort during a slow running task strongly suggests that central fatigue did limit the performance during the 24-hour running bout. These findings add support to the theories stating that the central nervous system is mainly responsible for exercise limitation in humans even if exact relationships between central fatigue and teleo-anticipation mechanisms still have to be determined.

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FIGURE LEGENDS

Figure 1: General view of experimental testing during the 24 TR (panel A) and summary of the neuromuscular evaluation testing (panel B). For the plantar flexor muscles, the superimposed tetanus was replaced by a superimposed single twitch for cramping reasons.

Figure 2: Typical torque trace during the knee extensor maximal voluntary contraction and determination of the maximal force production ability (panel A) and maximal activation level (panel B) at baseline (black line) and after the 24-hour treadmill run (black dashed line).

On panel A, black and white arrows indicate the timing of delivery of the superimposed tetanus (0.3s, 100 Hz). On panel B, black arrows indicate the timing of delivery of a single stimulation.

Figure 3: Evolution of running speed (panel A) and rating of perceived exertion (panel B) during the 24 TR.

Speed values were averaged over 4-hour periods and are mean ± SE. Ratings of perceived exertion were collected every two hours. Values are mean ± SE. Vertical labels, on the right-hand side of the charts, indicate a significant effect of time revealed by analysis of variance (###: $P < 0.001$). Significance level of pairwise comparisons between different instances, revealed by post-hoc analysis, are indicated by horizontal brackets (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$).

Figure 4: Evolution of maximal voluntary contraction (MVC) impairment on the knee extensor muscles (KE) in the control group (panel A), in the experimental group (panel B) and on the plantar flexor muscles (PF; panel C) in the experimental group during the 24 TR.

Values are expressed as a percentage (± SE) of baseline value (PRE). Vertical labels, on the right-hand side of the charts, indicate a significant effect of time × group interaction revealed by analysis of variance ($$$: $P < 0.001$). Significance level of pairwise comparisons between different instances, revealed by post-hoc analysis, are indicated by horizontal brackets (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$). Significance level of pairwise

comparisons between the two groups at different instances, revealed by post-hoc analysis, are indicated by horizontal brackets (§§: $P < 0.01$; §§§: $P < 0.001$).

Figure 5: Evolution of the maximal activation level (%VA) of the knee extensor (KE; panel A) and plantar flexor (PF; panel B) muscles during the 24 TR.

Values are expressed as a percentage (± SE) of baseline value (PRE). Vertical labels, on the right-hand side of the charts, indicate a significant effect of time revealed by analysis of variance (###: $P < 0.001$). Significance level of pairwise comparisons between different instances, revealed by post-hoc analysis, are indicated by horizontal brackets (**: $P < 0.01$; ***: $P < 0.001$).

Figure 6. Changes in the low-to-high frequency torque ratio ($P_{20} \cdot P_{80}^{-1}$) for the knee extensors (KE, panel A) and plantar flexors (PF, panel B) over the 24-hour treadmill run. *N.S.* non-significant. Values are expressed as a percentage (± SE) of rest value (PRE).

Figure 7. Changes in maximal absolute torque of the knee extensor muscles (KE) over the 24TR.

Vertical labels, on the right-hand side of the chart, indicate a significant effect of time revealed by analysis of variance (###: $P < 0.001$). Significance level of pairwise comparisons between different periods, revealed by post-hoc analysis, are indicated by horizontal brackets (**: $P < 0.01$; ***: $P < 0.001$). Values are expressed as a percentage (± SE) of rest value (PRE).

Figure 8. Changes in creatine kinase activities (CK) over the 24TR (panel A) and relationship between the maximal absolute torque and CK values at the end (POST) of the 24TR (panel B).

Individual maximal absolute torque values are expressed as a percentage of rest value (PRE).

Table 1. Mean (± SD) twitch peak force of knee extensor (KE) and plantar flexor (PF) muscles.

Data are expressed in percentage of initial values (% PRE).

(% PRE)	4h	8h	12h	16h	20h	POST
KE potentiated	94 ± 7	92* ± 12	87*** ± 11	80*** ± 12	78*** ± 10	75*** ± 10
PF potentiated	87** ± 15	82*** ± 9	74*** ± 8	79*** ± 13	78*** ± 10	77*** ± 9

Significantly different from PRE: *** $P < 0.001$; ** $P < 0.01$ and * $P < 0.05$.

Table 2. Mean (± SD) M-wave characteristics of Vastus Lateralis (VL) and Soleus (SOL) muscles.

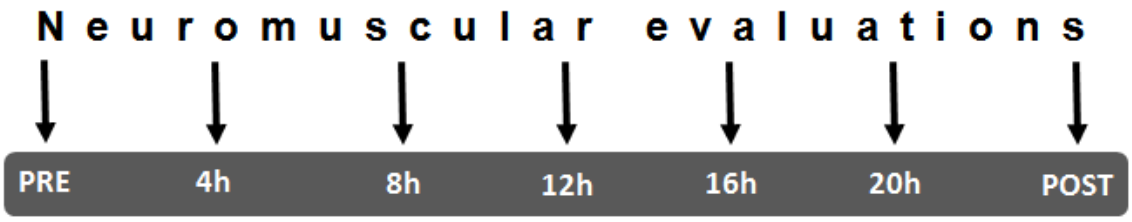
Data are expressed in percentage of initial values (% PRE).

	4h	8h	12h	16h	20h	POST
	Peak-to-peak Amplitude (% PRE)					
VL	89 ± 9	90 ± 24	83 ± 26	93 ± 23	100 ± 15	88 ± 24
SOL	77 ** ± 13	69 *** ± 25	72 *** ± 18	69 *** ± 23	68 *** ± 23	64 *** ± 22
	Peak-to-peak Duration (% PRE)					
VL	98 ± 8	103 ± 12	98 ± 5	99 ± 7	101 ± 15	96 ± 5
SOL	95 ± 10	99 ± 15	99 ± 15	99 ± 13	99 ± 15	102 ± 18

Significantly different from PRE: *** $P < 0.001$; ** $P < 0.01$ and * $P < 0.05$.

Figure 1

A.


Neuromuscular evaluations
PRE
4h
8h
12h
16h
20h
POST

B.

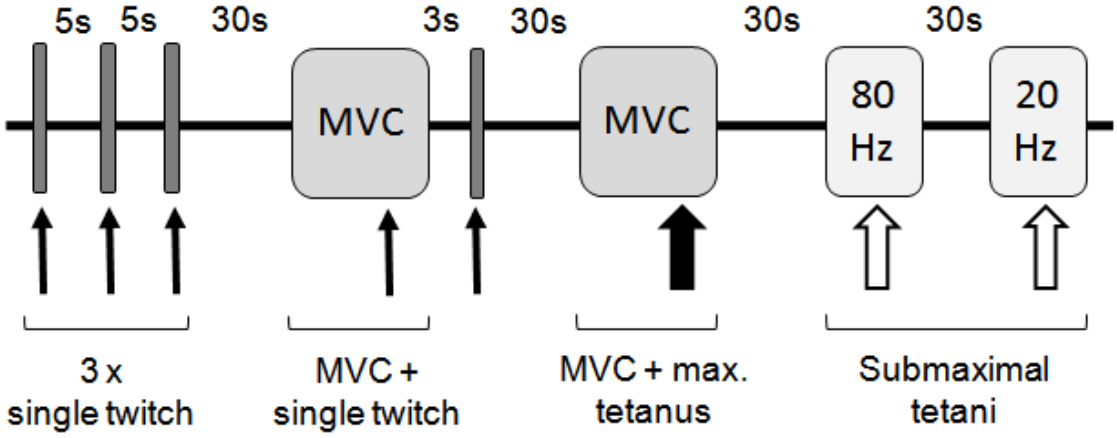

5s
5s
30s
3s
30s
30s
30s
MVC
MVC
80 Hz
20 Hz
3 x single twitch
MVC + single twitch
MVC + max. tetanus
Submaximal tetani

Figure 2

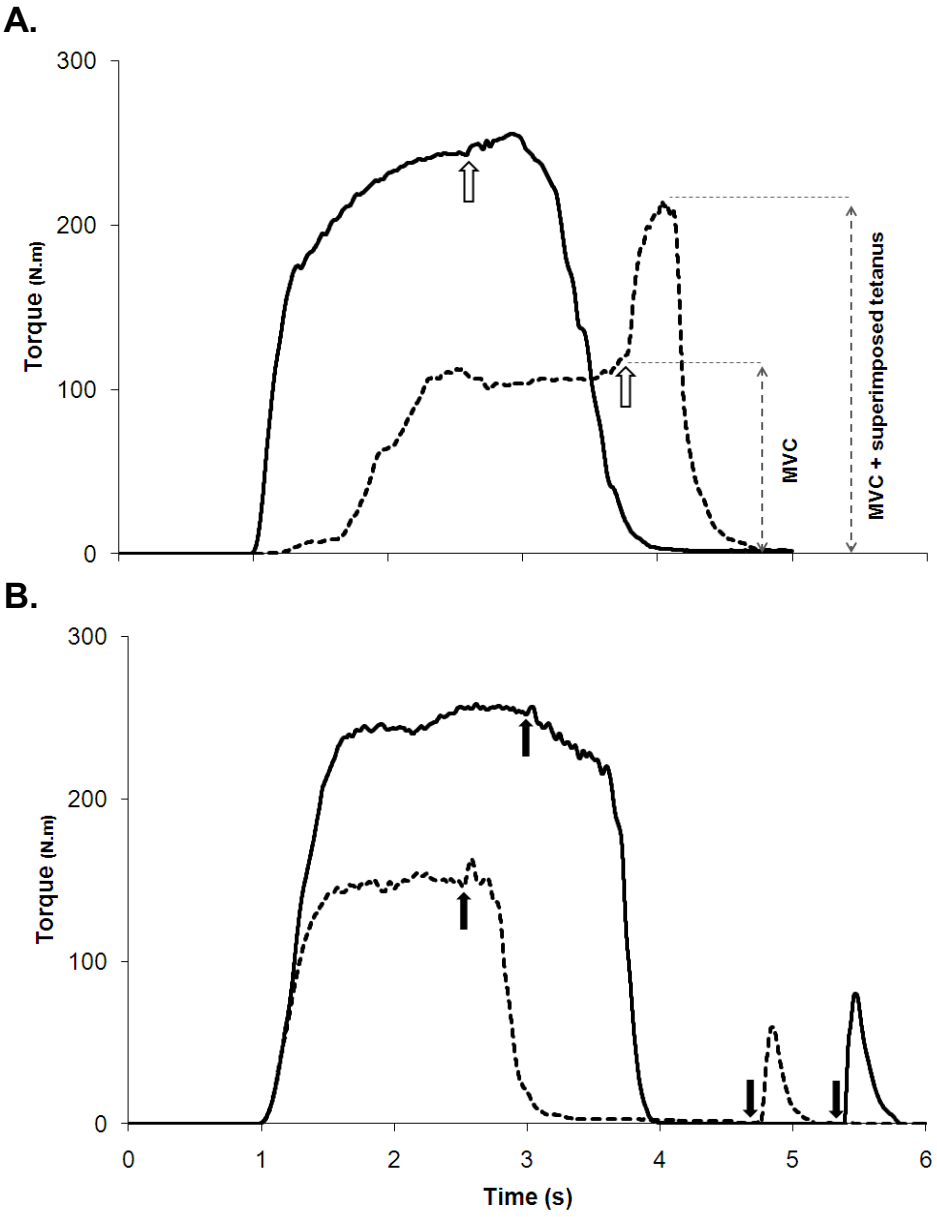

A.
300
200
100
0
Torque (N.m)
MVC
MVC + superimposed tetanus
B.
300
200
100
0
Torque (N.m)
0
1
2
3
4
5
6
Time (s)

Figure 3

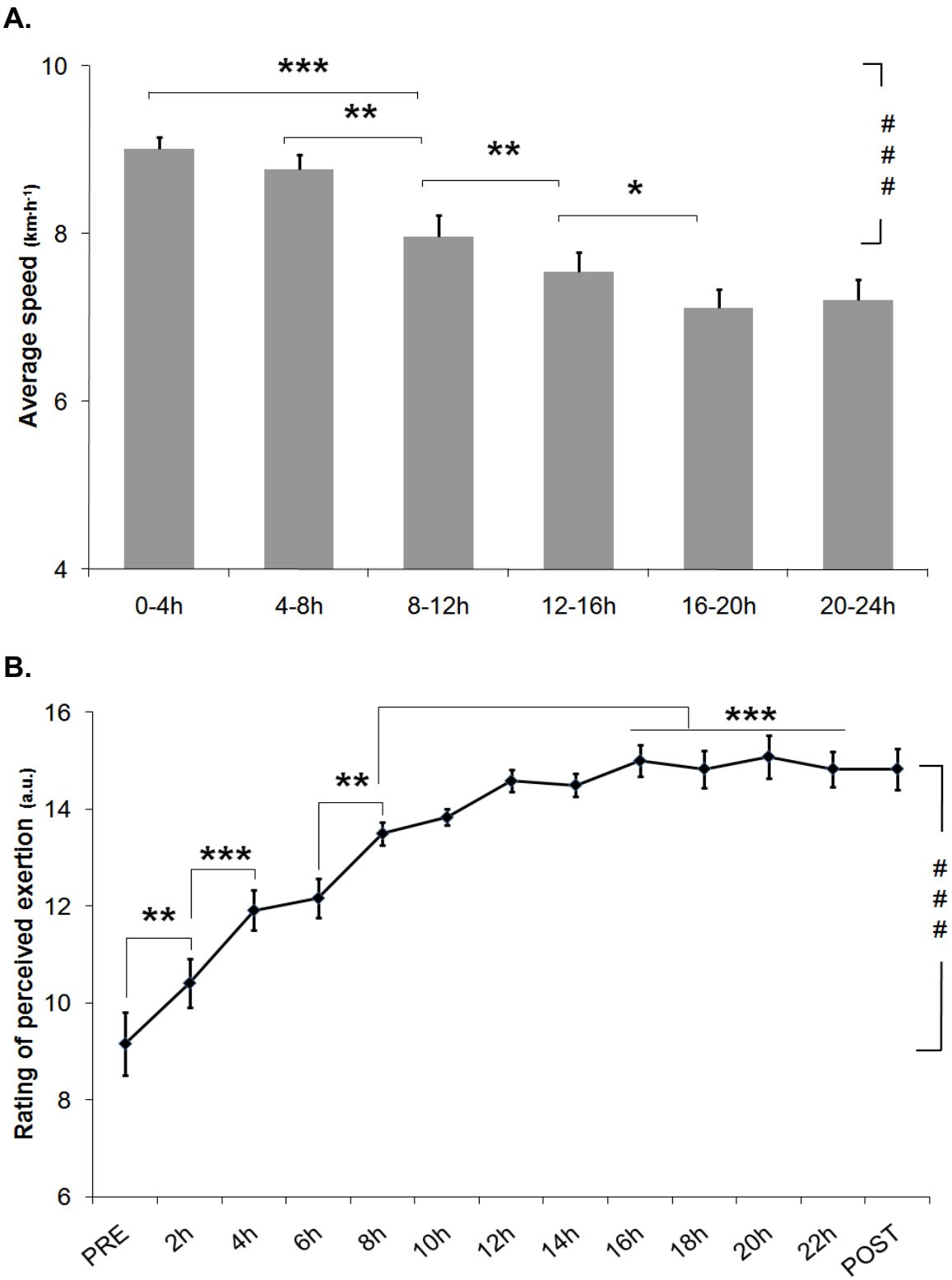

A.
Average speed (km·h⁻¹)
10
8
6
4
***
**
**
*
#
#
#
0-4h
4-8h
8-12h
12-16h
16-20h
20-24h
B.
Rating of perceived exertion (a.u.)
16
14
12
10
8
6
**
***
**
***
#
#
#
PRE
2h
4h
6h
8h
10h
12h
14h
16h
18h
20h
22h
POST

Figure 4

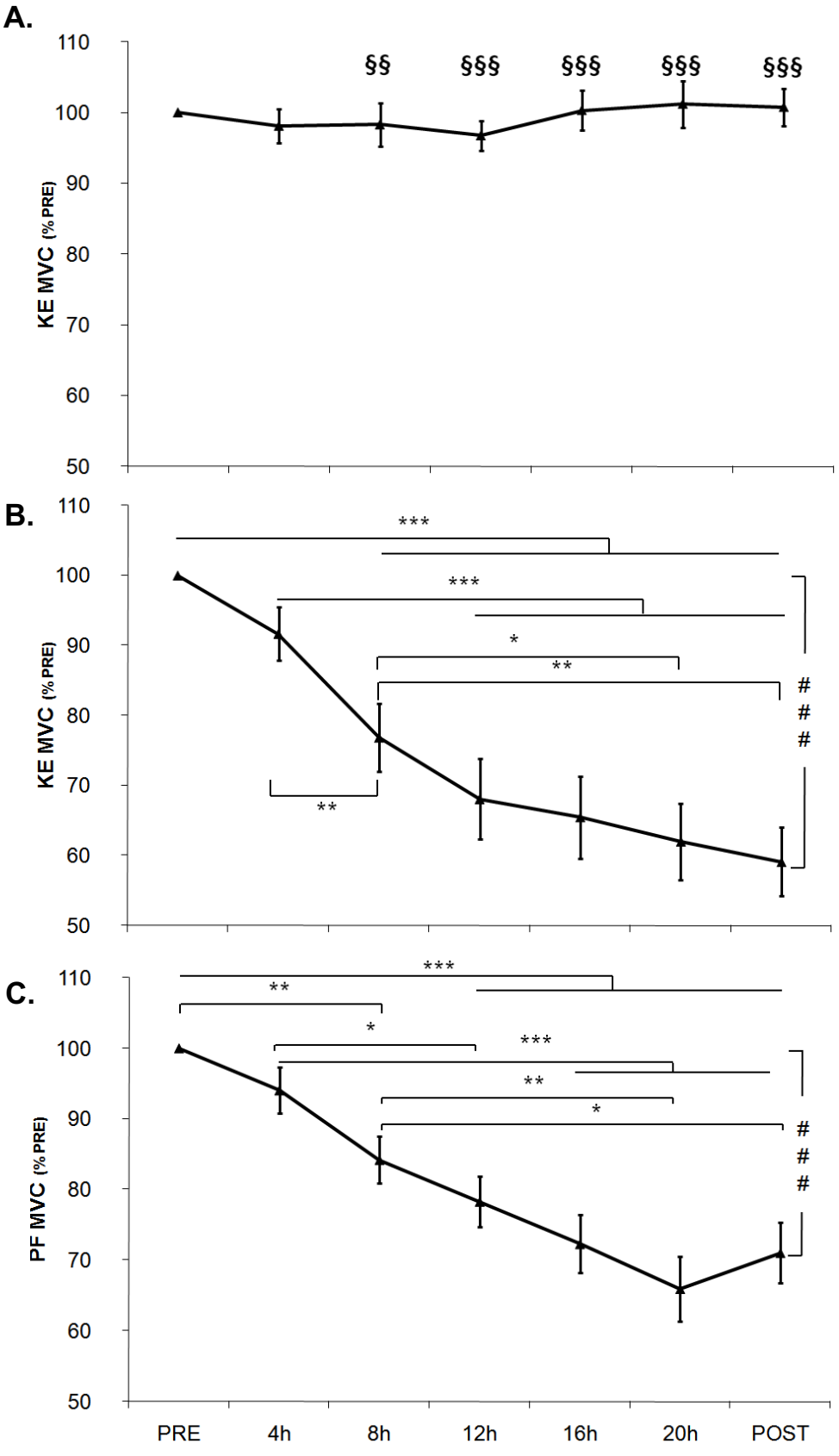

A.
B.
C.
KE MVC (% PRE)
PF MVC (% PRE)
110
100
90
80
70
60
50
§§
§§§
§§§
§§§
§§§
***
**
*
#
PRE
4h
8h
12h
16h
20h
POST

Figure 5

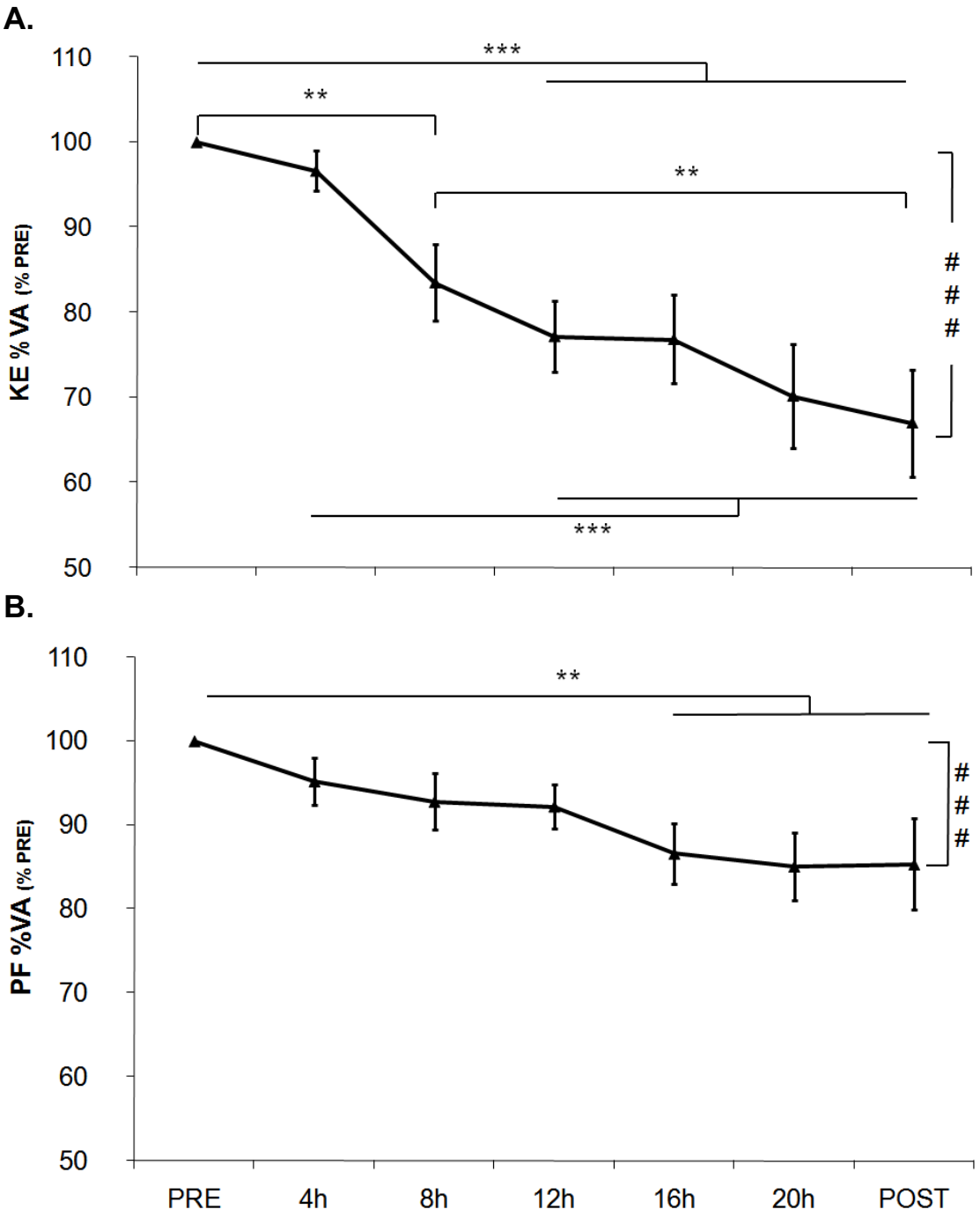

A.
KE % VA (% PRE)
110
100
90
80
70
60
50
***
**
**
***
#
#
#
B.
PF %VA (% PRE)
110
100
90
80
70
60
50
**
#
#
#
PRE
4h
8h
12h
16h
20h
POST

Figure 6.

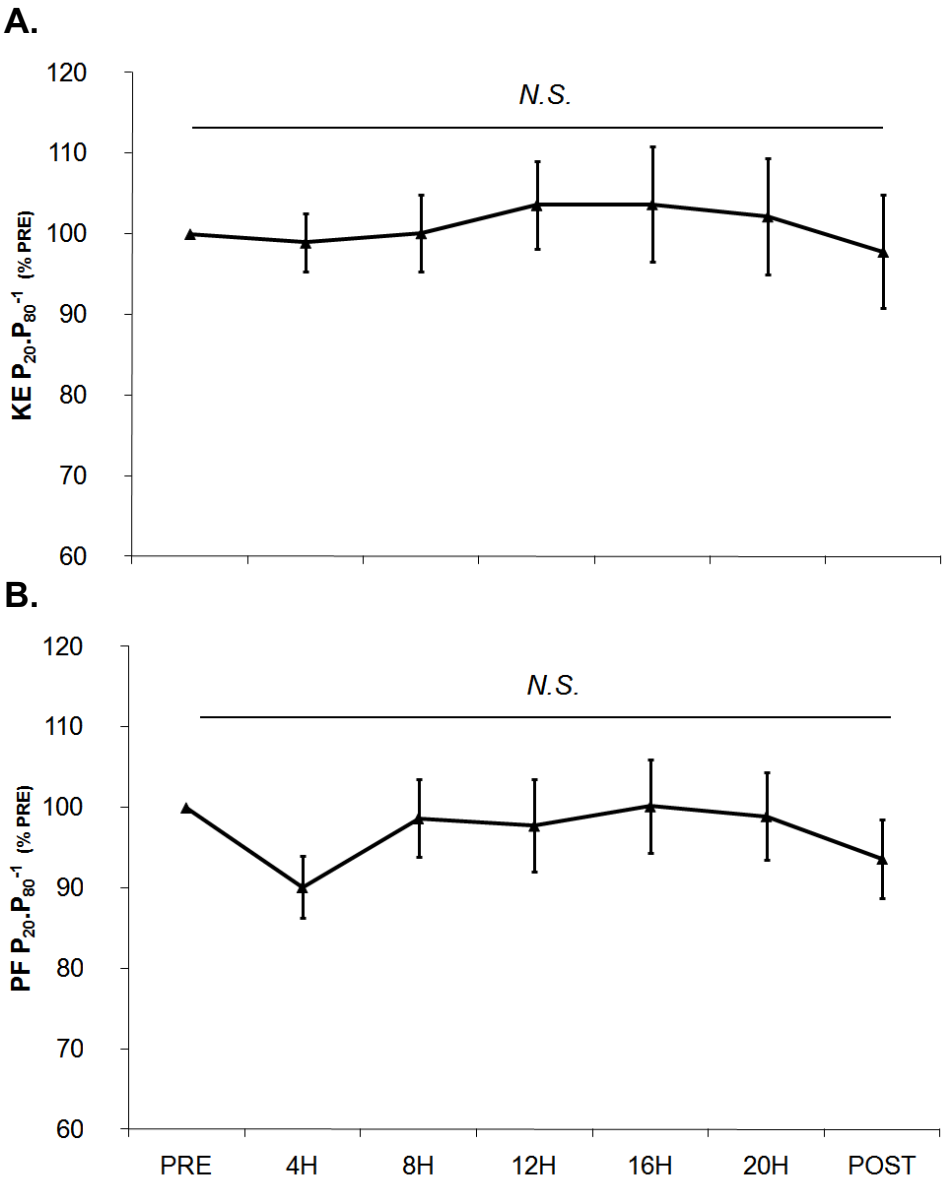

A.
120
110
100
90
80
70
60
N.S.
KE $P_{20}.P_{80}^{-1}$ (% PRE)
B.
120
110
100
90
80
70
60
N.S.
PF $P_{20}.P_{80}^{-1}$ (% PRE)
PRE
4H
8H
12H
16H
20H
POST

Figure 7.

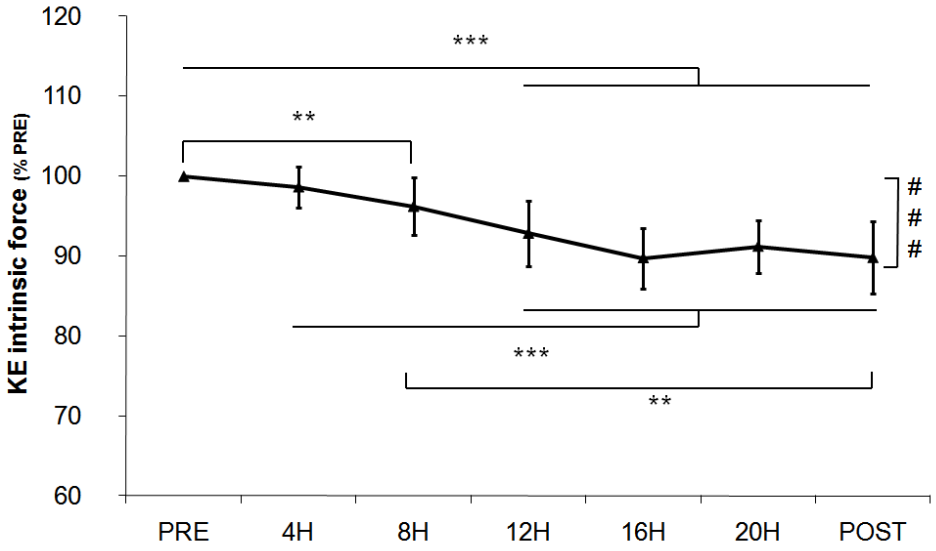
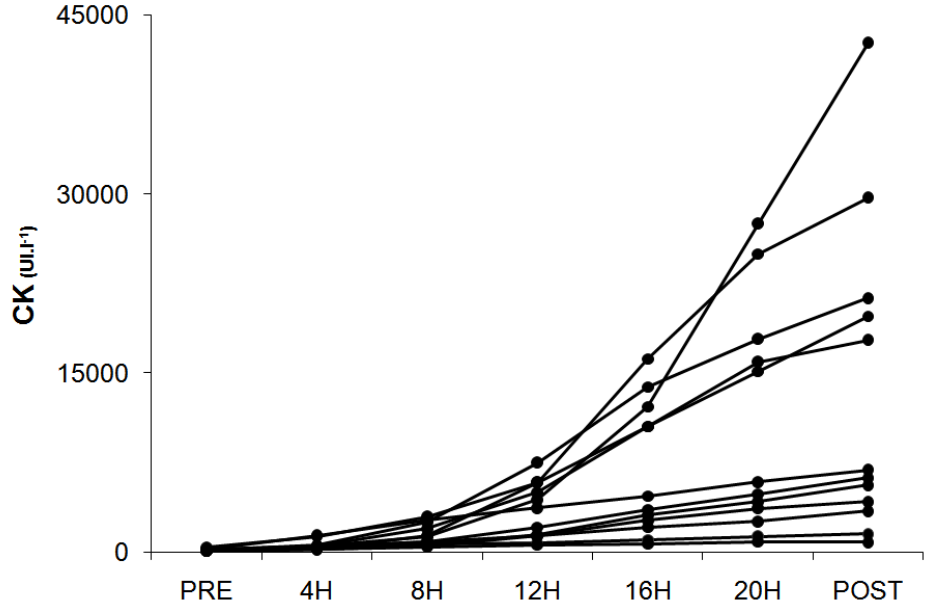
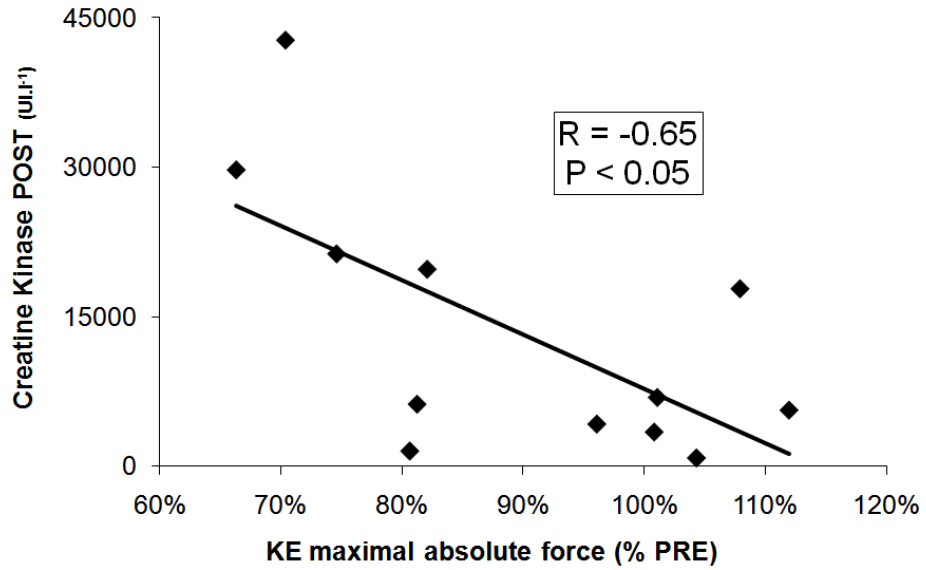

120
110
100
90
80
70
60
KE intrinsic force (% PRE)
***
**
#
#
#
***
**
PRE
4H
8H
12H
16H
20H
POST

Figure 8

A.


45000
30000
15000
0
CK (UI.l-1)
PRE
4H
8H
12H
16H
20H
POST

B.


45000
30000
15000
0
Creatine Kinase POST (UI.l-1)
R = -0.65
P < 0.05
60%
70%
80%
90%
100%
110%
120%
KE maximal absolute force (% PRE)