# Time Course of Neuromuscular Alterations during a Prolonged Running Exercise

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**ABSTRACT**

PLACE, N., R. LEPERS, G. DELEY, and G. Y. MILLET. Time Course of Neuromuscular Alterations during a Prolonged Running Exercise. *Med. Sci. Sports Exerc.*, Vol. 36, No. 8, pp. 1347–1356, 2004. **Purpose:** This study investigated the time course of contractile and neural alterations of knee extensor (KE) muscles during a long-duration running exercise. **Methods:** Nine well-trained triathletes and endurance runners sustained 55% of their maximal aerobic velocity (MAV) on a motorized treadmill for a period of 5 h. Maximal voluntary contraction (MVC), maximal voluntary activation level (%VA), and electrically evoked contractions (single and tetanic stimulations) of KE muscles were evaluated before, after each hour of exercise during short (10 min) interruptions, and at the end of the 5-h period. Oxygen uptake was also measured at regular intervals during the exercise. **Results:** Reductions of MVC and %VA were significant after the 4th hour of exercise and reached −28% ($P < 0.001$) and −16% ($P < 0.01$) respectively at the end of the exercise. The reduction in MVC was highly correlated with the decline of %VA ($r = 0.98$, $P < 0.001$). M-wave was also altered after the fourth hour of exercise ($P < 0.05$) in both vastus lateralis and rectus femoris muscles. Peak twitch was potentiated at the end of the exercise (+ 18%, $P = 0.01$); 20- and 80-Hz maximal tetanic forces were not altered by the exercise. Oxygen uptake increased linearly during the running period (+18% at 5 h, $P < 0.001$). **Conclusion:** These findings suggest that KE maximal voluntary force generating capability is depressed in the final stages of a 5-h running exercise. Central activation failure and alterations in muscle action potential transmission were important mechanisms contributing to the impairment of the neuromuscular function during prolonged running. **Key Words:** MAXIMAL VOLUNTARY CONTRACTION, ELECTROMYOGRAPHY, M-WAVE, CENTRAL FATIGUE, LONG-DURATION EXERCISE

Over recent years, there has been considerable research focused on examining the mechanisms contributing to muscle fatigue after long-duration exercise. Muscle fatigue, defined as a reduction in the maximal force generating capacity, was apparent after long-duration exercise including running, cycling, or cross-country skiing. For example, running events lasting 2–8 h have been shown to reduce maximal voluntary contraction (MVC) by 19–35% on the knee extensor (KE) muscles (4,14,19,26). Nevertheless, the origin and time course of muscle fatigue during prolonged exercise are not well established.

A recent study by Millet et al. (14) examined the changes in neuromuscular function after a 65-km ultramarathon race (~8 h 30 min). The reduction of KE MVC after exercise (~30%) was explained by a decrease in maximal voluntary activation. Similarly, Nybo (20) reported that after a 3-h cycling exercise performed at 60% of maximal oxygen uptake ($\dot{V}O_{2max}$) the 20% force loss during a 2-min sustained KE MVC was accompanied by a reduced level of central activation. However, when the same exercise was performed with a glucose supplementation, MVC was depressed by 10% and without central nervous system activation deficit. Furthermore, Lepers et al. (11) examined the neuromuscular properties of KE muscles during a 5-h cycling exercise and showed that central activation level was reduced only toward the end of the 5-h exercise. These data suggested that a reduction in efferent motor commands to the active muscles can induce a decline in neuromuscular function during prolonged exercise.

Mechanisms distal to the neuromuscular junction (peripheral mechanisms) will also contribute to muscle fatigue during and after long-duration exercise. For example, changes in the compound muscle action potential (i.e., M-wave) has been demonstrated for the vastus lateralis (VL) and vastus medialis (VM) muscles after prolonged cycling (11) and the VL muscle after a 3-h running race (15). Conversely, KE M-wave parameters were not significantly altered after running an ultramarathon (14). Furthermore, it has been shown that peak twitch (Pt) decreased by 24% after a 2-h cycling exercise (10) and by 16% after a 5-h cycling exercise (11), respectively. Together, these data suggest that the excitation-contraction (E-C) coupling process could also be altered by long-duration cycling exercise. In contrast, there is no consensus about Pt changes after long-duration running exercise. Pt has been shown to increase by 19% after a 65-km ultramarathon (14) but was reduced by 8% after a 30-km race (15).

Contractile properties from evoked tetanic contractions in response to long duration exercise are not well investigated.

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Only Millet et al. (15) observed a decrease in KE torque developed by 20- and 80-Hz stimulations (−10 and −9%, respectively) after a 30-km trail race. These alterations were accompanied by a reduction in maximal voluntary activation (−8%), suggesting that central and peripheral fatigue contributed to the MVC loss after a 30-km running event.

Although the mechanisms responsible for the alteration of neuromuscular function are well-identified at the termination of long-duration exercise, the time course of these alterations are not well understood. To the best of our knowledge, one study (11) has addressed this issue which consisted of a 5-h cycling exercise sustained at 55% of maximal aerobic power, including brief testing sessions at the end of each hour of exercise. At the end of the 5 h of cycling, the MVC had declined by 18%. This reduction was accompanied by an E-C process failure which was significantly altered after the first hour of cycling. In contrast, a decrease in central activation was only significant at the end of the exercise. Maximal electrically evoked torque was not assessed in that experiment. Furthermore, the time course of central and peripheral mechanisms involved in neuromuscular function failure have not been examined during prolonged running exercise, which differs from cycling in contraction types of the KE muscles.

Consequently, the aim of the study was to examine the time course of impairment in neural and contractile processes during a long-duration running exercise performed at a moderate intensity. To achieve this, we tested changes in voluntary and electrically induced (superimposed and on the relaxed muscle) contractions of the quadriceps muscle every hour during brief interruptions to a 5-h running exercise performed at 55% of the maximal aerobic velocity (MAV). The intensity and duration used were similar to that performed in a previous study in our laboratory that used cycling as the mode of exercise (11). This paper focused primarily on neuromuscular alterations during a prolonged running exercise, by using motor nerve transcutaneous stimulation. Based on the studies of Millet et al. (15) and Lepers (11), we hypothesized that 1) peripheral fatigue, as assessed by means of the M-wave (reflecting excitability), twitch/doublet (E-C coupling) and tetanic contractions (contractility) would be altered early in the exercise; and 2) central fatigue, assessed by voluntary activation levels, electromyographic activity of knee extensors and handgrip force, would appear in the latter stages of the 5-h running exercise.

## METHODS

### Approach to the Problem and Experimental Design

This experiment was conducted to understand the time course of neuromuscular alterations during long-duration running. To examine the central and peripheral components of fatigue, transcutaneous stimulation was performed at the end of each hour of exercise. Running economy and knee extensor EMG activity during running were also evaluated regularly during the exercise. All experiments were performed on the treadmill. The independent variable was the time at which the measure was taken. Dependent variables were MVC, evoked contractions (twitch, doublet, and tetanic stimulations), voluntary activation level, electromyographic measurements, oxygen uptake, stride duration, rate of perceived exertion (RPE), and heart rate.

### Subjects

Nine well-trained endurance runners or triathletes [age: 29.3 ± 6.4 (SD) yr; mass: 71.1 ± 6.2 kg; height: 176.7 ± 5.1 cm] volunteered to participate in this study after they were informed in detail of the experiment and risks. The subjects had regularly trained in running for 12 ± 6 yr before the study and were running 50–70 $km \cdot wk^{-1}$ during the last 3 months preceding the experiment. Personal records of half-marathon (21 km) were 77 ± 4 min. Written informed consent was given by each subject, and the local Committee on Human Research gave their approval for the project.

### Protocol and Experimental Procedures

**Preliminary session.** During an initial session that took place at least 7 d before the experiment, each of the nine subjects performed a continuous, incremental running test on a treadmill to determine their maximal aerobic velocity (MAV). Briefly, the test began with a warm-up at 10 $km \cdot h^{-1}$ for 5 min, after which the velocity was increased by increments of 1 $km \cdot h^{-1}$ every 2 min until volitional exhaustion. MAV was the highest velocity completed for 2 min (19 ± 1 $km \cdot h^{-1}$). $\dot{V}O_2$ measurements were not recorded during the preliminary session. Twenty minutes after this test, subjects familiarized themselves with the isometric measurement apparatus and with the transcutaneous stimulation.

**Running exercise.** The experiment consisted of sustaining 55% of MAV for 300 min on a treadmill (EF1800 model, medical development, Tec machine, Andrézieux-Bouthéon, France). This intensity was chosen to allow the comparison with a similar study conducted in our laboratory with cycling (11). In the present experiment, the velocity was constant throughout the 5-h running exercise. Average velocity of the subjects was 10.5 ± 0.4 $km \cdot h^{-1}$; the total distance covered was thus on average 52.5 ± 2 km. During the 5-h exercise, subjects had to ingest at least 700 $mL \cdot h^{-1}$ of a 7 $g \cdot 100\ mL^{-1}$ glucose polymer solution (Punch Power, France). Two fans were placed in front of the treadmill to reduce sweating during running. Each hour the subjects were stopped to perform neuromuscular tests. Such testing sessions lasted on average less than 10 min each.

**Neuromuscular performances.** A standardized warm-up was carried out by each subject before the testing procedure. It consisted of 10 min of cycling at 1.5 $W \cdot kg^{-1}$, followed by submaximal contractions. Subsequent to this warm-up protocol, neuromuscular tests were conducted. Subjects were first asked to perform two 5-s superimposed MVC with a 1-min rest between the trials; the subjects were strongly encouraged, and the best results were used for

further analysis. Electrical stimulation was then delivered in the order presented in Figure 1.

The three twitches and the two doublets were averaged for analyze (Fig. 2). After this stimulation protocol, handgrip force was measured as explained above. A strict timing regimen was adhered to so as to maintain constant conditions among repeated measurements. These neuromuscular tests were completed before and after 60 min (1 h), 120 min (2 h), 180 min (3 h), 240 min (4 h), and 300 min (5 h) of running and after a 30-min recovery period. Because of the painful nature of the high frequency tetanus, the 80-Hz stimulations were delivered only before and immediately after the 5-h exercise. However, three subjects could withstand 80-Hz stimulation each hour. KF maximal isometric contractions were performed only before (pre) and immediately after (5 h) exercise.

## MVC and Maximal Voluntary Activation

Maximal isometric torque at the right KE was recorded using an isometric ergometer which included a chair (Multiform, la Roque d'Anthéron, France) connected to a strain gauge (Allegro, Sallanches, France). The ergometer was located close to the treadmill, which allowed minimal time between running and testing at the end of each hour of exercise. Subjects were seated and the strain gauge was securely strapped around the ankle. The knee angle was fixed at 90°. Extraneous movement of the upper body was limited by two harnesses across the chest and the abdomen. KE maximal voluntary activation level (% VA) was estimated by using the interpolated-doublet technique. Briefly, two electrically evoked twitches (10 ms apart) were superimposed when the force had reached a plateau during the MVC. The ratio of the amplitude of the superimposed doublet over the size of the doublet in the relaxed muscle (control double shock) was then calculated to obtain %VA as follows:

$$\%VA = [1 - (\text{superimposed double shock} / \text{control double shock}] * 100$$

Control doublet was given 2 s after the end of the MVC and was thus potentiated; this technique was used to avoid any problems due to potentiation in the %VA calculation (1). Maximal isometric torque at the right knee flexors (KF) was also recorded with the knee angle fixed at 90°. Two trials, with a 1-min rest period, were executed.

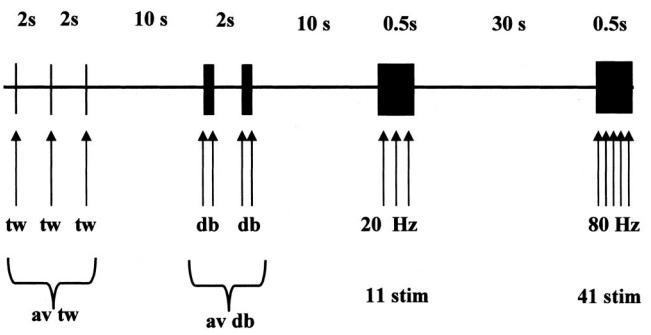


**FIGURE 1—Schematic view of the electrically induced contractions. tw, twitch; db, doublet; av tw, average twitch; av db: average doublet.**

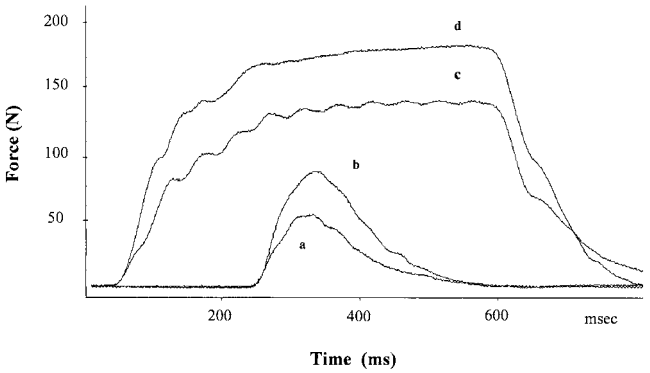


**FIGURE 2—Typical traces of electromyograms for a single twitch (a), a doublet (b), and the two tetanic stimulations evoked with the 20- (c) and 80-Hz (d) stimulation (P20 and P80, respectively)**

Right handgrip force was measured each hour using a mechanical hand dynamometer to study the changes in strength of muscles that were not directly involved in the prolonged running. Subjects were instructed to perform two maximal isometric contractions for a 3-s duration with a rest interval of ~ 30 s. The largest value was used for analysis.

## Electrically Evoked Contractions

All muscle contractile properties were conducted on the right KE muscles. Electrical stimulation was applied to the femoral nerve with a monopolar cathode ball electrode (0.5-cm diameter) pressed into the femoral triangle by the experimenter and using a high-voltage (maximal voltage 400 V) current-constant stimulator (model DS7, Digitimer, Hertfordshire, UK). The site of stimulation was marked on the skin so that placement of the electrode could be reliably repeated during the experiment. The anode was a 50 $cm^2$ (10 × 5 cm) rectangular electrode (Compex, Eclubens, Switzerland) located in the gluteal fold opposite the cathode. All motor units were considered recruited when an increase in current intensity did not induce a further increase in twitch amplitude and in the peak-to-peak amplitude of the vastus lateralis compound muscle action potential (M wave, see Electromyogram Recording). This intensity was used during the remainder of the experimental session. The E-C coupling properties were evaluated using single twitches (and doublets) and 0.5-s tetanic stimulation at frequencies of 20 and 80 Hz (i.e., 11 and 41 stimuli, respectively). Tetanic stimulation allowed us to assess muscle contractile function more accurately, as a single twitch may not be a good indicator of peripheral fatigue (15). We chose 20- and 80-Hz frequencies in order to compare with the results of Millet et al. (15,16). For the 80-Hz frequency stimulation, the torque reached during the tetanus corresponded to 87% of MVC.

The following parameters were analyzed from the mechanical response of the evoked twitch: 1) peak twitch tension (Pt), i.e., the highest value of twitch tension production; 2) twitch contraction time (CT), i.e., the time from the origin of the mechanical response to Pt; 3) half-relaxation time (HRT), i.e., the time to obtain half of the decline in twitch maximal force; 4) maximal rate of twitch tension

development (MRTD), i.e., maximal value of the first derivative of the force signal; and 5) maximal rate of twitch torque relaxation (MRTR), i.e., the most negative value of the first derivative of the torque signal. Peak torque was the only parameter analyzed on the doublet.

The highest values of tetanus torque production for the two frequencies were analyzed [20 Hz (P20) and 80 Hz (P80)], and the following P20/P80 and MVC/P80 ratios were calculated. Figure 2 shows typical trace of the mechanical torque recording.

### Electromyogram Recording

Electrical activity of the muscle [electromyograph (EMG)] for the vastus lateralis (VL), rectus femoris (RF), and the antagonistic biceps femoris (BF) muscles was recorded by means of two pairs of silver chloride circular (interelectrode distance = 20 mm) surface electrodes (Controle Graphique Medical, Brie-Comte-Robert, France) fixed lengthwise over the middle of the muscle belly. The skin was carefully prepared; low impedance ($<5$ k$\Omega$) at the skin-electrode surface was obtained by abrading the skin, and oil and dirt were removed from the skin by using alcohol. The reference electrode was attached to the wrist of the opposite arm. Subjects kept electrodes on their skin throughout all the duration of the experiment; nevertheless, the position of the electrodes was marked because some of them had to be replaced due to excessive sweating. Myoelectrical signals were amplified with a bandwidth frequency ranging from 1.5 to 500 Hz (common rejection ratio = 90 dB; impedance input = 100 M$\Omega$; gain = 1000) and simultaneously digitized on-line (sampling frequency 1 kHz) using a digital computer (IPC 486).

The following parameters were calculated from the M-wave of VL and RF muscles during the maximal twitches: peak-to-peak amplitude (PPA), peak-to-peak duration (PPD), and root mean square (RMS) of the M wave ($RMS_M$). During the MVC, EMG signals were quantified using the RMS, which was calculated over a 1-s period (for isometric KE and KF contractions) when the torque had reached a plateau. The knee extensors MVC RMS was normalized to the $RMS_M$ by using the ratio $RMS/RMS_M$, for both VL and RF muscles. A reduction in the MVC RMS without a reduction in $RMS_M$ was interpreted as a central activation failure.

VL, RF, and BF RMS were also calculated during running for seven of the nine subjects. Electrodes were maintained by a tubular elastic net bandage (Surgifix, France) wrapped around the thigh. Each record lasted 30 s, and 20 consecutive running bursts, corresponding to 20 strides, were further analyzed. The RMS was quantified for the three muscles every 20 min during running and was then averaged to obtain a value each hour. RMS was then normalized to the RMS obtained during the MVC before exercise. All the mechanical and EMG data were stored with commercially available software (Tida, Heka Elektronik, Lambrecht/Pfalz, Germany).

### Oxygen Uptake Measurements

Oxygen uptake ($\dot{V}O_2$) was recorded during running for 1 min every 20 min using an air flow meter (No. 17150, Parkinson-Cowen type, Vacumed, CA) and was then averaged to obtain a value each h. Gas exchanges were analyzed using an $O_2$ (No. 17515, Gold Edition, Vacumed, CA) and a $CO_2$ (No. 17518, Gold Edition, Vacumed) gas analyzers. Analyzers were calibrated beforehand and during the exercise (after the second or the third hour of running) using a calibration gas (No. 16914, ED Size, Vacumed).

### Heart Rate and RPE

Heart rate was monitored using a cardiofrequency meter (Polar Electro Oy, Finland). Subjects had also to indicate the perceived exertion value according to the Borg scale of 6–20 (3) every 10 min.

### Stride Duration

The time course of stride duration was assessed by using an accelerometer (FA201 model, FGP Instrumentation, France) fixed on the treadmill. Thus, foot contact was identified with the generated shock wave; a Butterworth low-pass filter at 3 Hz (the stride frequency being below 1.5 Hz during the exercise) was then applied to the signal because of high-frequency vibrations produced by the treadmill. The stride duration was calculated over 20 consecutive strides every 20 min during exercise and was averaged each hour.

### Statistical Analysis

All data presented are means ± SD (tables) and ± SE (figures). The data recorded during the exercise and recovery were statistically tested using a one-factor (time) ANOVA with repeated measures. When significant main effects were found, the Tukey test was used for *post hoc* analysis. The study variables measured before and immediately after exercise were compared with a Student's paired *t*-test. Correlation coefficients were calculated to determine the relationships between selected parameters. Reliability was determined for variables requiring several attempts by comparing the two or three trials performed in the same conditions (see Table 1). All intraclass correlation coefficients (ICC) were significant. Statistical power values have been calculated for various significant differences and ranged from 0.40 to 1 (see Table 1). For all statistical analyses, a $P$ value of 0.05 was accepted as the level of significance. The statistical analyses were undertaken by using the Sigmastat demo software for Windows (version 2.3, SPSS science, Chicago, IL).

## RESULTS

At the termination of the exercise, the RPE was 17.4 ± 2.1, indicating that the subjects had reached a high level of subjective fatigue. One subject had to stop the exercise after 4 h 30 min. Average energy intake during the 5-h exercise was 287 ± 55 kcal·h$^{-1}$. After exercise, the body mass loss

TABLE 1. Statistical power associated to the one-factor ANOVA. Size effect corresponds to the number of subjects needed to obtain respective power values at 0.80 (commonly accepted) for each variable.

| Variable | Measure | *P* | Power | Size Effect | ICC | CV (%) |
|---|---|---|---|---|---|---|
| MVC | Pre-4h | <0.01 | 0.67 | 12 | 0.879** | 2.7 |
| | Pre-5h | <0.001 | 0.61 | 14 | | |
| | Pre-post 30 | <0.001 | 0.69 | 12 | | |
| RMS/RMS M VL | Pre-4h | <0.001 | 0.91 | | 0.937*** | 6.6 |
| | Pre-5h | <0.001 | 0.86 | | | |
| | Pre-post 30 | <0.001 | 0.88 | | | |
| RMS/RMS M RF | Pre-5h | | | | 0.732* | 7.88 |
| VA | Pre-4h | <0.05 | 0.47 | 19 | 0.668* | 0.64 |
| | Pre-post 30 | <0.001 | 0.47 | 19 | | |
| MVC/P80 | Pre-5h | <0.05 | 0.46 | 20 | | |
| Stride duration | 1h–5h | <0.01 | 0.71 | 11 | | |
| Pt | Pre-5h | 0.01 | 0.48 | 18 | 0.987*** | 3.45 |
| CT | | 0.615 | | | 0.976*** | 3.08 |
| HRT | Pre-5h | 0.989 | | | 0.92** | 6.58 |
| MRTD | Pre-5h | 0.099 | | | 0.981*** | 4.43 |
| MRTR | Pre-5h | 0.051 | | | 0.965*** | 9.71 |
| Doublet | Pre-2h | <0.01 | 0.99 | 21 | 0.958*** | 4.18 |
| | Pre-4h | 0.01 | 0.5 | 17 | | |
| | Pre-5h | <0.05 | 0.5 | 17 | | |
| PPA VL | Pre-4h | <0.001 | 0.8 | 22 | 0.998*** | 3.4 |
| | Pre-5h | <0.001 | 0.82 | 17 | | |
| PPD VL | Pre-post 30 | <0.05 | 0.67 | 12 | 0.98*** | 1.58 |
| $RMS_M$ VL | Pre-4h | <0.05 | 0.71 | 11 | 0.997*** | 3.78 |
| | Pre-5h | <0.05 | 0.74 | 11 | | |
| PPA RF | Pre-5h | 0.128 | | | 0.998*** | 1.89 |
| | Pre-5h | 0.01 | 0.4 | 21 | 0.999*** | 1.1 |
| $RMS_M$ RF | Pre-4h | <0.05 | 0.6 | 14 | 0.994*** | 3.4 |
| | Pre-5h | <0.05 | 0.73 | 11 | | |
| | Pre-post 30 | <0.05 | 0.63 | 13 | | |
| $\dot{V}O_2$ | Pre-5h | <0.001 | 0.9 | | | |
| Heart rate | Pre-5h | <0.001 | 0.99 | | | |
| RPE | Pre-5h | <0.001 | 0.95 | | | |
| Handgrip strength | | 0.72 | | | 0.99*** | 4.74 |
| KF MVC | Pre-post | <0.01 | 0.74 | 11 | 0.934** | 6.62 |
| KF RMS MVC | Pre-post | <0.001 | 1 | | 0.964*** | 13.11 |

Data are presented when statistical significant differences ($P < 0.05$) were found ($N = 9$).
Intraclass correlation coefficients (ICC) and coefficients of variation (CV = SD/mean × 100) are displayed when at least two trials have been realized for corresponding variables ($N = 9$).
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

was 1.5 ± 0.4 kg, which was equivalent to 2.1 ± 0.5% of body weight.

**Oxygen uptake and heart rate.** $\dot{V}O_2$ increased linearly (r = 0.99) during the exercise, from 2.40 ± 0.2 $L \cdot min^{-1}$ at the beginning to 2.84 ± 0.3 $L \cdot min^{-1}$ at the end (+ 18 ± 11%, $P < 0.001$). Heart rate increased linearly during the 5-h (+13 ± 6%). The final average value was 154 ± 15 bpm, which represented 82 ± 5% of subjects' maximal heart rate.

**Muscular strength and voluntary activation.** Figure 3A shows that the knee extensor MVC were significantly ($P < 0.001$) reduced at the end of the exercise. Compared with "pre" values, MVC decreased progressively (−8 ± 6% at 1 h, −12 ± 9% at 2 h, −14 ± 15% at 3 h) and attained significantly lower levels after the fourth hour of exercise (−26 ± 23% at 4 h, $P < 0.01$; −28 ± 27% at 5 h, $P < 0.001$; Fig. 3A). MVC was still depressed after the 30-min recovery period (−30 ± 26%, $P < 0.001$).

$RMS/RMS_M$ for both VL and RF muscles are shown in Figures 3B and 3C, respectively. For the VL muscle, $RMS/RMS_M$ decline started after the first hour (−23 ± 26%) and remained constant after the second (−28 ± 16%) and third hours (−28 ± 18%) of exercise without being statistically significant. A second fall was then observed at the end of the fourth hour (−50 ± 21% at 4 h, $P < 0.001$; −45 ± 27% at 5 h, $P < 0.001$; −51 ± 30% at post 30 min, $P < 0.001$). For the RF muscle, $RMS/RMS_M$ decreased by 15 ± 19% after the exercise but did not reach significance ($P = 0.12$) due perhaps to a great intersubject variability.

The voluntary activation level, estimated by the doublet interpolation technique, remained unchanged during the first 3 h, but declined significantly after the fourth hour of running (−12 ± 17% at 4 h, $P = 0.03$; −16 ± 21% at 5 h, $P = 0.005$; −21 ± 28% at post 30 min, $P < 0.001$, see Fig. 3D). A strong correlation (r = 0.98; $P < 0.001$) was found between MVC loss and voluntary activation failure at 5 h (see Fig. 4).

Knee flexors MVC and maximal RMS were significantly reduced following the exercise by 13 ± 14%, $P < 0.01$ and 36 ± 17%, $P < 0.01$, respectively. However, handgrip strength did not significantly change during the 5-h exercise ($P = 0.72$).

**Evoked twitch.** Changes in the muscular twitch parameters are shown in Table 2. Pt gradually increased during the exercise (+18 ± 18% at 5 h, $P = 0.01$). After the 30-min recovery period, Pt returned close to preexercise values. All the other twitch parameters were not significantly altered by the exercise, despite a tendency for MRTR to increase during the exercise ($P = 0.051$). The maximal doublet torque was also potentiated during the running exercise

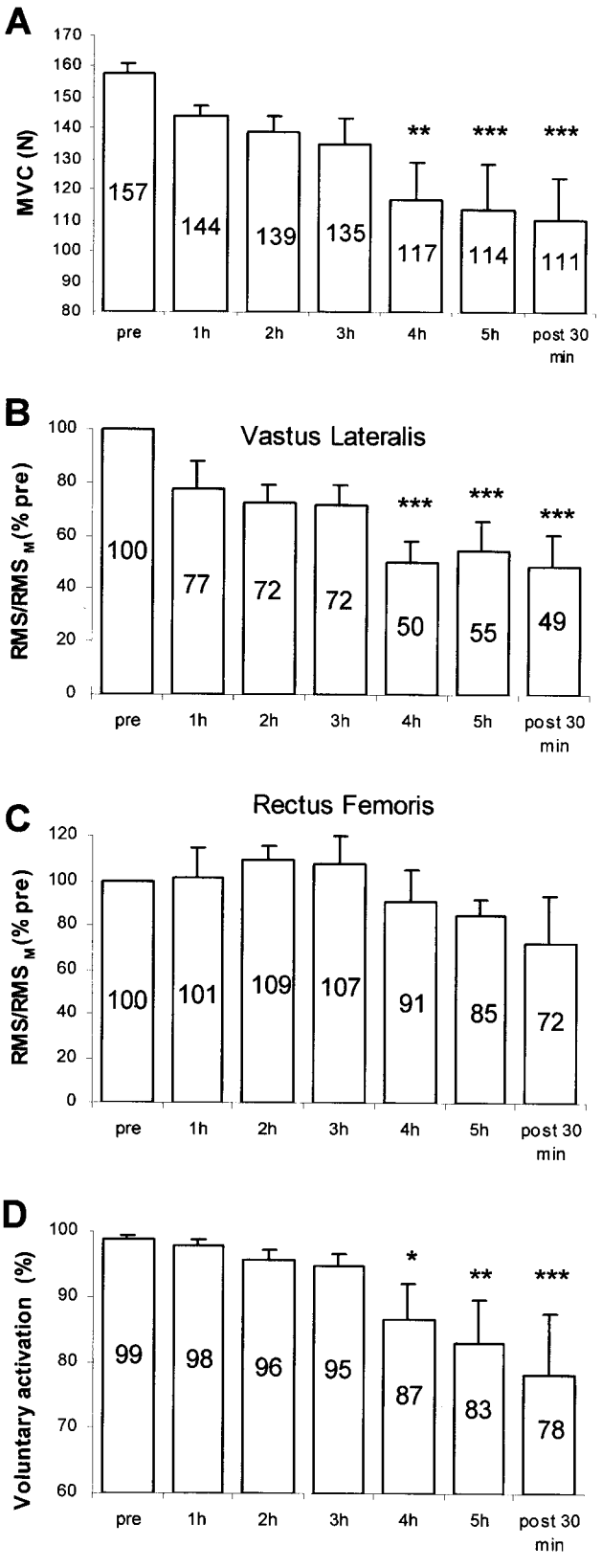


**FIGURE 3—Maximal voluntary contraction (A), electromyographic activity of the vastus lateralis muscle (B) electromyographic activity of the rectus femoris muscle (C), and voluntary activation level estimated by the superimposed doublet method (D) before (pre), during (1 h: 60th min, 2 h: 120th min, 3 h: 180th min, 4 h: 240th min), immediately after (5 h: 300th min), and 30 min after (post 30 min) the 5-h running exercise. Values are means ± SE. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$: statistically significant compared with preexercise values.**

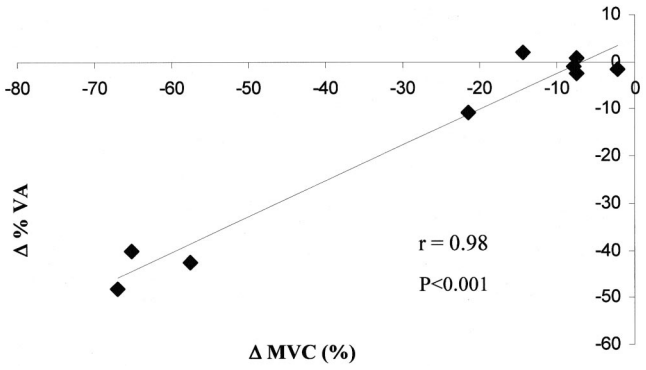


**FIGURE 4—Correlation between the KE maximal voluntary contraction (MVC) loss and the maximal voluntary activation level (% VA) loss at the end of the exercise.**

(+12 ± 5% at 2 h, $P < 0.05$; +15 ± 15% at 4 h, $P = 0.01$; and +14 ± 14% at 5 h, $P < 0.05$).

Alterations of M-wave characteristics are presented in Table 3. For the VL muscle, PPA decreased significantly at 4 h (−33 ± 21%) and 5 h (−34 ± 21%), as well as $RMS_M$ (−23 ± 18% and −24 ± 18% at 4 h and 5 h, respectively). PPD increased significantly (+ 20 ± 25%, $P < 0.05$) only 30 min after the end of the running event. For RF muscle, PPD diminished significantly only at 5 h (−18 ± 20%, $P < 0.05$), whereas $RMS_M$ was depressed at 4 h (−28 ± 26%, $P < 0.05$), 5 h (−33 ± 26%, $P = 0.01$), and post 30 min (−28 ± 25%, $P < 0.05$); PPA was not significantly altered for RF.

**Contractile properties.** Figures 5 A and B, show that P20 and P80 were not altered by the long-duration running exercise. Moreover, Figure 5C demonstrates that the P20/P80 ratio was unchanged at the end of the exercise ($P = 0.23$), which suggests the absence of both high- and low-frequency fatigue. The MVC/P80 ratio, an indirect indicator of central fatigue, decreased significantly after the 5-h exercise (−25 ± 31%; $P < 0.05$, see Fig. 5D).

**Muscle activity during running and stride duration.** RMS of VL, RF, and BF muscles did not change significantly during the 5-h running exercise ($P = 0.43$; $P = 0.83$ and $P = 0.23$, respectively, see Fig. 6A). During the exercise, stride-duration decreased linearly between the first and the last hour of exercise (−3.4%, $P < 0.01$, see Fig. 6B). Because running velocity was constant during the event, the stride frequency increased from 1.40 to 1.45 Hz.

## DISCUSSION

The main impetus of this research was to understand the time course of impairment in neural and contractile processes of the quadriceps muscle during a 5-h running exercise. The main findings were that: 1) MVC loss was highly correlated with central activation failure at the end of the exercise; 2) central fatigue occurred in the latest part of the exercise; and 3) despite a potentiation of the twitch, tetanic force was not reduced throughout the exercise. Thus, the running exercise was intense enough to observe alterations

TABLE 2. Twitch properties of the quadriceps muscle before (pre), during (1 h: 60th min, 2 h: 120th min, 3 h: 180th min, 4 h: 240th min), immediately after (5 h: 300th min), and 30 min after (post 30 min) the 5-h running exercise.

| | Pt (N) | CT (ms) | HRT (ms) | MRTD ($N \cdot ms^{-1}$) | MRTR ($N \cdot ms^{-1}$) | Doublet (N) |
|---|---|---|---|---|---|---|
| Pre | 39.2 ± 6.8 | 75.9 ± 13.2 | 69.1 ± 10.5 | 0.96 ± 0.35 | 0.52 ± 0.17 | 65.3 ± 8.4 |
| 1 h | 38.1 ± 5.3 | 82.2 ± 16.7 | 67.9 ± 13.2 | 0.93 ± 0.37 | 0.52 ± 0.17 | 67.9 ± 9.1 |
| 2 h | 41.6 ± 6.8 | 78.8 ± 13 | 67.3 ± 13.7 | 1.02 ± 0.37 | 0.58 ± 0.24 | 73.3 ± 8.2** |
| 3 h | 42 ± 6.1 | 78.8 ± 9.9 | 65.5 ± 12.7 | 1.07 ± 0.44 | 0.59 ± 0.21 | 71.7 ± 8.8 |
| 4 h | 42.9 ± 5.6 | 75.8 ± 11.8 | 68.2 ± 12 | 1.10 ± 0.35 | 0.62 ± 0.25 | 74.4 ± 7.4* |
| 5 h | 45.7 ± 7.2* | 76.8 ± 11 | 63.8 ± 9.4 | 1.16 ± 0.36 | 0.67 ± 0.18 | 73.7 ± 6.9* |
| Post 30 | 41.4 ± 5.1 | 71 ± 16.5 | 70.2 ± 14.8 | 1.15 ± 0.26 | 0.58 ± 0.12 | 67.5 ± 8.8 |

Pt, maximal twitch torque (N); CT, contraction time (ms); HRT, half relaxation time (ms); MRTD, maximal rate of twitch tension development ($N \cdot ms^{-1}$); MRTR, maximal rate of twitch torque relaxation ($N \cdot ms^{-1}$); doublet, maximal doublet torque (N).
Values are means ± SD.
* $P < 0.05$; ** $P < 0.01$: statistically significant compared with preexercise values.

in neuromuscular properties and physiological parameters such as $\dot{V}O_2$.

**Strength loss and central fatigue.** The present results show that KE MVC decreased by 28% at the end of the exercise, which is consistent with previous studies. Indeed, maximal isometric knee extension decreased by 30% after a 65-km ultramarathon (14), by 25% after 4 h of treadmill running at 65–70% $\dot{V}O_{2max}$ (4), and by 24% after a 30-km running race (15). The time course of MVC decline during the 5-h running exercise differs from that observed for previous cycling exercise performed at a similar duration and intensity in our laboratory (11). The time course of MVC reduction during the two 5-h exercise (cycling vs running) appears to be very similar throughout the first 3 h, but differences seem to occur after the fourth hour where MVC declines by 14% for running and by only 6% for cycling. The greater loss of maximal KE strength capacity after 5-h running (−28%) compared with 5-h cycling (−18%) could be explained by the difference in contraction mode of the quadriceps muscle. During running, eccentric contractions produce twice the tension per active muscle fiber compared with concentric contractions (25), leading therefore to greater muscular stress compared to cycling.

There was large intersubject variability in the magnitude of strength loss in the present study. Nevertheless, MVC reduction was highly correlated ($r = 0.98$) with the voluntary activation failure. Central activation decreased by 16% after the 5-h running exercise and this is consistent with other studies. For example, Millet et al. (14,15) found a reduction of central activation levels equal to 8% and 28% after 3-h and 8-h running races, respectively. In the present study, a reduction of central activation became significant only at the end of the fourth hour of running. However, this result might be interpreted cautiously, as the statistical power for the VA was not as high as required. But nevertheless, these data corroborate that from previous results in our laboratory (11), in which central fatigue occurred at the end of a 5-h cycling exercise. Moreover, as it has already been shown by Millet et al. (15) after a 30-km running race, the $RMS/RMS_M$ for the VL muscle and MVC/P80 ratios decreased significantly at the end of the exercise. However, neuromuscular properties of RF muscle seemed to be less altered than VL during the experiment; $RMS/RMS_M$ for the RF muscle slightly increased during the first 3 h and then decreased, but insignificantly. This discrepancy could be attributed to the anatomical functions of the muscles; VL is a monoarticular knee extensor muscle, whereas RF muscle is biarticular. These findings demonstrate that central fatigue occurs in the later stage of a prolonged running exercise.

According to Davis and Bailey (5), a reduction in central drive could result from either a reduction in corticospinal impulses reaching the motoneurons and/or an inhibition of motoneuron excitability by neurally mediated afferent feedback from the muscle. Decreased excitability of interneuronal circuits within the motor cortex has been shown after pull-ups until exhaustion (29). In contrast, Löscher and Nordlund (12) did not find any change in motor cortex excitability after a fatiguing task on the elbow flexors, despite a decreased voluntary activation. In the present study, the strength of a muscular group that was not directly involved in running (handgrip muscles) was evaluated during the exercise to further explore the origin of the lower KE activation. Millet et al. (15) hypothesized that grip strength

TABLE 3. M-wave characteristics before (pre), during (1 h: 60th min, 2 h: 120th min, 3 h: 180th min, 4 h: 240th min), immediately after (5 h: 300th min), and 30 min after (post 30 min) the 5-h running exercise for both vastus lateralis and rectus femoris muscle.

| | Vastus Lateralis | | | Rectus Femoris | | |
|---|---|---|---|---|---|---|
| | PPA (mV) | PPD (ms) | $RMS_M$ (mV) | PPA (mV) | PPD (ms) | $RMS_M$ (mV) |
| Pre | 11.40 ± 4.62 | 7.39 ± 0.66 | 3.41 ± 1.72 | 3.47 ± 1.32 | 12.06 ± 3.39 | 1.41 ± 0.57 |
| 1 h | 10.41 ± 4.48 | 7.56 ± 0.78 | 3.27 ± 1.67 | 2.89 ± 1.32 | 11.97 ± 3.69 | 1.24 ± 0.61 |
| 2 h | 10.13 ± 4.36 | 7.20 ± 0.63 | 3.29 ± 1.69 | 3.06 ± 1.11 | 11.36 ± 3.55 | 1.09 ± 0.34 |
| 3 h | 9.01 ± 4.17 | 7.11 ± 0.47 | 2.97 ± 1.56 | 2.89 ± 1.15 | 11.36 ± 3.42 | 1.02 ± 0.31 |
| 4 h | 7.40 ± 3.91*** | 7.72 ± 0.49 | 2.52 ± 1.40* | 2.85 ± 1.24 | 11.02 ± 3.64 | 0.91 ± 0.31* |
| 5 h | 7.05 ± 2.79*** | 7.75 ± 1.07 | 2.42 ± 1.05* | 2.58 ± 0.76 | 10.44 ± 4.20* | 0.88 ± 0.37* |
| Post 30 | 7.25 ± 3.24 | 8.94 ± 1.39* | 2.34 ± 1.21 | 3.00 ± 1.13 | 11.28 ± 4.20 | 0.93 ± 0.35* |

PPA, peak-to-peak amplitude; PPD, peak-to-peak duration; $RMS_M$, root mean square of the M wave.
Values are means ± SD.
* $P < 0.05$; *** $P < 0.001$: statistically significant compared with preexercise values.

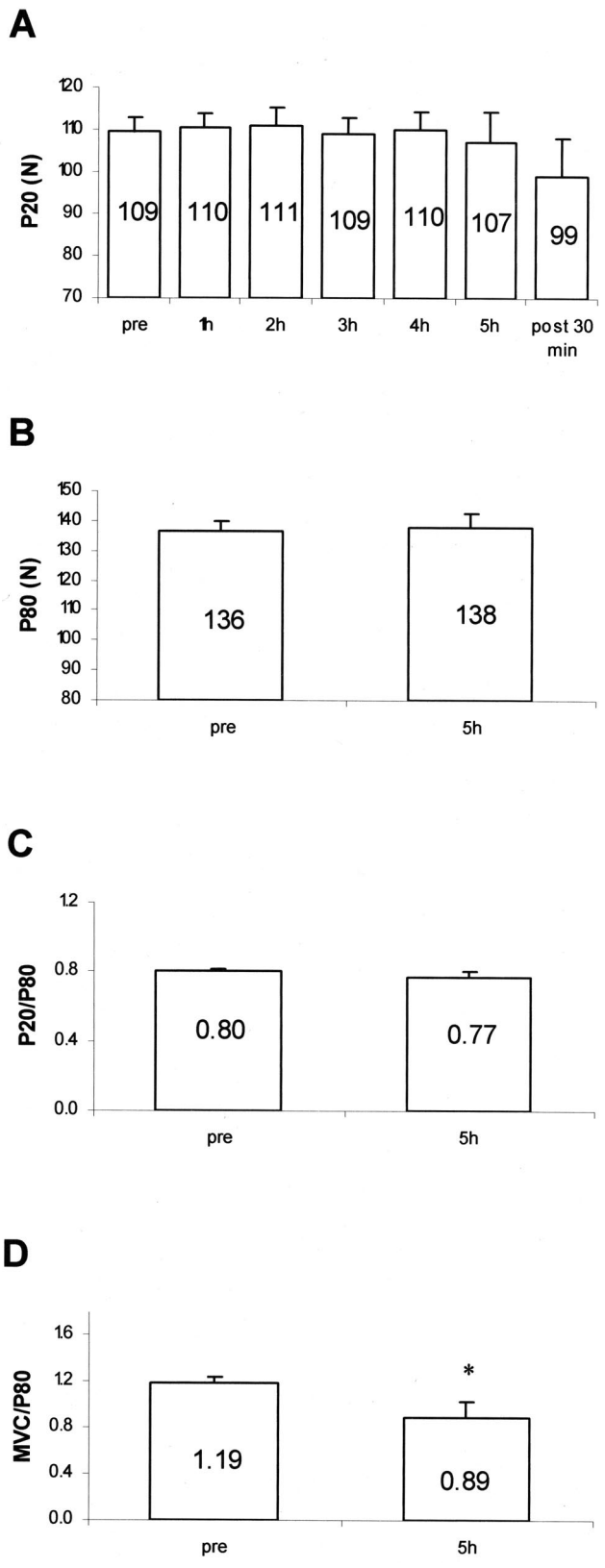


**FIGURE 5—A. Quadriceps contractile properties evoked by 20-Hz stimulation (P20) before (pre), during (1 h: 60th min, 2 h: 120th min, 3 h: 180th min, 4 h: 240th min), immediately after (5 h: 300th min), and 30 min after (post 30 min) the 5-h running exercise. B. Quadriceps contractile properties evoked by 80-Hz stimulation (P80), P20/P80 and MVC/P80 ratios before (pre) and immediately after (5 h: 300th min) the 5-h running exercise. Values are means ± SE. *$P < 0.05$: statistically significant compared with preexercise values.**

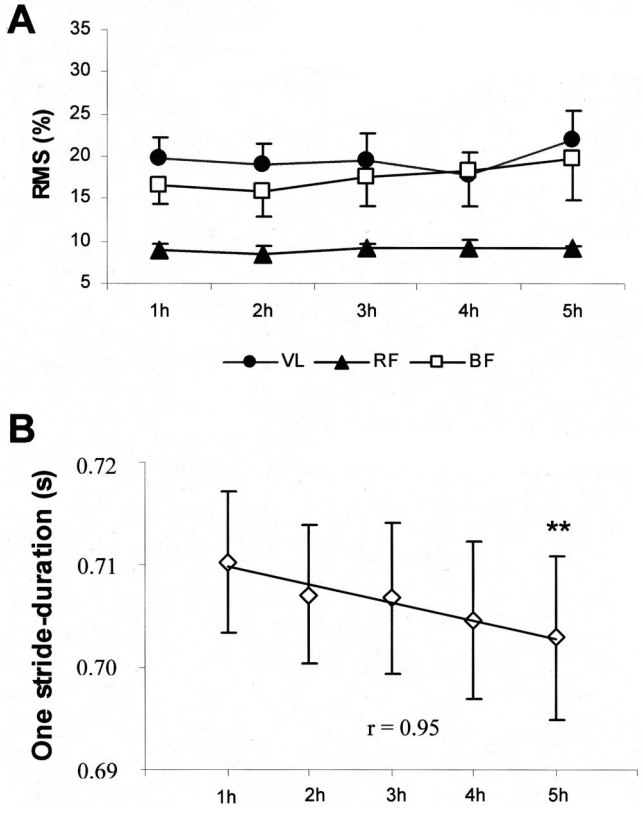


**FIGURE 6—A. Changes in vastus lateralis (VL), rectus femoris (RF), and biceps femoris (BF) EMG RMS during running, expressed as percent MVC RMS before exercise. Values were averaged each hour and are ± SE. B. Mean stride duration during the 5-h running exercise. **$P < 0.01$: statistically significant compared with 1-h values. Values were averaged each h and are ± SE.**

loss could be used as evidence of supraspinal fatigue. However, there was no significant loss of grip strength during or after the 5-h running exercise, and so we can not conclude there was the presence of supraspinal fatigue.

**Electrically evoked stimulation.** The amplitude and RMS values of the M-wave measured for the VL and RF muscles were significantly reduced by the 5-h running exercise. This is consistent with that found after a 30-km running event (15) and after a 2-h cycling exercise (10), but not observed in all studies. For example, Millet et al. (14) did not find any modifications in the KE M-wave characteristics following a 65-km ultramarathon.

Changes in M-wave parameters could represent both a failure in the neuromuscular transmission and/or a reduction in the ability to propagate action potentials (21). In the present study, M-wave duration was much less altered than amplitude or RMS values, suggesting a reduction in the action potential transmission rather than a propagation failure. The lower muscle fiber excitability could originate from an elevated blood ammonia concentration (18). A reduced chemical gradient for $Na^+$ and $K^+$ across the sarcolemma is another hypothesis frequently proposed. Consistent with this, an increase in plasma $K^+$ concentration has previously been found following marathon running (22). In the present study, M-wave alterations were found after the fourth hour of exercise, as has been previously shown after 5-h of

cycling (11). These results suggest that neuromuscular excitability is mainly affected in the latter part of a long-duration event.

Twitch and doublet forces were significantly potentiated at the end of the exercise. Pt potentiation has been previously reported after an ultramarathon (+ 19%; 14) or a ski-skating marathon (+ 7%; 16). In contrast, a 3-h running event has been shown to reduce Pt by 8% (15). The coexistence of potentiation and fatigue of a single twitch would make it difficult to quantify either process independently (23) and could explain the diversity of these results. Twitch potentiation has been found after prolonged events soliciting the stretch-shortening cycle (see above) but never after prolonged cycling exercises (2,10,11). Based on animal studies, it is now assumed that potentiation is due to regulatory myosin light chain phosphorylation (28). Phosphate incorporation is known to increase rate of force development (30) and $Ca^{2+}$ sensitivity (23); this could be an explanation for the higher Pt and doublet amplitude, and for the tendency to the greater MRTD observed in the present study. Pt potentiation has not been found after prolonged cycling exercises (10,11), suggesting that stretch-shortening cycle may also play a role in twitch potentiation observed during prolonged running exercises. Nevertheless, the statistical power values for peak twitch and doublets were not high enough; some of these results should also be interpreted with caution.

Force at the cross-bridges level for the quadriceps muscle, examined here by P80, was not significantly affected by the exercise. To the best of our knowledge, only one study (15) examined maximal tetanic force after a 3-h running exercise. These authors reported 10% and 9% losses for P20 and P80, respectively, and an unchanged P20/P80 ratio. In contrast, Davies and Thompson (4) did not observe any change in tetanic stimulation at different frequencies after a 4-h running exercise. However, these last results must be compared with caution, because the stimulation intensity was submaximal. We used individual optimal intensity and not a supramaximal intensity of stimulation, so as to minimize the pain associated with tetanic stimulation. Hence, the optimal intensity of stimulation may have altered slightly during the exercise. However, peak twitch and peak doublet forces increased, indicating that supramaximal stimulation may have enhanced potentiation, but probably not have changed the direction of the results. In contrast to Millet et al. (15) study, the absence of contractile alterations in the present experiment could be explained by a lower muscular stress due to greater shock absorption when running on the treadmill compared to the ground. Also, the course of the race supporting the study of Millet et al. (15) was hilly, that is, eccentric contractions were probably more intense.

In the present study, P20 did not change throughout the exercise. The running exercise could have potentiated P20 and consequently may have masked any low-frequency fatigue (24). Nevertheless, this seems improbable because potentiation lasts only a few minutes (17), and P20 was not reduced 30 min after the end of the exercise. Low-frequency fatigue is due to a reduction in $Ca^{2+}$ release, and may be a consequence of some damage to the structure of the muscle fiber and the E-C coupling (8). Because structural damage has been shown to occur during prolonged running (9), low-frequency fatigue was anticipated in the present study. Nevertheless, no low-frequency fatigue was observed during the 5-h running exercise, but was observed after an intense stretch-shortening cycle exercise (27) and cycling exercise performed at 75% $\dot{V}O_{2max}$ until exhaustion (2). In our study, the potentiated twitch provided evidence for the lack of reduction in $Ca^{2+}$ release during the running exercise and could explain the absence of low-frequency fatigue.

Similarly, P80 was not altered at the end of the 5-h exercise, neither during the exercise in the three subjects to whom 80-Hz stimulation was applied each hour. These findings suggest that the MVC loss reported during the 5-h running exercise was not due to impaired force at the cross-bridge level but rather to a decrease in central activation and/or muscle excitability.

**Running pattern and economy.** EMG activity of the VL and RF muscles was analyzed during the 5-h running exercise. Results showed that EMG activity was not altered during the 5 h of running. To our knowledge, only one study has been interested in the VL muscle EMG during prolonged running (7). These authors showed a ~ 22% increase in the integrated EMG flow during the last 45 min of a marathon and attributed this finding to an increase in motor unit recruitment and/or in their discharge rate. The discrepancy between their study and the present may be due to the different exercise intensities and durations (75% vs 55% of MAV). During submaximal running, muscle contractions could be performed by only a portion of the muscle motor unit pool. A change of activity between different motor units might occur with fatigue and could contribute to the stability of EMG activity in the present study.

Stride duration slightly decreased throughout the exercise, indicating that stride frequency increased by the same proportion, running velocity being constant during the exercise. This result is in agreement with previous findings obtained during a marathon. Hausswirth et al. (6) observed an 11% decline in the stride length, whereas Kyrolaïnen et al. (9) found a 4% increase in stride frequency. The present data confirm the hypothesis, which suggests that biomechanical pattern changes during prolonged running may be evidence for a compensation for an impaired neuromuscular function (9). Indeed, because MVC of the knee extensors was reduced, the intensity of contractions at each stride represented a higher % of MVC. Thus, this altered locomotor pattern could have been an adaptation to the muscle fatigue, i.e., the decrease of knee extensors maximal force.

In the present study, running economy decreased linearly during the 5-h exercise by 18%. Davies and Thompson (4) reported a 9% increase in $\dot{V}O_2$ after a 4-h running exercise, whereas a 16% increase was observed during a marathon (9). In contrast, Millet et al. (13) found no change in oxygen cost of running 15 min after the end of an ultramarathon. Increase of fat as an energy substrate, raised demand of body temperature regulation, and changes in running pattern

are the most common explanations for the increase in the oxygen requirement during exercise.

In summary, this study examined the time course of neural and contractile alterations of the quadriceps muscles during a 5-h running exercise. In contrast to our hypothesis, the contractile function evaluated by means of maximal tetanic stimulations was unaffected throughout the exercise in well-trained subjects. A reduced voluntary activation and a decreased excitability, both occurring in the latter stage of the exercise, may have been responsible for the reduction in maximal voluntary force-generating capacity. An increase in oxygen uptake throughout the exercise was associated with slight changes in running pattern, which could be attributed to an adaptation to impaired neuromuscular function. Finally, the location of central impairment (spinal vs supraspinal) requires further investigation for long-duration exercises.

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