Comparison of electrical and magnetic stimulations to assess quadriceps muscle function

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Verges S, Maffiuletti NA, Kerherve H, Decorte N, Wuyam B, Millet GY. Comparison of electrical and magnetic stimulations to assess quadriceps muscle function: electrical and magnetic quadriceps stimulations. J Appl Physiol 106: 701–710, 2009. First published 28 August 2008; doi:10.1152/japplphysiol.01051.2007.—This study aimed to 1) compare electrical and magnetic stimulations for quadriceps muscle function assessment, and 2) ascertain whether the ratios of the second twitch elicited by supramaximal electrical and magnetic femoral nerve stimulation at 10 and 100 Hz (T210:100) and the total twitch force elicited by the same types of stimulations (Fpaired10:100) are equivalent to the standard low-–high-frequency force ratio associated with submaximal electrical tetanic stimulations (Ftet10:100). Quadriceps force and vastus lateralis EMG were recorded at rest (n = 21 subjects), immediately after, and 30 min after a 30-min downhill run (n = 10) when 1) supramaximal electrical nerve stimulation (ENS), 2) magnetic nerve stimulation (MNS) and 3) submaximal electrical muscle stimulation (EMS) were delivered in random order at 1 (single stimulation), 10, and 100 Hz (paired stimulations). Ten- and 100-Hz 500-ms tetani were also evoked with EMS to determine Ftet10:100. Before exercise, contractile properties with single and paired stimulations were similar for ENS and MNS (all intraclass correlation coefficients k > 0.90), but smaller for EMS (P < 0.001). M-wave characteristics were also similar for ENS and MNS (all k > 0.90). After exercise, changes in all parameters did not differ between forces. With fatigue, the changes in Ftet10:100 were inconsistently correlated with the changes in T210:100 (r² = 0.24–0.73, P = 0.002–0.15) but better correlated with the changes in Fpaired10:100 (immediately after exercise: r² = 0.80–0.83, P < 0.001; 30 min after exercise: r² = 0.46–0.82, P = 0.001–0.03). We conclude that ENS and MNS provide similar quadriceps muscle function assessment, while Fpaired10:100 is a better index than T210:100 of low- to high-frequency fatigue of the quadriceps in vivo.

TO ASSESS MUSCLE FUNCTION, artificial stimulation is widely used both in research and clinical settings. Many studies have specifically investigated the quadriceps femoris because it is a primary locomotor muscle. Percutaneous electrical stimulation was used originally, mainly by placing large stimulating elec-

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of the phrenic nerve in human and of isolated rat diaphragmatic strips, Yan et al. (31) showed that the force evoked by paired stimulations can be viewed as the sum of two successive responses and that the ratio of the second twitch (T2) obtained by subtracting the force response to a single twitch from the force response to paired stimulations at 10 Hz over 100 Hz (T2_{10:100}) may provide similar information as tetanic force at 10 Hz over 100 Hz (F_{tet10:100}). Paired stimulations were used in subsequent studies to detect low and high frequency fatigue (1, 2, 11, 15, 23, 24), but to our knowledge paired stimulations have never been compared with tetanic stimulations for quadriceps function assessment.

We aimed to compare the outcomes of electrical and magnetic stimulations before and after an eccentric exercise to establish the degree of agreement between different methods and indices commonly used to assess quadriceps muscle function. We systematically examined quadriceps force and vastus lateralis M-wave responses obtained with ENS, MNS, and EMS with either single or paired stimulations. Moreover, we compared the T2_{10:100} ratio obtained with paired stimuli via ENS, MNS, and EMS to the F_{tet10:100} ratio obtained with tetanic quadriceps stimulation via submaximal EMS as commonly performed in the literature (see Electrical Nerve Stimulation). The experimental setup and stimulation protocol are described in Fig. 1. Three single stimulations were first delivered via ENS, MNS, and EMS, in a random order. The same procedure was repeated for paired stimulations at 10 Hz (100-ms interstimulus interval) and then at 100 Hz (10-ms interstimulus interval). Afterward, three stimulation trains at 10 Hz and three trains at 100 Hz were delivered via EMS. All stimulations were separated by 20-s resting intervals. Lastly, the subjects performed three maximal voluntary contractions (MVC) to assess MVC force. Subjects were instructed to reach maximal force in 1 s and then to maintain this level for 4 s while receiving strong verbal encouragements. No visual feedback was provided.

All subjects were investigated in the unfatigued state. Ten subjects were also assessed 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 (S 2500, Tecmachine, Andrezieux-Bouthéon, France) (18). Because potentiated twitches were shown to be more sensitive to fatigue than unpotentiated twitches (13), each MVC was followed by three single stimulations to obtain potentiated twitches, which were delivered every 4 s via ENS, MNS, and EMS, in a random order.

**MATERIALS AND METHODS**

**Subjects**

Twenty-one healthy and physically active male subjects completed the study. The average (± SD) age, body mass, and height were 30 ± 6 y, 78 ± 7 kg, and 181 ± 6 cm, respectively. The study was approved by the local ethics committee and performed according to the Declaration of Helsinki. All subjects gave their written informed consent to participate in the study.

**Experimental Setup**

All the assessments were performed on the right quadriceps femoris muscle under isometric conditions. Subjects lay supine on a table with the right knee joint angle set at 90° of flexion. A noncompliant strap connected to a strain gauge (SBB 200 kg, Tempo Technologies, Taipei, Taiwan) was attached around the subject’s shank, 3–5 cm above the tip of the lateral malleolus. The subjects were secured to the table with noncompliant straps to minimize body movement. Subjects were at rest for at least 20 min before the first measurements, permitting skin preparation, electrode placing, and determination of the stimulation intensities (see Electrical Nerve Stimulation).Fig. 1. *A*: Experimental setup: subject was secured to the table with noncompliant straps, and a cut pad was placed underneath right thigh to allow biceps femoris electromyographic electrode placement. *B*: Stimulation protocol: single (1 Hz) and paired (10 Hz and 100 Hz) stimulations were delivered via electrical nerve stimulation (ENS), magnetic nerve stimulation (MNS), and electrical muscle stimulation (EMS). Then, 500-ms stimulation trains at 10 Hz and 100 Hz were delivered with EMS (EMS_{10:100}). Finally, maximal voluntary contractions (MVC) were performed, followed by single stimulations (1 Hz) with ENS, MNS, and EMS. See MATERIALS AND METHODS for details. *Pre-post exercise measurements only.*

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Electrical Nerve Stimulation

ENS was delivered percutaneously to the femoral nerve by a cathode electrode (20-mm diameter) pressed in the femoral triangle, 3–5 cm below the inguinal ligament. The anode, a 10.2-cm × 5.2-cm gel pad electrode (Compex SA, Ecublens, Switzerland), was located over the gluteal fold. For both single and paired stimulations, square-wave pulses (1-ms duration) were produced via a high-voltage (maximal voltage 400 V) constant-current stimulator (Digitimer DS7, Hertfordshire, UK). For all stimulus modalities, stimulation intensity corresponded to ~120% of the optimal intensity (range 20–90 mA), i.e., the stimulus intensity at which the maximal amplitude of both twitch force and concomitant vastus lateralis M-wave (see Electromyographic Recordings) were reached.

Magnetic Nerve Stimulation

Femoral nerve stimulation was performed with a 45-mm figure-of-eight coil powered by two linked Magstim 200 stimulators (peak magnetic field strength 2.5 T, stimulation duration 0.1 ms; Magstim, Whitland, Dyfed, United Kingdom). The linking circuitry (Bistim Module, Magstim) was capable of precisely controlling the inter-stimulus interval between 1 and 999 ms to an accuracy of within 0.05 ms. One stimulator was used for single stimulations, while both stimulators were used for paired stimulations. All stimuli were given at maximum stimulator output (22). The stimulating coil head was positioned high in the femoral triangle just lateral to the femoral artery; the best spot allowing the maximal force and concomitant vastus lateralis M-wave was determined with minor adjustments and then marked on the skin for the remainder of the experiment.

Electrical Muscle Stimulation

EMS was delivered percutaneously via two 5.1-cm × 5.1-cm gel pad electrodes (Compex SA) placed on the motor point of the vastus lateralis and vastus medialis muscles and one 5.1-cm × 10.2-cm dispersive electrode positioned proximally, ~5 cm below the femoral triangle (see Fig. 1A). For both single and paired stimulations, square-wave pulses (1-ms duration) were produced via the same constant-current stimulator as for ENS. Individual stimulation intensity was progressively increased until peak twitch force attained 50% of the peak twitch force obtained via ENS (range 65–100 mA). For stimulation trains, 500-ms tetani (EMS_{tet}) were delivered, since this duration allows a force plateau to be reached (18).

Electromyographic Recordings

The electromyographic (EMG) signal was recorded from the right vastus lateralis (as a surrogate for quadriceps muscles, see Ref. 20) and biceps femoris muscles (as a surrogate for antagonist hamstring muscles) with two pairs of silver chloride surface electrodes of 20 mm diameter (universal ECG electrode, Control Graphic Medical, Brie Comte Robert, France) during ENS and MNS (electromyographic signals are not available during EMS due to interference with the electrical stimulation). Low resistance (<10 kΩ) between the two electrodes was obtained by light abrasion of the skin and cleaning with alcohol. Recording electrode locations were based on SENIAM recommendations (10) with an inter-electrode distance of 25 mm. The reference electrode was fixed over the patella. 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). Electromyographic data together with force signals were digitized online at a sampling frequency of 2,000 Hz and recorded by acquisition card (DAQCard-6062E, National Instruments, Austin, Texas), administered by the Imago software developed under Labview (National Instruments).

Data Analysis

The following parameters were calculated from the mean force response obtained for each type of stimulation (i.e., the average of the three single or paired stimulations): peak force; time to peak force, i.e., the time elapsed between the twitch onset and the peak force; and maximal rate of force development (MRFD) and relaxation (MRFR), i.e., respectively, the highest and the lowest value of the first derivative of the force signal. The following parameters were calculated from the M-wave traces: peak-to-peak duration, peak-to-peak amplitude, and area. These force and electromyographic parameters were calculated to assess any difference in contractile and M-wave characteristics between stimulation modalities.

The effect of the interstimulus interval on the amplitude of the force elicited by the second stimulus (T2) was obtained by digitally subtracting the mean force response of the single twitches from the mean of the paired responses at a given interstimulus interval (21, 31). The T210:100 was obtained by division of the T2 force response at 10 Hz by the T2 force response at 100 Hz. The T210:100 ratios obtained with ENS, MNS, and EMS were compared with the ratio of the tetanic forces measured with EMStet for stimulation trains at 10 Hz and 100 Hz (Ftet10:100). The ratios of paired stimulation peak forces at 10 Hz over 100 Hz (Fpaired10:100) were also compared with Ftet10:100.

All descriptive statistics presented are mean values ± SD. Each variable was compared between stimulation methods (ENS, MNS, EMS) by means of a two-factor ANOVA with repeated measures (methods × stimulation frequencies or methods × time). When significant main effects were found, the Fischer’s p-test was used for post hoc analysis. To quantify the agreement between the measurements obtained with the different stimulation techniques (ENS, MNS, EMS) when no statistical difference was observed, intraclass correlation coefficients and Bland-Altman plots (3) were used. Intraclass correlation coefficients (type 2,1; k) were calculated with a two-way random effects model with single-measure reliability in which variance over the repeated session is considered (25). Pearson’s product-moment correlations were used to assess the agreement between Ftet10:100 and T210:100 ratios and between Ftet10:100 and Fpaired10:100. Fisher’s R-to-Z test was used to determine the statistical significance of the correlations. All statistical calculations were performed on standard statistics software (Statview 5.0, SAS Institute, Cary, North Carolina). Significance was set at P < 0.05.
RESULTS

Force and electromyographic recordings for ENS, MNS, and EMS are shown in Figs. 2 and 3 from one representative subject.

Measurements in the Unfatigued State (n = 21)

Peak forces. Mean quadriceps peak forces for ENS, MNS, and EMS are shown in Fig. 4. Similar quadriceps forces were obtained with ENS and MNS (1 Hz, k = 0.91; 10 Hz, k = 0.94; 100 Hz, k = 0.94), while EMS induced significantly smaller forces compared with both ENS and MNS.

Peak forces during ENS, MNS, and EMS were: 20 ± 5%, 19 ± 5%, and 10 ± 3% of MVC at 1 Hz; 34 ± 8%, 33 ± 9%, and 18 ± 4% of MVC at 10 Hz; and 41 ± 7%, 41 ± 8%, and 23 ± 4% of MVC at 100 Hz, respectively. Figure 5 shows Bland-Altman plots of individual peak forces obtained with ENS and MNS at 1, 10, and 100 Hz.

T2 peak forces at 10 Hz (ENS: 203.2 ± 50.7 N; MNS: 188.9 ± 52.0 N; EMS: 114.3 ± 30.4 N) and 100 Hz (ENS: 197.8 ± 35.8 N; MNS: 189.9 ± 44.6 N; EMS: 121.1 ± 30.7 N) were similar for ENS and MNS (1 Hz: k = 0.93, 0.94, and 0.95, respectively; 10 Hz: k = 0.94, 0.94, and 0.95, respectively; 100 Hz: k = 0.93, 0.98, and 0.92, respectively), while MRFD and MRFR were significantly lower for EMS compared with both ENS and MNS.

Contractile properties. Quadriceps contractile properties associated with single and paired stimulations are shown in Table 1. Time to peak force, MRFD, and MRFR were similar for ENS and MNS (1 Hz: k = 0.93, 0.94, and 0.95, respectively; 10 Hz: k = 0.94, 0.94, and 0.95, respectively; 100 Hz: k = 0.93, 0.98, and 0.92, respectively), while M-wave characteristics. Vastus lateralis M-wave duration (ENS: 9.5 ± 2.4 ms; MNS: 9.4 ± 2.3 ms; k = 0.99), amplitude (ENS: 54.8 ± 15.4 mV; MNS: 54.3 ± 15.0 mV; k = 0.98), and
Measurements in the Fatigued State (n = 10)

Contractile properties associated with single and paired stimulations as well as M-wave characteristics for ENS, MNS, and EMS before and after exercise are shown in Fig. 7, Table 2, and Table 3, respectively. After exercise, peak force, time to peak force, M-wave duration, and area were significantly reduced, while MRFD, MRFR, and M-wave amplitude were not significantly modified. No differences were observed between ENS and MNS parameters, while peak force, MRFD, and MRFD were significantly lower for EMS compared with ENS and MNS. Postexercise changes expressed as a percentage of pre-exercise values were not significantly different between methods (all \( P > 0.05 \)).

\( T_{210:100} \) obtained via ENS, MNS, and EMS as well as \( F_{tet10:100} \) were significantly reduced after exercise (Fig. 8). The reduction in \( T_{210:100} \) for ENS and MNS was significantly smaller than the reduction in \( T_{210:100} \) for EMS. Also, the reduction in \( F_{tet10:100} \) after exercise was significantly higher than the reduction in \( T_{210:100} \) for ENS, MNS, and EMS. With all types of stimulation, \( F_{paired10:100} \) was reduced after exercise to an extent similar to \( T_{210:100} \) (all \( P > 0.1 \)) but less than \( F_{tet10:100} \) (all \( P < 0.001 \)).

Correlations between postexercise changes in \( F_{tet10:100} \) and postexercise changes in \( T_{210:100} \) or \( F_{paired10:100} \) are shown in Table 4. Immediately after exercise, the change in \( F_{tet10:100} \) was significantly correlated with the changes in \( T_{210:100} \) for

### Table 1. Quadriceps contractile properties associated with single and paired stimulations in the unfatigued state

<table>
<thead>
<tr>
<th>Time to peak force, ms</th>
<th>MRFD, N/ms</th>
<th>MRFR, N/ms</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>ENS</td>
<td>MNS</td>
</tr>
<tr>
<td></td>
<td>ENS</td>
<td>MNS</td>
</tr>
<tr>
<td></td>
<td>ENS</td>
<td>MNS</td>
</tr>
<tr>
<td>1 Hz</td>
<td>87.5 ± 12.0</td>
<td>88.0 ± 12.2</td>
</tr>
<tr>
<td>10 Hz</td>
<td>168.7 ± 10.3</td>
<td>168.5 ± 9.4</td>
</tr>
<tr>
<td>100 Hz</td>
<td>108.7 ± 12.9</td>
<td>110.8 ± 13.5</td>
</tr>
</tbody>
</table>

Values are means ± SD, n = 21. 1 Hz, single stimulations; 10 and 100 Hz, paired stimulations; ENS, electrical nerve stimulation; MNS, magnetic nerve stimulation; EMS, electrical muscle stimulation; MRFD, maximal rate of force development; MRFR, maximal rate of force relaxation. *\( P < 0.05 \) compared with ENS and MNS.
MNS and EMS as well as with the changes in \( F_{\text{paired}10:100} \) for all types of stimulation. Thirty minutes after exercise, changes in \( F_{\text{tet}10:100} \) were not significantly correlated with changes in \( T_{2,10:100} \) but were still significantly correlated with \( F_{\text{paired}10:100} \) for all types of stimulation.

**DISCUSSION**

To our knowledge, this is the first study to compare electrical and magnetic stimulation methods to assess quadriceps function. Force and M-wave characteristics associated with single and paired stimulations were similar for ENS and MNS, both before and after a fatiguing exercise. Also, the exercise-induced reduction in \( F_{\text{tet}10:100} \) did not consistently correlate with the reductions in \( T_{2,10:100} \) for the three stimulation modalities. Rather, while the amplitude of the changes are different, the reduction in \( F_{\text{tet}10:100} \) after exercise was better related to the reduction in the low- to high-frequency ratio calculated from peak forces during paired stimulations (\( F_{\text{paired}10:100} \)).

**Magnetic Stimulation to Assess Quadriceps Muscle Function**

The use of MNS was described ten years ago as a new method to assess quadriceps function. Since then, it has been widely used in research settings as well as in clinical practice (1, 9, 15, 17, 24, 29). However, the specificity of magnetic stimulation may present some limitations for muscle function assessment. First, stimulation supramaximality, i.e., the fact that further increase in stimulation intensity gives no further response in both electrical and mechanical response of the muscle, is critical when artificial stimulation is used to assess muscle function. Due to limited power output of commercially available magnetic stimulators, supramaximal stimulation may be difficult to achieve, since maximal muscle response may be reached at or near the maximal power output of the stimulator (9). Second, because the magnetic field is relatively wide and goes deep into the tissue (17), stimulation is less focalized and therefore risks of coactivation may arise. Wragg et al. (30), for example, reported significantly smaller twitch transdiaphragmatic pressure with electrical compared with magnetic phrenic nerve stimulation, potentially due to coactivation of other muscle groups with the latter modality (14). Conversely, with ENS, supramaximal stimulation can be easily achieved because stimulation intensity can be increased without technical limitations. Moreover, the electric field can be selectively applied to the femoral nerve by pressing a small electrode in the femoral triangle. By comparing ENS to MNS, the present results showed that quadriceps force and vastus lateralis M-wave characteristics of single and paired stimulations were very similar for ENS and MNS, as shown by \( k \) values > 0.90 and Bland-Altman analysis (Figs. 4 and 5). Therefore, despite
the fact that supramaximaly of MNS in the present study was verified only a posteriori, these findings confirm that stimulation at 100% of the magnetic stimulator power output can be supramaximal as previously assumed in our and other laboratories (7, 15, 22, 24, 29). Moreover, the quasi-absence of biceps femoris electromyographic activity during both ENS and MNS suggested that potential coactivation of the antagonist muscles did not affect contractile properties assessment.

Furthermore, changes in force and M-wave characteristics via single and paired stimulations following exercise were similar for ENS and MNS, suggesting that both methods are equally sensitive to exercise-induced fatigue. A critical point when twitches are used to assess muscle fatigue following voluntary contractions (16, 22). In the present study, stimulations performed immediately after running were potentially influenced by the potentiation effect of exercise. However, we believe that this potential effect does not influence our results regarding comparisons of different quadriceps stimulations modalities, because 1) stimulation modalities were performed in random order and were therefore similarly influenced by the exercise-induced potentiation, 2) comparisons between stimulation modalities provide similar results immediately after exercise and 30 min after exercise (i.e., when the effect of potentiation was abolished), and 3) comparison of potentiated single stimulations also shows no difference between ENS and MNS. Hence, the present results indicated that ENS and MNS provide similar outcomes regarding quadriceps muscle function. While subjects’ spontaneous reports also indicated no major discomfort with ENS and MNS, MNS may have the advantage of being easier to handle in clinical practice (17). Also, while voluntary muscle activation was not evaluated in the present study, recent results suggest that electrical and magnetic stimulations of the quadriceps provide similar results regarding quadriceps activation levels (19). Thus these results confirm that magnetic stimulation is an attractive tool to assess muscle function both for clinical and research purposes and further developments, as increase in maximal stimulator power output or repetitive stimulations (27) may improve the potential of this technique.

### Paired Stimulations to Assess Quadriceps Muscle Function

The use of paired stimulation to deduce the low- to high-frequency force and fatigue in isolated muscle is long established (5). Figure 4 shows that the increment in force when stimulation frequency increases differ when measured with paired stimulations or stimulation train. Previous studies showed indeed that force-frequency relationships depend on the number of impulses in the train (31). Despite these differences, paired stimulations on muscles in vivo have been proposed as a surrogate for stimulation trains to assess force-frequency ratio (21, 31). Yan et al. (31) suggested that the ratio of T2 at 10 Hz over 100 Hz (T2_{10:100}) may provide similar information compared with tetanic force at 10 Hz over 100 Hz (Ftet_{10:100}). These authors indeed observed that T2_{10:100} and

### Table 3. Vastus lateralis M-wave characteristics for ENS and MNS

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>Post30</th>
</tr>
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<tbody>
<tr>
<td>ENS</td>
<td>8.5±2.0</td>
<td>7.3±2.1</td>
<td>8.0±2.2</td>
</tr>
<tr>
<td>MNS</td>
<td>8.4±1.9</td>
<td>7.3±2.0</td>
<td>7.9±2.0</td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENS</td>
<td>65.0±9.4</td>
<td>61.6±8.1</td>
<td>60.5±9.5</td>
</tr>
<tr>
<td>MNS</td>
<td>63.4±7.5</td>
<td>61.5±8.1</td>
<td>60.4±9.7</td>
</tr>
<tr>
<td>Area, mV·s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENS</td>
<td>0.48±0.09</td>
<td>0.41±0.06</td>
<td>0.41±0.04</td>
</tr>
<tr>
<td>MNS</td>
<td>0.47±0.08</td>
<td>0.41±0.07</td>
<td>0.41±0.04</td>
</tr>
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</table>

Values are means ± SD, n = 10.
Ftet10:100 ratios measured before and after fatiguing contractions were highly correlated in isolated rat diaphragm strips. However, paired stimuli and stimulation trains were not compared on the diaphragm in vivo, because phrenic nerve stimulation trains are very painful. In the present study, T210:100 and Ftet10:100 were compared on the quadriceps in vivo. In the unfatigued state, although values comparable to previous studies were obtained (21, 24, 31), correlation between these ratios were weak when T210:100 was measured via EMS and MNS (r² < 0.3) and moderate when T210:100 was measured via EMS (r² = 0.50). After exercise, the reduction in T210:100 was smaller than the reduction in Ftet10:100. Also, exercise-induced changes in T210:100 and Ftet10:100 were inconsistently correlated, with significant correlations immediately after exercise with T210:100 measured via MNS and EMS only but no significant correlations 30 min after exercise. Therefore, these results taken as a whole do not confirm that T210:100 and Ftet10:100 ratios are equivalent for low-frequency fatigue detection can be performed with Ftet10:100. This result is important in a clinical perspective, as stimulation trains inducing discomfort in patients and unfeasible with most of the available magnetic stimulators could be replaced by paired stimulations while providing similar information.

In conclusion, the present study indicated that ENS and MNS provided similar force and M-wave responses to single and paired stimulations both in the unfatigued and fatigued quadriceps muscle. Therefore, contrary to other muscle groups like inspiratory muscles, both stimulation modalities can be considered as equivalent for quadriceps muscle function assessment. While previous results on isolated muscles suggest that T210:100 and Ftet10:100 may be equivalent to percentage changes in low- to high-frequency ratio of peak forces for paired stimulations is a better surrogate for stimulation trains than T2 ratio to assess low-frequency fatigue in the human quadriceps in vivo. In other words, while amplitude changes (both absolute and relative) are different between Ftet10:100 and Ftet10:100 after exercise, low-frequency fatigue detection can be performed with Ftet10:100. Changes in ratio of peak forces measured at 10 and 100 Hz with tetanic EMS (T210:100) and in ratio of peak forces during second twitch stimulation measured at 10 and 100 Hz (Ftet10:100) are expressed as percentage change from before exercise to immediately after exercise (Pre-Post) and to 30 min after exercise (Pre-Post30). Changes in ratio of peak forces measured at 10 and 100 Hz with tetanic EMS were compared with changes in ratio of peak forces during second twitch stimulation measured at 10 and 100 Hz (T210:100) and in ratio of peak forces during paired stimulations measured at 10 and 100 Hz (Ftet10:100). Changes are expressed as percentage change from before exercise to immediately after exercise (Pre-Post) and to 30 min after exercise (Pre-Post30).

**Table 4. Correlations between the postexercise changes in ratios of peak forces**

<table>
<thead>
<tr>
<th></th>
<th>T210:100</th>
<th>Ftet10:100</th>
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<tbody>
<tr>
<td></td>
<td>r²</td>
<td>p</td>
<td>r²</td>
<td>p</td>
</tr>
<tr>
<td>Pre-Post</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENS</td>
<td>0.35</td>
<td>0.072</td>
<td>0.81</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MNS</td>
<td>0.64</td>
<td>0.005</td>
<td>0.80</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>EMS</td>
<td>0.73</td>
<td>0.002</td>
<td>0.83</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pre-Post30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENS</td>
<td>0.24</td>
<td>0.148</td>
<td>0.46</td>
<td>0.031</td>
</tr>
<tr>
<td>MNS</td>
<td>0.37</td>
<td>0.062</td>
<td>0.66</td>
<td>0.005</td>
</tr>
<tr>
<td>EMS</td>
<td>0.30</td>
<td>0.104</td>
<td>0.82</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Changes in ratio of peak forces measured at 10 and 100 Hz with tetanic EMS were compared with changes in ratio of peak forces during second twitch stimulation measured at 10 and 100 Hz (T210:100) and in ratio of peak forces during paired stimulations measured at 10 and 100 Hz (Ftet10:100). Changes are expressed as percentage change from before exercise to immediately after exercise (Pre-Post) and to 30 min after exercise (Pre-Post30).
assess low- to high-frequency force, inconsistent correlations between these two ratios were observed in the present study, and therefore it cannot be confirmed on human muscle in vivo that $T_2/10:100$ can be used as a surrogate to tetanus for low- to high-frequency force and fatigue assessment. A simple method based on low- to high-frequency ratio of peak forces may be more appropriate.

ACKNOWLEDGMENTS

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