M-wave potentiation after voluntary contractions of different durations and intensities in the tibialis anterior

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Rodriguez-Falces J, Duchateau J, Muraoka Y, Baudry S. M-wave potentiation after voluntary contractions of different durations and intensities in the tibialis anterior. J Appl Physiol 118: 953–964, 2015. First published February 12, 2015; doi:10.1152/japplphysiol.01144.2014.—The study was undertaken to provide insight into the mechanisms underlying the potentiation of the muscle compound action potential (M wave) after conditioning contractions. M waves were evoked in the tibialis anterior before and after isometric maximal voluntary contractions (MVC) of 1, 3, 6, 10, 30, and 60 s, and after 3-s contractions at 10, 30, 50, 70, 90, and 100% MVC. The amplitude, duration, and area of the first and second phases of the M wave, together with the median frequency (Fmedian) and muscle fiber conduction velocity (MFCV) were recorded. Furthermore, twitch force, muscle fascicle length, and pennation angle were measured at rest, before, and 1 s after the conditioning contractions. The results indicate that only the amplitude of the second phase of the M wave was significantly increased after conditioning contractions. The extent of this potentiation was similar for MVC durations ranging from 1 to 10 s and augmented progressively with contraction intensity from 30 to 70% MVC. After these conditioning contractions, the duration and area of the two M-wave phases decreased (P < 0.05), whereas MFCV and Fmedian increased (P < 0.05). For all of these parameters, the greatest changes occurred 1 s after the conditioning contraction. Changes in MFCV after the contractions were correlated with those in M-wave second-phase amplitude (r² = 0.42; P < 0.05) and Fmedian (r² = 0.53; P < 0.05). In contrast, fascicle length and pennation angle did not change after the conditioning contractions. It is concluded that the potentiation of the second phase of the M wave is mainly due to an increased MFCV.

It is well known that both the contractile and membrane excitability properties of the skeletal muscle are history dependent (19, 36, 47). One of the most studied aspects of contractile history is the potentiation of the electrically induced mechanical response (twitch) and muscle compound action potential (M wave) after a conditioning contraction (2, 23). Twitch potentiation has been extensively investigated, with numerous studies assessing the extent and time course of twitch enlargement after contractions of different durations and intensities (21, 36, 45). Comparatively, much less research has been devoted to the study of how M-wave potentiation is influenced by the history of previous contractions and how this potentiation decays over time (24, 46). This is surprising because the M wave is often used to assess change in neuromuscular propagation (5, 11, 13, 17) or to control the stimulation during the experimental session (4, 41).

A first parameter that influences the extent of M-wave potentiation is the duration of a conditioning contraction. The available scientific literature is not conclusive in this regard, partly because of the different protocols and muscles investigated. For example, Hicks and colleagues (23) reported that, after a 3-s maximal voluntary contraction (MVC), M-wave amplitude increased by 15 and 8% in the abductor pollicis brevis and extensor digitorum brevis, respectively, whereas an increase of 7% was observed in the quadriceps muscles after a 10-s MVC (21). Moreover, if a brief conditioning contraction induces M-wave potentiation, fatigue-related mechanisms may partly counteract potentiation during long-lasting contractions. Results of such long-duration MVCs on M-wave amplitude remain, however, controversial. Indeed, some studies reported a decrease in M-wave amplitude compared with baseline values after a 1-min MVC (14, 39, 41), whereas others reported no change after a similar duration of contraction (5, 6).

A second parameter that can modulate M-wave potentiation, is the intensity of the conditioning contraction. If the mechanisms responsible for M-wave potentiation resided in the muscle fiber membrane [as suggested by Hicks and McComas (22)], then the degree of M-wave enlargement would be related to the number of motor units recruited during the conditioning contraction and their firing rate. On this basis, it would be expected that the extent of potentiation increases with the intensity of the conditioning contraction. However, to date, no study has detailed the magnitude of M-wave potentization as a function of the intensity of the contraction.

Several factors have been proposed to contribute to M-wave potentiation: 1) an increase in electrogenic Na+/K+ pumps (22); 2) a broadening of the individual transmembrane action potentials (11); 3) a more synchronous generation of muscle fiber action potentials (12); 4) an increased muscle fiber conduction velocity (MFCV) (20, 33); and 5) a change in muscle architecture (11, 34). Although the most accepted explanation for M-wave enlargement is the enhancement of the electrogenic pump, the role of MFCV in this phenomenon should be taken into account, since both experimental and simulated data indicate that an increase in MFCV can also enlarge the M wave (11, 25). In this regard, recent studies have shown that a progressive increase in MFCV occurs during the first few discharges of a motor unit that starts to fire after a short period of inactivity (7, 29). Accordingly, conduction velocity might increase when muscle fibers are stimulated after a conditioning contraction. In addition to the changes in MFCV, alterations in muscle fiber architecture (such as changes in fiber length and pennation angle) might persist during a few seconds after a
contraction (17) and, therefore, could be partly responsible for the M-wave enlargement.

The objective of the present work was to examine the extent and time course of M-wave potentiation in the tibialis anterior after conditioning contractions of different durations and intensities. The involvement of MFCV in M-wave potentiation was evaluated by investigating the possible associations between changes in MFCV and M-wave amplitude after the conditioning contraction. In addition, ultrasonography was used to assess the possible effect of changes in muscle architecture on M-wave potentiation. The present study was designed to document the influence of a conditioning contraction on M-wave parameters and to provide insight into the mechanisms underlying M-wave potentiation in human skeletal muscle. We hypothesize that M-wave enlargement after a conditioning contraction is mainly due to an increase in conduction velocity, but could also be influenced by changes in muscle architecture.

MATERIAL AND METHODS

Participants. Eleven young participants (four women), aged between 22 and 40 yr (mean ± SD: 31.8 ± 9.3 yr), volunteered to participate in this study. Their average height and weight were 176.7 ± 7.6 cm and 69.2 ± 6.8 kg, respectively. Written, informed consent was obtained from all participants before the experimental session. None of the participants reported current or recent (at least 6 mo before the study) neuromuscular or musculoskeletal disorders. Approval for the project was obtained from the local Ethics Committee, and all procedures used in this study conformed to the Declaration of Helsinki.

Mechanical recordings. Each subject sat on an adjustable chair in a slightly reclined position with the right foot strapped to a footplate of an ankle ergometer, as described previously (4). The plate was inclined at an angle of 45° relative to the floor, and the seat was adjusted so that ankle and knee joint angles were at 90 and 120°, respectively. The foot was held in place by a heel block and secured to the footplate via three straps placed around the instep and the foot. The isometric dorsiflexion torque was calculated by multiplying the force signal, recorded by a strain-gauge transducer (Kulite, TC 2000-500, sensitivity: 0.018 V/N · m; linear range: 0-200 N · m, Basingstoke, UK) attached under the footplate at the level of the metatarsophalangeal of the big toe, by the corresponding lever arm. The force signal was sampled at 200 Hz using an analog-to-digital conversion system (MP150; Biopac, Goleta, CA).

Electromyographic recordings. Surface electromyogram (EMG) signal was recorded from the tibialis anterior by means of silver disk electrodes (recording diameter of 8 mm) filled with gel. Before the electrodes were placed, the skin was shaved when necessary and cleaned with a solution of alcohol, ether, and acetone to reduce the impedance at the skin-electrode interface. Surface EMG signals were amplified (×1,000) and filtered (10 Hz to 1 kHz) by a custom-made differential amplifier and sampled at 5 kHz using an analog-to-digital conversion system (MP150; Biopac, Goleta, CA).

The recording electrodes were placed relative to the motor point, identified as the location of the skin area over the muscle in which an electrical pulse evoked a mechanical twitch with the lowest stimulus intensity (8, 12). The direction of the muscle fibers was identified using a linear grid, consisting of 16 silver bar electrodes (5 × 1 mm, 5-mm interelectrode distance), which was connected to a multichannel amplifier (OT Bioelettronica, Torino; bandwidth 10–500 Hz). Thereafter, two pairs of electrodes were placed in a belly-tendon configuration, with the proximal electrodes located ~3 cm (first pair) and 6 cm (second pair) distal from the motor point (Fig. 1). This particular placement of the proximal electrodes increased the likelihood of recording two M waves with distinct first phases (each one containing one single peak), separated by a clear time interval, which reflected propagation of the action potentials along the fibers (see Fig. 1B). The two proximal electrodes were placed along a line parallel to the orientation of the muscle fibers to accurately estimate the conduction velocity.

Fig. 1. A: experimental arrangements for the electrical stimulation of the fibular nerve, recording surface electromyogram (EMG) activity from the tibialis anterior, and the placement of the ultrasound probe. Two pairs of electrodes were placed in a belly-tendon configuration. The proximal electrodes of these configurations were aligned with the muscle fiber direction to estimate the conduction velocity of muscle fibers. The distal electrodes were placed close together over the tendon of the tibialis anterior. B: representative traces of the proximal and distal M waves obtained in one subject. Note the time delay between the first (positive) peak of the two M waves. C: measurement of the M-wave parameters: amplitude, duration, and area of the first (AmplFIRST, DurFIRST, and AreafIRST) and second (AmplSECOND, DurSECOND, and AREafSECOND) phases.
The distal electrodes were placed, close together, over the tendon of the tibialis anterior, as depicted in Fig. 1. The ground electrodes were placed over the tibia. The belly-tendon configuration gives a signal that is similar to the theoretical monopolar M wave. The major advantage of monopolar signals is that they contain the entire informative content of the propagating potential, that is, a monopolar M wave is the “genuine” representation of the electrical potential generated by the muscle (35). In addition, the belly-tendon configuration has been often adopted for the study of M-wave potentiation (11, 23).

The tibialis anterior was chosen for the following reasons: 1) EMG and torque responses induced by electrical stimulation are relatively easy to record; 2) the phenomenon of twitch potentiation has often been examined in the tibialis anterior (4, 45), and so it appears pertinent to characterize the potentiation of the M wave in the same muscle; 3) because of its length, estimation of conduction velocity can be easily determined; and 4) as superficially located, muscle architecture can be easily assessed by ultrasonography.

Ultrasonography. Ultrasonography was used to verify whether the architectural characteristics of the tibialis anterior were similar before and after the conditioning contractions at the time when the stimulus was delivered. Longitudinal images were obtained using real-time B-mode ultrasonographic apparatus (Prosound F75, Aloka, Japan) with a 6-cm-width linear-array probe positioned at the proximal one-third of the tibialis anterior (Fig. 1). Once at least one muscle fascicle was clearly identified, the position of the probe was firmly held in place using a custom-made resin sheath strapped to the skin. The restraint ensured a constant orientation and pressure of the probe on the skin. The probe was coated with a water-soluble transmission gel to provide adequate acoustic contact. Recordings were performed before and immediately after (1 s) the conditioning MVC, when the muscle was completely relaxed, as verified by the absence of EMG activity.

Stimulation procedure. Electrical stimulus (0.2-ms duration) applied to the deep branch of the fibular nerve was delivered by a constant-current stimulator (DS7A, Digitimer, Hertfordshire, UK), triggered by a digital timer (Master-8, AMPI, Jerusalem, Israel). Stimulating surface electrodes (silver-silver chloride electrodes; 8-mm diameter) were fastened with adhesive tape to the skin at the knee level of the right leg. The cathode was attached to the skin over the deep fibular nerve (5 cm close to the neck of the fibula, and the anode was fastened on the opposite side of the leg. The absence of peroneal muscle activity was assessed by palpation and shape of the mechanical response. A distortion in the shape of the twitch signal reflected mechanical contribution of the peroneal muscle.

The maximal stimulus intensity was determined by gradually increasing the stimulation intensity up to a level at which the M-wave amplitude and peak twitch torque reached a plateau. This level of intensity was then further increased by 20% to ensure that the stimulation remained supramaximal throughout the experimental session.

Experimental procedure. The experimental session consisted of two parts performed in a counterbalanced order across subjects. In the first part, the effect of contraction intensity on M-wave potentiation was examined. To do this, each participant was asked to perform conditioning contractions of 3 s, in random order, at the following intensities: 10, 30, 50, 70, 90, and 100% of MVC. Each contraction was separated by resting periods of 5 min. Before each conditioning contraction, three electrical stimulations (control responses) were evoked at rest with a 5-s interval between stimulations. Thereafter, the conditioning contraction was performed, and, subsequently, single electrical stimuli (starting 1 s after the end of the conditioning contraction) were applied to the fibular nerve at 2-s intervals for the subsequent 22 s.

In the second part of the experiment, the effect of MVC duration on M-wave potentiation was assessed. To that end, the participants were required to perform, in random order, conditioning MVCs of 1, 3, 6, and 10 s. In addition, 30- and 60-s MVCs were performed (in this order) after the previous MVCs to avoid a potential effect of these long contractions on the data obtained with briefer conditioning contractions. This range of MVC durations was chosen to determine the shortest MVC that induced a recognizable M-wave potentiation and also to investigate the influence of long-lasting MVC on M-wave parameters. Each MVC was separated by a resting period of at least 10 min. At the end of this resting period, the amplitude of the M wave was checked to ensure that it did not differ from the control values obtained at the beginning of the session. If a difference greater than 5% was observed, the resting period was then prolonged by 5 min. In addition, the MVC torque was measured during a 1-s epoch and compared across sequences. The torque did not differ between MVCs (one-way ANOVA, \( P = 0.63 \)), indicating that the resting period was long enough to permit a complete recovery. Before each conditioning contraction, three electrical stimulations (control responses) were evoked at rest with a 5-s interval. This was followed by the conditioning MVC, after which electrical nerve stimulation were triggered at 1 s, 5 s, 9 s, 13 s, 17 s, 21 s, 33 s, 43 s, 53 s, 2 min, and 10 min after the MVC.

Data analysis. Data were recorded with commercially available software (AcqKnowledge, Biopac Systems, Goleta, CA), and the EMG signal was monitored online for any abnormality in the M-wave shape that would have prevented a clear determination of the first (positive) peak and second (negative) peak. Subsequently, data were exported to Matlab (version R2012b; The Math-Works, Natick, MA) for quantitative analysis using ad hoc scripts.

For each M-wave response, the amplitude, duration, and area of the first (AmplFIRST, DuraFIRST, and AreaFIRST) and second (AmplSECOND, DuraSECOND, and AreaSECOND) phases were measured (see Fig. 1C). The onset of DuraFIRST was determined by a deviation >2 SDs of the baseline noise from the baseline, whereas the end corresponded to the baseline-crossing point. This point marked the onset of the second phase (DuraSECOND). The end point of the second phase was determined by a deviation <2 SDs of the baseline noise from the baseline. The area parameters were calculated as the integral of the absolute value of the M wave above the above-defined phases. The spectral analysis of the M wave was performed over a window length that comprised the whole M-wave time course (excluding the stimulus artifact). A fast Fourier transformation of 512 points (Hamming window processing) was applied on this window length. The M-wave median frequency (Fmedian) was calculated according to the definition of Stulen and DeLuca (40). Changes in AmplFIRST, AmplSECOND, AreaFIRST, and AreaSECOND were expected to reflect changes in the amplitude of individual muscle fiber action potentials (22) and/or alterations in the dispersion between the constituent motor unit potentials of the M wave. Changes in DuraFIRST and DuraSECOND were assessed to analyze possible alterations in MFCV (33). Similarly, changes in Fmedian were assumed to be mainly caused by variations in MFCV (6, 35). All of these parameters were measured on the proximal M wave (see Fig. 1). The peak torque of the twitch was measured before and 1 s after the contraction. In each of the sequences of the experimental protocol, the three responses evoked before the conditioning contraction were averaged and used as control values. All M-wave and twitch parameters recorded after the conditioning contractions were expressed as percentage of the control responses.

Estimation of MFCV was done according to the procedure used by Cupido et al. (11). Briefly, the method consisted in detecting the M wave at two different locations along the muscle fiber direction and to estimate the time delay between these two signals. The two recorded M waves were approximately similar in shape, but shifted in time (Fig. 1B). MFCV was determined by dividing the distance between the two proximal recording electrodes of each pair by the time interval between the negative peak of the M waves recorded at the two locations.

Muscle fascicle length and pennation angle of the tibialis anterior were measured at rest, before, and immediately after the conditioning MVC to determine whether briefer M waves after the conditioning...
contractions could be related to changes in muscle architecture (shortened fascicle and increased pennation angle). Muscle fascicle was defined as a clearly visible fiber bundle lying between the two aponeuroses (superficial and deep) and was measured along the marked fiber bundle, from the deep to the superficial aponeurosis (1). Pennation angle was defined as the angle formed by the tracked fascicle and the deep aponeurosis. Both parameters were computed using a public domain image program (Image J, National Institutes of Health). When the end of the fascicle extended off the acquired ultrasound image, fascicle length was estimated by trigonometry using the law of sines. The error due to the linear extrapolation has been estimated to be 2–7% (1).

Statistics. Kolmogorov-Smirnov tests confirmed that each of the M-wave parameters analyzed in the present study was normally distributed. Differences in the control values of the M-wave parameters among the MVC duration and contraction-intensity protocols were examined using a one-way ANOVA. The changes in the M-wave parameters during the first 23 s following the conditioning contraction of different intensities were analyzed with a two-way repeated-measures ANOVA (contraction intensity × time after the contraction). The changes in the M-wave parameters during the 10-min recovery following the conditioning MVCs of different durations were analyzed with a two-way repeated-measures ANOVA (MVC duration × time after the contraction). When main effects or interactions were significant, Student-Newman-Keuls post hoc tests for pairwise comparisons were applied. The coefficient of determination ($r^2$) was calculated to determine the degree of association between MFCV, AmpliSECOND, DurSECOND, and Fmedian. Differences between twitch torque before and after the conditioning contractions were examined using a two-way ANOVA and Student-Newman-Keuls post hoc test when main effects or interactions were significant. Significance was set at $P < 0.05$. Data are presented as means ± SD in the text and Tables 1–3 and as means ± SE in the Figs. 3, 4, 6, and 7.

RESULTS

M-wave potentiation after conditioning contractions of different intensities. Figure 2 provides representative examples of M waves recorded in one subject before (control) and after conditioning contractions of different intensities. Except for the 10% MVC, the M wave evoked 1 s after the contraction was briefer relative to the control M wave. This narrowing was accompanied by an increase in the AmpliSECOND, with no obvious change in the AmpliFIRST. As the time elapsed after the conditioning contraction increased, the differences in amplitude and duration between the pre- and postconditioning M waves decreased gradually. The differences between the control and postconditioning M waves were already apparent after the 30% MVC (Fig. 2B) and increased with the intensity of contraction up to 70% MVC (Fig. 2, D–F).

Figure 3 shows the time course of the changes in the AmpliFIRST, DurFIRST, and AreaFIRST (first column) and AmpliSECOND, DurSECOND, and AreaSECOND (second column) after the conditioning contractions of different intensities for the whole study group. In Fig. 3A, it can be seen that, regardless of the contraction intensity, AmpliFIRST did not increase significantly compared with control values (no time effect, $P > 0.05$). In contrast, AmpliSECOND increased immediately after the contraction (with the maximum values
being reached 1 s after the contraction) and reached statistical significance for all conditions, except for the 10% MVC (time × contraction intensity, \(P < 0.001\), Fig. 3A). During the first 5 s following the cessation of the conditioning contraction, the extent of increase in Ampli\(_{\text{SECOND}}\) was greater for the 70, 90, and 100% MVC compared with the 10% MVC (Student-Newman-Keuls, \(P < 0.05\)). Noteworthy, the time course of change for Ampli\(_{\text{SECOND}}\) after the 70, 90, and 100% MVCs was almost identical.

Dur\(_{\text{FIRST}}\), Dur\(_{\text{SECOND}}\), Area\(_{\text{FIRST}}\), and Area\(_{\text{SECOND}}\) decreased immediately after the conditioning contraction and then returned to control values within 23 s (Fig. 3, C–F), with the greatest extent of change observed 1 s after the conditioning contraction. During the first 7 s following the cessation of

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Fig. 3. A–F: time course of Ampli\(_{\text{FIRST}}\), Ampli\(_{\text{SECOND}}\), Dur\(_{\text{FIRST}}\), Dur\(_{\text{SECOND}}\), Area\(_{\text{FIRST}}\), and Area\(_{\text{SECOND}}\), respectively, after conditioning contractions of different intensities. All data are expressed in percentage of control values and reported as means ± SE (n = 11). *Significant difference with control (\(P < 0.05\)). †Significant difference: 70, 90, and 100% MVC vs. 10% MVC (\(P < 0.05\)). ‡Significant difference: 70, 90, and 100% MVC vs. 30% MVC (\(P < 0.05\)).
accompanied by a marked increase in the AmpliSECOND, the M wave evoked 1 s after the conditioning MVC was significantly greater after the 6- and 10-s MVC (Student-Newman-Keuls, P < 0.05).

For all contraction intensities, except the 10% MVC, Fmedian and MFCV increased immediately after the conditioning contraction (with the maximum values being reached 1 s after the contraction) and then returned to baseline values within 23 s (contraction intensity × time, P < 0.001, Fig. 4, A and B). During the first 9 s after the cessation of the contraction, the increase in Fmedian and MFCV was greater for the 70, 90, and 100% MVC compared with the 10% MVC (Student-Newman-Keuls, P < 0.05). Noteworthy, the time course of both Fmedian and MFCV was practically identical after the 70, 90, and 100% MVCs. The increase in MFCV 1 s after the conditioning contraction was significantly correlated with the increase in Fmedian (r² = 0.52, P < 0.001) and the increase in AmpliSECOND (r² = 0.43, P = 0.008).

Before the conditioning contraction, twitch torque was similar across the protocols, consisting in contractions of different intensities (P > 0.05, Table 1). Twitch torque increased significantly after contractions, with intensities ≥50% MVC (P < 0.05, Table 1).

M-wave potentiation after conditioning MVCs of different durations. Figure 5 provides representative examples of M waves recorded in one subject before (control) and after conditioning MVCs of different durations. Except for the 60-s MVC, the M wave evoked 1 s after the conditioning MVC was briefer relative to the control M wave. This change was accompanied by a marked increase in the AmpliSECOND, whereas the AmpliFIRST remained nearly the same. These changes in amplitude and duration of the postconditioning M waves were gradually abolished during the first minute following the conditioning MVC. The effects of the conditioning MVC were already apparent after the 1-s MVC (Fig. 5A) and increased with the duration of the MVC up to 10-s MVC (Fig. 5, C and D) to decrease for longer MVCs (Fig. 5, E and F).

Figure 6A shows that, regardless of the MVC duration, AmpliFIRST did not increase significantly compared with control values (time effect, P > 0.05). In contrast, AmpliSECOND increased immediately after the MVC (with the maximum values being reached 1 s after the MVC), with this increase being statistically significant for all conditioning contractions, except for the 60-s MVC (MVC duration × time, P < 0.001, Fig. 6B). The extent of potentiation of AmpliSECOND was similar after MVC durations ranging from 1 to 10 s, but was reduced after the 30-s MVC. After this transient initial increase, AmpliSECOND returned to baseline values within 15–20 s. During the first 9 s after the cessation of the MVC, the extent of the increase in AmpliSECOND was greater for the 6- and 10-s MVC compared with the 60-s MVC (Student-Newman-Keuls, P < 0.05).

Both DurFIRST and DurSECOND decreased abruptly immediately after the MVC and then returned to control values within 30 s (Fig. 6, C and D). For both parameters, the greatest decline was reached 1 s after the conditioning MVC. During the first 13 s after the cessation of the MVC, the extent of the decrease in DurFIRST and DurSECOND was greater for the 6- and 10-s MVC compared with the 60-s MVC (Student-Newman-Keuls, P < 0.05). AreaFIRST and AreaSECOND decreased significantly immediately after the MVC (with the greatest changes observed 1 s following the MVC) and then returned to baseline values within 30–40 s (Fig. 6, E and F). During the first 9 s after the cessation of the MVC, the extent of the decrease of AreaSECOND was significantly greater after the 6- and 10-s MVCs compared with the 60-s MVC (Student-Newman-Keuls, P < 0.05).

Table 1. Peak twitch torque before and 1 s after a 3-s conditioning contraction of different intensities

<table>
<thead>
<tr>
<th>Peak Twitch Torque</th>
<th>10% MVC</th>
<th>30% MVC</th>
<th>50% MVC</th>
<th>70% MVC</th>
<th>90% MVC</th>
<th>100% MVC</th>
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<tr>
<td>Before</td>
<td>2.28 ± 1.12</td>
<td>2.26 ± 0.90</td>
<td>2.39 ± 0.71</td>
<td>2.37 ± 0.84</td>
<td>2.21 ± 0.93</td>
<td>2.23 ± 0.92</td>
</tr>
<tr>
<td>After</td>
<td>2.27 ± 1.03</td>
<td>2.59 ± 0.93</td>
<td>3.21 ± 0.98*</td>
<td>4.86 ± 1.94*</td>
<td>5.58 ± 1.79*</td>
<td>5.46 ± 1.64*</td>
</tr>
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</table>

Values are means ± SD in N·m. MVC, maximal voluntary contraction. *Significant difference before vs. after the contraction for the same contraction intensity at P < 0.05.
MVC compared with the 60-s MVC (Student-Newman-Keuls, $P < 0.05$).

Figure 7 shows the time course of changes in $F_{\text{median}}$ and $M_{\text{FCV}}$ after the conditioning MVCs of different durations for the whole group. Except for the 60-s MVC, both $F_{\text{median}}$ and $M_{\text{FCV}}$ increased abruptly after the conditioning MVCs (with the maximum values being reached 1 s after the MVCs) and then returned toward baseline values within 20–30 s (MVC duration × time, $P < 0.001$, Fig. 7, A and B). During the first 13 s after the cessation of the MVC, the extent of the increase in $F_{\text{median}}$ and $M_{\text{FCV}}$ were greater for the 6- and 10-s MVC compared with the 60-s MVC (Student-Newman-Keuls, $P < 0.05$). The increase in $M_{\text{FCV}}$, 1 s after the contraction, was significantly correlated with the increase in $F_{\text{median}}$ ($r^2 = 0.62$, $P < 0.001$) and in Ampli$\text{SECOND}$ ($r^2 = 0.45$, $P < 0.001$).

The average torque at the end of the 30- and 60-s MVCs declined by, respectively, 33 ± 13 and 58 ± 17% of the torque recorded at the onset of the MVCs (unfatigued state).

Before the conditioning MVC, twitch torque was similar across the protocols consisting of MVCs of different durations ($P > 0.05$, Table 2). Twitch torque was significantly increased after conditioning, regardless of MVC duration ($P < 0.05$, Table 1).

Muscle architecture and fascicle length after conditioning MVCs of different durations. The average fascicle length before the conditioning contractions was 71.4 ± 9.1 mm and did not change 1 s after the conditioning MVC, regardless of its duration (mean value: 71.5 ± 9.2 mm) (MVC duration × time, $P > 0.05$, Table 3). Similarly, when collapsed across MVC durations, pennation angles did not differ before and after the conditioning contractions (9.3 ± 1.7 vs 9.4 ± 1.8°) (MVC duration × time, $P > 0.05$, Table 3).

**DISCUSSION**

The main finding of the present study is that, regardless of the duration and intensity of the conditioning contractions, the Ampli$\text{FIRST}$ was not potentiated, whereas the Ampli$\text{SECOND}$ underwent a marked increase. The potentiation of the M-wave second phase augmented progressively with contraction intensity from 30 to 70% MVC and was similar for MVC durations ranging from 1 to 10 s. After these conditioning contractions, the duration and area of the first and second phases was significantly decreased, whereas $M_{\text{FCV}}$ and $F_{\text{median}}$ were increased. The peak changes of these parameters occurred only 1 s after the conditioning contractions before returning to their initial values within 15–30 s. Interestingly, these changes were observed at a time when muscle architecture did not differ from the precontraction conditions.

**M-wave potentiation after conditioning contractions of different intensities and durations.** Surprisingly, we found that Ampli$\text{FIRST}$ was not potentiated, regardless of the duration and intensity of the conditioning contraction. In contrast, a 3-s conditioning contraction of 30% MVC was sufficient to increase significantly Ampli$\text{SECOND}$. The magnitude of potentiation of Ampli$\text{SECOND}$ increased progressively with the intensity of the conditioning contraction up to 70% MVC before leveling off for greater intensities. This suggests that the extent of
M-wave potentiation might be related to the number of activated motor units during the contraction as, in the tibialis anterior, most motor units are recruited at contraction intensities of 70–80% MVC (9, 27, 42). Furthermore, the magnitude of potentiation of Ampli\textsubscript{SECOND} did not differ across MVCs ranging from 1 to 10 s. However, the enlargement of Ampli\textsubscript{SECOND} was significantly reduced after the 30-s MVC, most likely due to the detrimental fatigue-related effects on the neuromuscular propagation (14) that partially offset the potentiation effect (32, 49). The presence of fatigue is evidenced by Fig. 6.

Fig. 6. A–F: time course of Ampli\textsubscript{FIRST}, Ampli\textsubscript{SECOND}, Dur\textsubscript{FIRST}, Dur\textsubscript{SECOND}, Area\textsubscript{FIRST}, and Area\textsubscript{SECOND}, respectively, after conditioning MVCs of different durations. All data are expressed in percentage of control values and reported as means ± SE (n = 11). *Significant difference with control (P < 0.05). †Significant difference between 6- and 10-s MVC compared with 60-s MVC (P < 0.05). ‡Significant difference between 6- and 10-s MVC compared with 30-s MVC (P < 0.05).
the reduction in twitch potentiation after the 30-s MVC compared with the 10-s MVC. This counteracting effect of fatigue is further supported by the absence of M-wave potentiation after a 60-s MVC. In parallel to the increase in Ampli\textsubscript{SECOND}, contraction intensities ≥30% MVC and MVC durations ≤30 s also induced a pronounced reduction in the duration of the M wave, with greater changes in the M-wave second phase (Dur\textsubscript{SECOND}). The similar amount of M-wave potentiation after 1-s MVC compared with 10-s MVC suggests that the underlying mechanisms responsible for M-wave potentiation almost reached their maximal capacity within the first second of an MVC. In contrast, twitch potentiation required additional time (~10 s) to reach is maximal value.

**Possible mechanisms underlying M-wave potentiation.** The concurrent changes in the amplitude and duration of the M wave after a conditioning contraction could be attributed to several factors. Changes in muscle architecture could account for the M-wave enlargement. This factor was first proposed by Cupido and colleagues (11), who attributed the decrease in M-wave duration to the “shortening of the contracted fibers.” However, this possibility seems unlikely in view of the lack of differences in muscle architecture (fascicle length and pennation angle) before and after the conditioning contraction at the time of the stimulation. The rapid return (<1 s) of fascicle length and pennation angle to normal values after a submaximal or maximal contraction observed in our experimental conditions discounts a role of muscle fiber architecture in M-wave changes. Although the length of the muscle-tendon unit in the resting condition may influence the time taken for the muscle architecture to return to the initial condition after the conditioning contraction, this aspect should not have affected our data, because the ankle angle was kept at 90° throughout the experimental session, a position that does not place muscle fascicles in slack condition. Moreover, the classical explanation for M-wave potentiation, namely that the enhanced electrogenic Na\textsuperscript{+}-K\textsuperscript{+} pumping results in an increased amplitude of the muscle fiber action potentials (22), cannot account for the different increase in Ampli\textsubscript{FIRST} and Ampli\textsubscript{SECOND}. Indeed, an increase in individual action potentials after a conditioning contraction should have induced a comparable increase in the first and second phases of the M wave. Another argument that questions the validity of the enhanced electrogenic Na\textsuperscript{+}-K\textsuperscript{+} pumping hypothesis to explain M-wave potentiation is that membrane hyperpolarization was found to be maximal 4 min after a 5-min intermittent stimulation at 20 Hz (22), which is in contrast to the rapid recovery of Ampli\textsubscript{SECOND} reported here. Finally, it is unlikely that a contraction-induced increase in muscle temperature was a major contributing factor to the M-wave enlargement.

The most likely candidate to explain the enlargement of the M wave and the decrease of its duration after a conditioning contraction is a transient increase in conduction velocity occurring during the recovery phase. Support for this hypothesis comes from the observation that MFCV and M-wave duration changed to a similar extent with MVC duration, but in opposite directions. Indeed, a comparable increase in MFCV was observed after MVCs ranging from 1 to 10 s, and this is consistent with the fact that M-wave duration decreased to a similar extent after MVCs shorter than 10 s. Furthermore, the increase in MFCV was less prominent after the 30-s MVC compared with shorter conditioning contractions, which is in agreement with the lesser change observed in Dur\textsubscript{FIRST} and Dur\textsubscript{SECOND} after this MVC duration. Moreover, after the 60-s MVC.

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**Table 2. Peak twitch torque before and 1 s after conditioning MVCs of different durations**

<table>
<thead>
<tr>
<th>Peak Twitch Torque</th>
<th>1-s MVC</th>
<th>3-s MVC</th>
<th>6-s MVC</th>
<th>10-s MVC</th>
<th>30-s MVC</th>
<th>60-s MVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>2.20 ± 1.23</td>
<td>2.29 ± 1.09</td>
<td>2.15 ± 1.04</td>
<td>2.12 ± 1.05</td>
<td>2.05 ± 1.01</td>
<td>2.29 ± 1.27</td>
</tr>
<tr>
<td>After</td>
<td>3.42 ± 1.22*</td>
<td>5.34 ± 1.85*</td>
<td>6.29 ± 1.65*</td>
<td>7.53 ± 1.67*</td>
<td>6.31 ± 1.68*</td>
<td>3.54 ± 1.13*</td>
</tr>
</tbody>
</table>

Values are means ± SD in N·m. *Significant difference before vs. after the MVC for the same MVC duration at \( P < 0.05 \).

---

**Fig. 7. Time course of changes in M-wave F\textsubscript{median} (A) and MFCV (B) after conditioning MVCs of different durations. All data are expressed in percentage of control values and reported as means ± SE \(( n = 11)\). †Significant difference with control \(( P < 0.05)\). ‡Significant difference between 6- and 10-s MVC compared with 60-s MVC \(( P < 0.05)\). ‡‡Significant difference between 6- and 10-s MVC compared with 30-s MVC \(( P < 0.05)\).**
MVC, MFCV remained unchanged compared with control values and so did DurFIRST and DurSECOND. Moreover, in the absence of changes in muscle architecture, the increase in Fmedian after contractions intensities >30% MVC and MVC durations <30 s most likely reflects an increased conduction velocity (35). There are other lines of evidence supporting a strong association between MFCV and AmpliSECOND. First, the increase in MFCV measured 1 s after the contraction was significantly associated with the increase in AmpliSECOND (r² = 0.42, P = 0.002). In addition, we found that the increase in AmpliSECOND after a contraction was associated (r² = 0.82) with a decrease in DurSECOND, supporting the idea that the M-wave potentiation is related to an increased MFCV (3). It is well known that the area of the two phases of the M wave is determined by the duration of each phase and the size of the individual muscle fiber action potentials (6). The observation that AreaSECOND decreased after contraction intensities >50% MVC, despite the increase in AmpliSECOND, further supports that the main factor affecting the M waves after the contraction was an increase in conduction velocity.

The above results indicate that the extent of reduction in M-wave duration after conditioning contractions likely depends, at least in part, on the degree of MFCV enhancement. This suggestion is supported by the work of McGill and Lateva (29) showing a progressive increase in MFVC during the first few discharges of a motor unit starting to fire after a period of inactivity of ~1 s. The authors found that MFCV increases by ~10% after a brief period of rest. Consistent with this finding, previous studies have also reported an augmented MFCV at the onset of electrical stimulation (7, 12, 20, 38). It has been suggested that the history-dependent “supernormality” of MFCV might be due to sarcolemmal depolarization brought about by the accumulation of potassium in the t-tubule system (48). This means that, during several seconds following a contraction, the sarcoplemma remains depolarized and thus closer to threshold, explaining thereby the increased MFCV when the fiber fires again after a few seconds of inactivity. In addition, the diameter of a muscle fiber increases slightly but quickly after contractions elicited by trains of 20- and 40-Hz stimuli (26). Therefore, the possibility that fiber swelling also contributed to the augmentation of MFCV cannot be completely excluded.

At first, the present results appear to contradict previous research showing that MFCV declines rapidly over the course of an MVC (49) and after an MVC (44, 45). The explanation for these apparently contradictory findings should reside in the technical differences between the methods employed to estimate MFCV. First, we did not assess conduction velocity from the voluntary EMG generated while the muscle was being contracted (as in the classical methods), but from the synchronized EMG signal evoked by electrical nerve stimulation after the voluntary contractions ended. Second, in the classical methods, MFCV is computed solely from the active motor units, some of which may stop firing because of the fatigue developed during a prolonged MVC (10, 13, 31). In the present study, the estimation of MFCV was based on the maximal M wave, which theoretically integrates the contribution from most motor units in the muscle, regardless of the duration of the conditioning MVC. Finally, further support for the MFCV findings reported here comes from the observation that the increase in MFCV after contractions of different durations and intensities followed closely that of M-wave Fmedian (r² = 0.53, P < 0.001).

Differences in potentiation between the M-wave first and second phases. As stated earlier, the behavior of the first and second phases of the M wave after the conditioning contractions of varying durations and intensities exhibited important differences. First, whereas AmpliSECOND was significantly potentiated after nearly all conditioning contractions tested, AmpliFIRST was not increased, regardless of the contraction duration and intensity. Moreover, the extent of potentiation of AmpliSECOND was strongly dependent on the characteristics of the conditioning contraction, whereas AmpliFIRST did not change. Although M-wave potentiation is a common observation (23, 28), the present study is one of the first to show that M-wave potentiation was mainly confined to the second phase of the potential. Previous studies dealing with M-wave potentiation did not allow distinguishing the unequal enlargement of AmpliFIRST and AmpliSECOND, mainly because they considered the peak-to-peak amplitude as the reference parameter (21, 23, 24, 46).

Discrepancies in the extent of potentiation between AmpliFIRST and AmpliSECOND might be related to the fact that the M-wave first phase results from the propagation of the excitation source along the fiber, whereas the second phase mainly reflects the extinction of this source at the aponeurosis/tendon (15). Therefore, if changes in muscle architecture had persisted for a few seconds after the contraction, these changes would have influenced the M-wave second phase to a greater degree compared with the first phase (35, 37). However, this explanation seems unlikely, given the rapid return of fascicle length and pennation angle to resting values after a contraction. The unequal degree of potentiation of AmpliFIRST and AmpliSECOND could be explained by the fact that changes in MFCV have a greater impact on the second than the first phase of the M wave (35). Indeed, a maximal M wave represents the summation of the surface potentials of most of the motor units in the muscle (25), which are dispersed in time due to their different

Table 3. Fascicle length and pennation angle recorded in tibialis anterior before and immediately (1 s) after the conditioning MVC of different durations

<table>
<thead>
<tr>
<th></th>
<th>1 s-MVC</th>
<th>3 s-MVC</th>
<th>6 s-MVC</th>
<th>10 s-MVC</th>
<th>30 s-MVC</th>
<th>60 s-MVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fascicle length, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Before</strong></td>
<td>71.5 ± 9.4</td>
<td>71.6 ± 9.2</td>
<td>71.1 ± 9.2</td>
<td>71.3 ± 8.4</td>
<td>71.4 ± 9.1</td>
<td>71.8 ± 9.5</td>
</tr>
<tr>
<td><strong>After</strong></td>
<td>71.5 ± 9.5</td>
<td>71.3 ± 8.9</td>
<td>71.3 ± 9.5</td>
<td>71.8 ± 8.7</td>
<td>71.8 ± 9.4</td>
<td>71.1 ± 9.3</td>
</tr>
<tr>
<td>Pennation angle, º</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Before</strong></td>
<td>9.4 ± 1.7</td>
<td>9.1 ± 1.5</td>
<td>9.3 ± 1.7</td>
<td>9.1 ± 1.6</td>
<td>9.4 ± 1.7</td>
<td>9.5 ± 1.7</td>
</tr>
<tr>
<td><strong>After</strong></td>
<td>9.4 ± 1.7</td>
<td>9.2 ± 1.7</td>
<td>9.4 ± 1.9</td>
<td>9.2 ± 1.5</td>
<td>9.3 ± 1.9</td>
<td>9.6 ± 1.9</td>
</tr>
</tbody>
</table>

Values are means ± SD.
conduction velocities. The increase in MFCV observed after a conditioning contraction would reduce the dispersion between the constituent motor unit action potentials, thereby resulting in a more efficient summation (less cancellation) of the electrical activity, that is, in increased M-wave amplitude (12, 20). The time taken for the action potentials to travel from the innervation zone to the region below the proximal electrode (generation of the first phase) is much shorter than the time taken to reach the distal tendon or aponeurosis (generation of the second phase). Consequently, any increase in MFCV would increase comparatively more the synchronization between the second phases of the surface motor unit potentials than between the first phases of these potentials (33, 35). This assumption is further reinforced by the finding that the average increase in the Ampli\textsubscript{FIRST} of the distal M wave 1 s after MVCs ranging between 1 and 10 s was significantly greater than that observed in the Ampli\textsubscript{FIRST} for the proximal M wave (mean value: 6.8 vs. 2.1%; \( P < 0.001 \)).

**Time course of change in M-wave characteristics after a conditioning contraction.** A consistent finding of the present study is that Ampli\textsubscript{SECOND} increased immediately after the conditioning contraction, with the maximal potentiation being observed at the first time point measured (1 s after the cessation of the contraction). This rapid change applied not only to the M-wave amplitude, but also to the other parameters investigated. These observations are in contrast with previous studies reporting that the peak of potentiation occurs several seconds or even minutes after the cessation of the conditioning contraction (30). For instance, Jubeau et al. (24) found that, for the rectus femoris muscle, M-wave potentiation was maximal 6 s after a 40% MVC of 10-s duration, whereas, for the vastus lateralis, M-wave potentiation was at its maximum 20 s after a similar conditioning contraction. In the same line, West and colleagues (46) reported that M-wave amplitude reached its largest value 1 min after a 30% MVC of 3 min in the vastus medialis. Moreover, the aforementioned studies reported a slow time course for the change in M-wave amplitude during the recovery phase, whereas we found a rapid recovery of all of the parameters within 15–30 s after the cessation of the conditioning contractions. Differences in methodology (tetanic vs. voluntary contractions, time at which measurements were done after the postconditioning contraction) might explain, at least partly, the discrepancies between studies. Regardless of the mechanisms responsible for M-wave potentiation, it can be concluded that, in our experimental conditions, they should reach their full capacity immediately after the conditioning contraction before declining rapidly afterwards.

In conclusion, our study indicates that, regardless of the duration and intensity of the conditioning contractions, the Ampli\textsubscript{FIRST} was not enlarged. Potentiation of the M-wave second phase was maximal after MVC durations ranging from 1 to 10 s and contraction intensities within the range 70–100% MVC. The greatest changes in all of the studied parameters occurred 1 s after each conditioning contraction. The present findings indicate that it is unlikely that the enhanced electrogenic Na\textsuperscript{+}/K\textsuperscript{+} pumping could account solely for M-wave potentiation. In contrast, the unequal potentiation of the first and second M-wave phases, together with the absence of change in muscle architecture after the conditioning contractions, suggest that the increase in conduction velocity plays a key role in M-wave potentiation. Functionally, but although small, such transient increase in conduction velocity would influence the initial summation of the muscle mechanical responses to a train of activation pulses. The results further suggest that the peak amplitude of the M-wave first phase is more accurate than the peak-to-peak amplitude to monitor potential changes in the stability of the electrical stimulation conditions.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


