Assessment of low-frequency fatigue with two methods of electrical stimulation

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Martin, V., G. Y. Millet, A. Martin, G. Deley, and G. Lattier. Assessment of low-frequency fatigue with two methods of electrical stimulation. J Appl Physiol 97: 1923–1929, 2004. First published July 16, 2004; doi:10.1152/japplphysiol.00376.2004.-The aim of this study was to compare the use of transcutaneous vs. motor nerve stimulation in the evaluation of low-frequency fatigue. Nine female and eleven male subjects, all physically active, performed a 30-min downhill run on a motorized treadmill. Knee extensor muscle contractile characteristics were measured before, immediately after (Post), and 30 min after the fatiguing exercise (Post30) by using single twitches and 0.5-s tetani at 20 Hz (P20) and 80 Hz (P80). The P20-to-P80 ratio was calculated. Electrical stimulations were randomly applied either maximally to the femoral nerve or via large surface electrodes (ES) at an intensity sufficient to evoke 50% of maximal voluntary contraction (MVC) during a 80-Hz tetanus. Voluntary activation level was also determined during isometric MVC by the twitch-interpolation technique. Knee extensor MVC and voluntary activation level decreased at all points in time postexercise (P <0.001). P20 and P80 displayed significant time \times gender \times stimulation method interactions (P < 0.05 and P < 0.001, respectively). Both stimulation methods detected significant torque reductions at Post and Post30. Overall, ES tended to detect a greater impairment at Post in male and a lesser one in female subjects at both Post and Post30. Interestingly, the P20-P80 ratio relative decrease did not differ between the two methods of stimulation. The low-to-high frequency ratio only demonstrated a significant time effect (P < 0.001). It can be concluded that low-frequency fatigue due to eccentric exercise appears to be accurately assessable by ES.

low- and high-frequency electrical stimulation; central activation; gender; body fat percentage

LOW-FREQUENCY FATIGUE (LFF), i.e., the preferential loss of force at low frequencies of electrical stimulation, is a prominent characteristic of exercises involving lengthening contractions of the active muscles such as eccentric- and stretchshortening cycle-type exercises (for a review see Ref. 13). Almost all studies related to eccentric and stretch-shortening cycle exercises have used electrical stimulation of the muscle (ES), i.e., stimulation of the exercised muscles via large surface electrodes, to evaluate LFF (24, 26, 28, 29) with evoked torque levels ranging from 33 to 75% maximal voluntary contraction (MVC) at high stimulation frequencies. However, the use of submaximal evoked torques may include a number of limitations. First, ES depolarizes axons located near the stimulating electrode, especially the larger ones (16). As a consequence, fast-twitch motor units are preferentially recruited by ES (31). Because these motor units are more fatigable than their slowtwitch counterparts, this preferential recruitment could lead to an overestimation of the whole muscle fatigability. Second, axonal recruitment thresholds may vary so that different motor units can be tested in different trials (10). Third, it has been suggested that muscle damage is partly responsible for LFF after damaging exercises (13). Because muscle damage is heterogeneously distributed in the whole muscle group, the use of ES, which recruits only a fraction of the muscle group, could misestimate the extent of LFF (32). Fourth, Binder-Macleod et al. (3) demonstrated that the force-frequency relationship was shifted to the left at an intensity sufficient to evoke 80% MVC compared with lower submaximal intensities. As a result, at lower submaximal intensities, the low-to-high frequency ratio is lower compared with the same ratio obtained with an intensity sufficient to evoke 80% MVC. According to Binder-Macleod et al., this change in the force-frequency relationship at 80% MVC may be due to the recruitment of deeper heads of the muscle that display a different relationship between their contractile rates and force-frequency relationships than the more superficial heads. Finally, Davies and White (4) demonstrated that electrically elicited torque loss is voltage dependent and underlined the difficulty of using submaximal forces when low-to-high frequency ratios are employed to assess muscle function after exercise in humans. Rather, they suggested the use of stimulation, which ensures the complete activation of the muscle. Nevertheless, the discomfort induced by submaximal stimulation via ES is very limited, and this is the reason why this type of stimulation is widely used in clinical practice and laboratory testing.

Tetanic stimulation of the motor nerve is effective in recruiting almost maximally the knee extensor muscle group (KE) (7, 22) but induces discomfort so that one cannot use this method with patients or aged subjects. However, neurostimulation (NES) can be considered as the best currently available method to study LFF. Rutherford et al. (25) previously addressed this issue of transcutaneous vs. motor nerve stimulation of the quadriceps for the interpolated twitch technique and did not find any difference between the two methods. As far as we know, this comparison has never been made for the evaluation of LFF. Therefore, the purpose of this experiment was to compare the results provided by these two methods of electrical stimulation, i.e., ES and NES, after eccentric-type exercise to test the validity of submaximal elicited torques in the evaluation of LFF. A secondary objective was to examine any gender differences in the responses to ES and NES, because the higher percentage body fat observed in female subjects may alter the response to ES.

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METHODS

Subjects. Twenty physically active subjects (9 women and 11 men) completed the study. Their morphological characteristics are presented in Table 1. All were familiar with electrical stimulation and with testing procedures. The study was conducted according to the Declaration of Helsinki and approval for the project was obtained from the local Committee on Human Research. Additionally, each subject gave written, informed consent.

Muscle function measurements. Before the experiment, each subject performed a 10-min warm-up on a bicycle ergometer (Excalibur, Lode, Groningen, The Netherlands) at a self-selected power output. Muscle contractile characteristics were measured before, immediately after, and 30 min after a 30-min downhill run performed at a speed of 10 km/h with a 20% negative slope on a motorized treadmill (EF 1800, Tecmachine, Andrézieux-Bouthéon, France). During the testing procedures, the participants were seated in a strength-training device (modified leg extension machine) where the mechanical response was recorded by a strain gauge (SBB 200 kg, Tempo Technologies, Taipei, Taiwan). The subjects were secured firmly with Velcro straps across the hips and chest to minimize upper body movement. Subjects were also asked to keep their arms crossed over the chest during voluntary efforts. All measurements were taken from the subject's right leg, which was flexed at 90° from full extension. The isometric contractions performed during the experiment included MVCs and electrically evoked torque measurements. During all the testing procedures, the subjects were strongly encouraged.

MVCs. MVC testing involved three conditions. Two trials per condition were required with a 1-min rest between each trial. For each condition, the best result was used for further analysis. In the first condition, the contractions were performed without electrical stimulation. These two MVC attempts were only performed before the fatiguing exercise because the best value was used to determine ES stimulation intensity (see below). The second and third conditions involved MVC with a double pulse (time interpulse: 10 ms) superimposed to the isometric plateau, by either ES or NES, in a randomized order. The stimulation intensity was the same as that set for the electrically evoked torque measurements (see below). The double pulse superimposition technique, based on the interpolated-twitch method (20), enabled us to estimate the KE maximal voluntary activation level (%VA). The ratio of the amplitude of the superimposed double pulse over the size of a double pulse in the relaxed muscle (control doublet) was then calculated to obtain %VA as follows:

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

A control double pulse was delivered 2 s after each MVC. This procedure provides the opportunity to obtain a potentiated mechanical response, which helps reduce the variability in %VA values (15). The superimposed doublet was preferred to a superimposed twitch because it is effective in increasing the signal-to-noise ratio and thereby in detecting small changes in %VA (11). In a few cases, in which the doublet was applied when the torque level was already slightly declining, a correction was applied in the original equation, as suggested by Strojnik and Komi (30). The correction was only used for

Table 1. Physical characteristics of the subjects

	Age, yr	Height, cm	Mass, kg	Body fat percentage, %
Male	23.8±3.5	178.1±6.9	71.5±7.5	12.2±3.1%
Female	24.6 ± 4.3	166.3±4.1*	59.7±6.1*	24.4±4.0%*

Values are means \pm SD. Significantly different from male data: **P* < 0.001.



Fig. 1. Typical trace of the tetani from a representative subject. Typical trace of the torque evoked with the 20-Hz and 80-Hz stimulation (P20 and P80, respectively) using either electrical stimulation applied to the femoral nerve (NES; A) or electrical stimulation applied with large surface electrodes (ES; B).

trials in which torque level at the time of stimulation was superior or equal to 95% MVC. Other trials were discarded from analysis.

Electrically evoked torque measurements. After MVC measurements, electrical stimulation of resting KE was performed by using either ES or NES. Square-wave pulses with a width of 0.5 ms at a maximal voltage of 400 V were produced by two stimulators (both were Digitimer DS7, Welwyn Garden City, United Kingdom).

ES was delivered percutaneously via two 5.1 cm \times 10.2 cm gel pad electrodes (Compex SA, Ecublens, Switzerland) placed proximally and distally on the anterolateral thigh. The stimulation intensity, ranging from 36 to 112 mA, was set by progressively increasing the stimulus intensity of a 500-ms tetanus at a stimulation frequency of 80 Hz until the stimulation train was sufficient to evoke 50% of the subject's MVC on the day of testing. This 500-ms tetanus duration is sufficient to reach a plateau during the tetanus (Fig. 1).

NES was applied percutaneously to the femoral nerve by using a ball probe cathode pressed in the femoral triangle, 3–5 cm below the inguinal ligament. The anode, a 10.2 cm \times 5.2 cm gel pad electrode (Compex SA), was located in the gluteal fold. The stimulation intensity, ranging from 46 to 110 mA, corresponded to 110% of the optimal intensity, i.e., the stimulus intensity at which the maximal isometric twitch torque and concomitant vastus lateralis compound muscle action potential (M wave, see below) were reached. The individual NES and ES stimulation intensities were used for the fatigued condition.

The electrically evoked torque measurements comprised three single twitches and two 0.5-s tetani at a frequency of 80 and 20 Hz for each stimulation method. The stimulations were applied in the order presented in Fig. 2. A P20-to-P80 ratio (P20/P80) was also calculated; any decrease in this ratio is commonly interpreted as an index of low-frequency fatigue. The three twitches were averaged and the resulting mean responses were considered for the comparison between rest, fatigue (Post), and recovery (Post30) conditions. The following parameters were obtained from the mean twitch response: peak torque, twitch contraction time, half-relaxation time, maximal rate of



Fig. 2. Schematic view of the electrical stimulation protocol. The electrically evoked torque measurements comprised 3 single twitches and 2 0.5-s tetani at a frequency of 80 or 20 Hz (i.e., 41 and 11 stimuli, respectively). Each type of mechanical response was evoked successively with first one stimulation method and then the other. Temporal delays are displayed.

torque development, i.e., maximal value of the first derivative of the mechanical signal divided by peak torque, maximal rate of torque relaxation, i.e., maximal value of the first derivative of the mechanical signal divided by peak torque. These twitch characteristics were measured to assess any difference in motor unit recruitment between genders and/or stimulation method.

Finally, the efficiency of the stimulation method (17) was calculated for the 20 and 80 Hz tetani, as follows:

Stimulation efficiency = torque/stimulation intensity

To take into account possible gender differences in subcutaneous fat content, normalized stimulation efficiency was calculated taking into account the lean body mass:

Normalized stimulation efficiency

= torque \cdot lean body mass⁻¹ \cdot stimulation intensity⁻¹

Electromyogram recording. The EMG signal of the right vastus lateralis was recorded by using bipolar silver chloride surface electrodes of 10-mm diameter (type 0601000402, Contrôle Graphique Medical, Brie-Comte-Robert, France) during MVC and electrical stimulation. The recording electrodes were fixed lengthwise over the muscle belly with an interelectrode distance of 25 mm. The position of the electrodes was marked on the skin to fix them at the same place postexercise. The reference electrode was attached to the opposite patella. Low impedance (Z) at the skin-electrode surface was obtained $(Z < 5 \text{ k}\Omega)$ by abrading the skin with emery paper and cleaning with alcohol. Myoelectrical signals were amplified (custom-made amplifier: common mode rejection ratio = 90 dB, Z input = 1,000 M Ω , gain = 1,000) with a bandwidth frequency ranging from 1.5 Hz to 2 kHz and simultaneously digitized online (Tida ITC 16, Heka Elektronik, Lambrecht/Pfalz, Germany; sampling frequency 2,000 Hz). The root mean square (RMS) value of the M-wave (RMS_M) obtained with NES was determined during the maximal twitches. The window size was adjusted to the size of the M-wave, to take into account the distinct phases of the M-wave, i.e., all the modifications of the resting potential. In addition, an RMS value was calculated during the MVC trials (RMS_{MVC}) over a 0.5-s period after the torque had reached a plateau and before the surperimposed stimulation was evoked. All mechanical and EMG data were stored with commercially available software (Tida 5.0, Heka Elektronik).

Stimulation-induced pain. Pain associated with 20- and 80-Hz stimulation using either ES or NES was evaluated by use of visual analog scales consisting of a 100-mm horizontal line with an item at each extremity: from "no pain" to "very very sore." Subjects were asked to put a vertical mark on the horizontal line to describe the pain associated with each frequency and each type of stimulation. The distance between the beginning of the line and the vertical mark was used as the pain score.

Body fat percentage. Body fat percentage, based on skinfold thickness measurements, was assessed by using a Harpenden skinfold caliper (Harpenden skinfold caliper, British Indicators, Burgess Hill, West Sussex, Great Britain). Skinfolds measured at bicipital, tricipital, subscapular, and suprailiac sites (5) were used to determine body density from which body fat percentage was calculated (27). All skinfolds were the average of three measurements assessed on the right side of the body, always by the same observer.

Statistics. All descriptive statistics presented are mean values \pm SD. Normal distribution was checked by using a Shapiro-Wilk test of normality. Each study variable was then compared between rest, Post, and Post30 conditions by a three-way (gender \times stimulation type \times time) ANOVA with repeated measures. Newman-Keuls post hoc tests were applied to determine between-means differences if the ANOVA revealed a significant main effect for time or interaction of stimulation type \times time or gender \times stimulation type \times time. Morphological variables were compared with a single-tailed Student's *t*-test. For all statistical analyses, a *P* value of 0.05 was accepted as the level of significance.

RESULTS

Male and female subjects differed on number of morphological variables (Table 1), especially regarding body fat percentage, which was twice as high in female compared with male subjects. Stimulation intensity did not differ either between ES and NES, or between genders (female ES and NES: 75.6 ± 21.1 mA vs. 73.4 ± 14.5 mA, respectively; male ES and NES: 84.3 ± 18.5 mA vs. 73.0 ± 16.3 mA, respectively). NES supramaximal stimulation evoked torque levels equal to $94.5 \pm 8.1\%$ MVC during high-frequency tetanus. ES-evoked torque levels reached $55.2 \pm 3.2\%$ MVC.

For the 80-Hz-stimulation at rest, stimulation efficiency displayed significant gender × stimulation efficiency interaction (P < 0.05). Whereas no gender difference was observed for ES (male: 1.11 ± 0.25 vs. female: 1.00 ± 0.41 N·m/mA), male subjects displayed greater stimulation efficiency for NES compared with female subjects (2.26 \pm 0.51 vs. 1.65 \pm 0.63 N·m/mA; P < 0.05). The same tendency was observed for the 20-Hz stimulation, but the interaction failed to reach the significance level (P = 0.09). These tendencies disappeared when stimulation efficiency was normalized to lean body mass. Normalized stimulation efficiency only displayed a stimulation method effect: NES proved to be more efficient in evoking torque than ES, both for the 80-Hz (0.037 \pm 0.011 vs. 0.020 \pm 0.008 N·m·kg lean body mass⁻¹·mA⁻¹; P < 0.001) and the 20-Hz stimulation (0.028 \pm 0.01 vs. 0.014 \pm 0.006 N·m·kg lean body mass⁻¹·mA⁻¹; P < 0.001).

Voluntary contractions. MVC data displayed a significant time effect (P < 0.001). Torque decreased at all points in time postexercise with no significant recovery between Post and Post30 (Table 2). This significant impairment occurred concurrently with a significant reduction of %VA and RMS_{MVC} over time (P < 0.001 and P < 0.01, respectively; see Table 2). At Post30, RMS_{MVC} decline just failed to reach the significance level (P = 0.06). None of these variables showed any gender difference when the relative decrease was considered. In addition, estimation of %VA did not differ according to the method of stimulation.

Electrically evoked contractions. Interestingly, the P20/P80 relative decrease did not differ between the two methods of

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Table 2. Relative decrements	in maximal voluntary
contraction torque, voluntary	activation, and root mean
square value after and 30 mil	n after the eccentric exercise

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	MVC, %	%VA (ES)	%VA (NES)	EMG RMS _{MVC} , %
Male				
Post	-13.6 ± 5.5 †	$-3.0\pm5.1*$	$-1.9\pm5.0*$	$-11.1\pm16.4*$
Post30	-13.6 ± 4.8 †	-3.7 ± 5.4 †	-0.9 ± 2.9 †	-9.7 ± 18.4
Female				
Post	-16.4 ± 11.5 †	$-5.7\pm4.7*$	$-5.2\pm7.9*$	$-16.9\pm22.7*$
Post30	-17.0 ± 8.6 †	-5.4 ± 5.2 †	-5.7 ± 7.3 †	-6.8 ± 22.2

Values are means \pm SD. Relative decrements in maximal voluntary contraction torque (MVC), voluntary activation level [%VA; obtained either with transcutaneous (ES) or motor nerve stimulation (NES)], and electromyographic signal root mean square value (EMG RMS_{MVC}) of the vastus lateralis muscle. These measurements were performed in female and male subjects after (Post) and 30 minutes after (Post30) the fatiguing exercise. Significantly different from rest condition: *P < 0.01; †P < 0.001. No gender or stimulation method difference was observed.

stimulation. The low-to-high frequency ratio only demonstrated a significant time effect (Fig. 3). It was reduced at Post (P < 0.001) and Post30 (P < 0.001) and significantly recovered between these points in time (P < 0.001).

Mechanical response to 20-Hz and 80-Hz tetani displayed significant time \times gender \times stimulation method interaction (P < 0.05 and P < 0.001, respectively; Fig. 4). Both stimulation methods detected significant P20 and P80 reductions at Post and Post30. Overall, compared with NES, ES tended to detect a greater impairment at Post in male and a lesser one in female subjects at both Post and Post30.

At rest, twitch characteristics did not show any stimulation method \times gender interaction (see Table 3).

M-wave characteristics. RMS_M demonstrated a significant time × stimulation method combined effect (P < 0.01). RMS_M tended to decrease at Post ($-8.0 \pm 11.3\%$; NS). At Post30, this decrement was significant ($-14.6 \pm 12.1\%$; P < 0.01).

Stimulation discomfort. As shown in Fig. 5, the stimulationinduced perceived discomfort demonstrated significant stimulation method effect (P < 0.001) and gender \times frequency interaction (P < 0.01). ES was 1.5 to 2 times more comfortable than NES. For men, low-frequency stimulation was less painful than high frequency. This difference was not apparent in female subjects.

DISCUSSION

Our results suggest that, after a 30-min downhill run, relative low-to-high frequency ratio decreases evoked via ES and NES were similar. When evoked torque decrements were evaluated, both ES and NES were able to detect neuromuscular fatigue effects but were not consistent on the magnitude of these reductions. As expected, submaximal surface muscle stimulation appeared more comfortable than supramaximal percutaneous nerve stimulation.

Stimulation-induced discomfort. ES appeared less painful than NES. On the one hand, this cannot be the result of stimulation intensity, because ES and NES did not differ in the intensity level set to evoke muscle contractions. On the other hand, the current density is more diffuse with larger electrodes. Thus the larger size of ES stimulation electrodes, compared with NES, could have contributed to the minimization of electrically induced pain (1).

Exercise-induced fatigue: comparison with the literature. The 30-min downhill run induced a 15% MVC decrease together with a slight decline of %VA and RMS_{MVC} . The fatiguing exercise also induced a significant reduction in the P20/P80 over time, together with a general impairment of electrically evoked torques. M-wave characteristics were also altered after the downhill run. These results are in line with previous work on neuromuscular fatigue after eccentric-type exercise involving KE (19). In this study, we demonstrated that both central, i.e., decline of voluntary activation level, and peripheral, i.e., low-frequency fatigue, components contributed to the 19.6% KE MVC decline observed after an intermittent one-legged downhill run. If low-frequency fatigue is commonly observed after eccentric exercise (8, 23), the experimental evidence for the presence of central fatigue is rather scarce (2, 18, 19, 21). However, it is generally believed that the contribution of this central component to MVC decline may be weak. Peripheral fatigue, i.e., low-frequency fatigue and decreased sarcolemmal excitability, explains most of the MVC decrement in the present study. It is also worth noting that the estimation of %VA was independent of gender and stimulation method. This result is consistent with previously published literature on twitch-interpolation methodology (25).

Electrically evoked contractions. To our knowledge, the present report is the first to compare ES and NES in the

Fig. 3. Low-to-high frequency ratio (P20/P80) modifications after the fatiguing exercise. P20/P80 relative decrements were obtained in female and male subjects by using either electrostimulation (ES) or neurostimulation (NES) immediately after (Post) and 30 min after (Post30) the downhill run. Values are means \pm SD. Significant difference with rest condition: ***P < 0.001. Significant difference between Post and Post30: $\dagger \dagger P < 0.001$.



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Fig. 4. Evoked mechanical responses under fatigued and recovery conditions evaluated by ES and NES. Relative decrements of mechanical responses to 80-Hz (P80; *A*) and 20-Hz stimulation (P20; *B*). Variables were measured immediately after (Post) and 30 min after (Post30) the downhill run. Values are means \pm SD. Significant differences with rest condition: *P < 0.05; **P < 0.01; ***P < 0.001. Significant differences between stimulation methods: \$P < 0.05; \$\$P < 0.01; \$\$\$P < 0.001. Gender differences are unmarked.

evaluation of low-frequency fatigue. Previous attempts were aimed at comparing torques evoked at different voltages using ES (4, 6, 9, 12). However, all these studies have only used submaximal torques (<70%MVC). As stated in the introduction, this choice may involve a number of limitations.

In the present study, the comparison of transcutaneous vs. motor nerve stimulation of the quadriceps revealed that the P20/P80 behavior under fatigued and recovery conditions was influenced neither by the method employed to elicit muscle contraction nor by gender. As a result, low-frequency fatigue appeared to be accurately assessable using ES. This result can be linked to the report by Edwards et al. (6) on sternomastoid muscle in which the P20/P50 ratio was demonstrated to be stable across a range of submaximal voltages under fatigued conditions. Hanchard et al. (12) drew the same conclusions for the tibialis anterior muscle using torque levels comprising between 10 and 33% MVC. However, conflicting results were

 Table 3. Main mechanical characteristics of the evoked

 twitch before the fatiguing exercise

	Pt, N·m	CT, ms	HRT, ms	MRTD, s ⁻¹	MRTR, s ⁻		
Male							
EMS	19.3 ± 5.4^{e}	91.9 ± 17.3	67.2 ± 23.8^{d}	26.5 ± 5.3	14.9 ± 4.1		
NES	40.1 ± 9.9	96.2 ± 18.7	77.2 ± 13.7	27.0 ± 3.1	12.9±1.9		
Female							
EMS	$14.3 \pm 3.5^{\text{be}}$	98.0 ± 16.6	57.5 ± 17.6^{d}	22.4 ± 4.7^{a}	15.7 ± 3.6		
NES	30.6 ± 7.9^{b}	93.5 ± 13.9	74.5 ± 13.2	$21.9\!\pm\!4.5^{\mathrm{a}}$	13.7 ± 3.0		

Values are means \pm SD. Peak torque (Pt), twitch contraction time (CT), half relaxation time (HRT), maximal rate of torque development (MRTD), and maximal rate of torque relaxation (MRTR) were obtained from male and female subjects by either the ES or NES method. Significantly different from male data: ^a P < 0.05; ^b P < 0.01. Significantly different from NES method: ^c P < 0.05; ^d P < 0.01; ^e P < 0.001.

obtained by Davies and White (4) on the plantarflexor muscles. The reasons for such a discrepancy are unclear, but it is possible that voltage (or intensity) dependency may differ between muscle groups (12).

If both methods similarly detected the presence of lowfrequency fatigue, they also revealed a general alteration of electrically evoked torques. Qualitatively, both stimulation methods detected an impairment in P20 and P80 but were not in agreement on the magnitude of these decreases. Overall, ES tended to detect a greater impairment at Post in male and a lesser one in female subjects at both Post and Post30. The reasons for these differences are unclear. A possible explanation may be that different motor unit pools are recruited when male and female subjects are stimulated



Fig. 5. Perception of electrically induced discomfort. Electrically induced discomfort perceived by male and female subjects was measured for electrostimulation at 80-Hz (ES 80) and 20-Hz (ES 20) frequencies. Discomfort was also evaluated for neurostimulation at 80 Hz (NES 80) and 20 Hz (NES 20). Values are means \pm SD. Significant difference between ES and NES: \$\$P < 0.001. Significant difference between 80- and 20-Hz frequencies: \$\$P < 0.001.

by ES. However, the analysis of the twitch characteristics does not support this hypothesis. Another possibility is that the greater subcutaneous fat content in women may have influenced their response to ES stimulation. It is worth noting that stimulation intensities did not differ between genders. One could suppose that the higher subcutaneous fat content in female subjects may have altered stimulation efficiency in this group of subjects. However, it must be remembered that men have a greater muscle mass. Because cross-sectional area was not measured in the present study, we normalized evoked torque to lean body mass. When evoked torque was expressed in newton-meters per kilogram of lean body mass, there was no longer any difference between genders for a given stimulation intensity, suggesting that the stimulation efficiency did not differ between men and women, whatever the stimulation method. According to Lieber and Kelly (17), the ability to evoke muscle torque via transcutaneous stimulation is not simply a matter of applying high currents to small and lean individuals. Geometric factors related to the location of the recruited motor units with respect to the stimulating electrodes as well as superficial patterns of motor nerve branching could also influence muscle response to ES (3, 14). In the present study, differences in muscle architecture could have contributed to gender and stimulation method differences in the evaluation of P20 and P80 decreases after the fatiguing exercise, although this is not supported by any scientific evidence. In any case, it is important to note that these differences did not affect the P20/P80, which did not differ, either between ES and NES or between genders.

In conclusion, the results presented here suggest that, after a 30-min downhill run, relative decrements in low-to-high frequency ratios evoked via transcutaneous and motor nerve stimulation were not different. Thus submaximal torques evoked via ES proved to be valid in evaluating low-frequency fatigue. In estimating evoked torque decrements, both ES and NES were able to detect fatigue effects, although results from the two methods were not in agreement on the magnitude of these reductions. The reasons for this discrepancy remain unclear, but differences in muscle architecture might be involved. Because ES proved to be less painful that NES, these results may have important practical implications for the evaluation of low-frequency fatigue in sensitive populations such as patients or aged subjects.

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