Potentiation of the first and second phases of the M wave after maximal voluntary contractions in the biceps brachii muscle

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ABBREVIATIONS

$Ampli_{FIRST}$: Amplitude of the first phase of the M-wave

$Ampli_{SECOND}$: Amplitude of the second phase of the M-wave

$Ampli_{PP}$: Amplitude resulting from the sum of $Ampli_{FIRST}$ and $Ampli_{SECOND}$

$Area_{FIRST}$: Area of the first phase of the M-wave

$Area_{SECOND}$: Area of the second phase of the M-wave

$Area_{TOTAL}$: Area resulting from the sum of $Area_{FIRST}$ and $Area_{SECOND}$

Dur_{FIRST}: Duration of the first phase of the M-wave

Dur_{SECOND}: Duration of the second phase of the M-wave

Dur_{PP}: Time interval between the first and second peaks of the M wave

EMG: Electromyography

MVC: Maximal voluntary contraction

M wave: Compound muscle action potential

RF: Rectus femoris

SD: Standard deviation

SE: Standard error of the mean

VL: Vastus lateralis

VM: Vastus medialis

ABSTRACT

***Purpose*:** The study was undertaken to examine separately the potentiation of the first and second phases of the M wave in *biceps brachii* after conditioning maximal voluntary contractions (MVCs) of different durations.

***Methods*:** M waves were evoked in the *biceps brachii* muscle before and after isometric MVCs of 1, 3, 6, 10, 30 and 60s. The amplitude, duration, and area of the first and second phases of monopolar M waves were measured during the 10-min period following each contraction.

***Results*:** Our results indicated that the amplitude and area of the M-wave first phase increased after MVCs of long (≥30s) duration ($P<0.05$), while it decreased after MVCs of short (≤10s) duration ($P<0.05$). The enlargement after the long MVCs persisted for 5 min, whereas the depression after the short contractions lasted only for 15s. The amplitude of the second phase increased immediately (1s) after all MVCs tested ($P<0.05$), regardless of their duration, and then returned rapidly (10s) to control levels. Unexpectedly, the amplitude of the second phase decreased below control values between 15s and 1 min after the MVCs lasting ≥6s ($P<0.05$).

***Conclusions*:** Our results reinforce the idea that the presence of fatigue is a necessary condition to induce an enlargement of the M-wave first phase and that this enlargement would be greater (and occur sooner) in muscles with a predominance of type II fibers (*quadriceps* and *biceps brachii*) compared to type-I predominant muscles (*tibialis anterior*). The unique findings observed for the M-wave second phase indicate that changes in this phase are highly muscle dependent.

INTRODUCTION

It is well known that the compound muscle action potential (M wave), as evoked by applying a transcutaneous electrical stimulus to a peripheral nerve, increases transiently after a brief (<10s) muscle contraction [18]. This phenomenon is normally referred to as "M-wave potentiation" and, although it has been studied under a variety of conditions [17, 27], the underlying mechanisms are not well understood [33]. Previous research on M-wave potentiation have considered the M wave as a whole, i.e., without analyzing separately its first and second phases [3, 10, 12, 18, 19]. By doing this, these studies assumed that the enlargement of the first and second M-wave phases were caused by common mechanisms. However, in a series of recent studies we have shown that the phenomenon of M-wave potentiation is more complex than originally thought, as the amplitudes of the first and second phases change in a different manner (see below) after a maximal voluntary contraction (MVC), irrespective of its duration [35, 37]. While these results are surprising, they have been demonstrated only in pennate muscles (*tibialis anterior* and *quadriceps femoris*) and whether or not the different potentiation of the first and second M-wave phases can be generalized to muscles with a different architecture has not been tested. Thus, in the present experiments, we extend this line of investigation to a fusiform muscle, such as the *biceps brachii*.

One factor that can influence the electrical formation of the first and second M-wave phases (and thus their potentiation) is the geometric arrangement of fibers in the muscle (pennate vs fusiform). In particular, in pennate muscles, due to the inclination of the fibers, the M-wave first phase is primarily composed by the electrical activity of the most superficial (close to the skin) portion of the fiber, whereas, in fusiform muscles, a longer portion of the fiber makes a significant contribution to the M-wave first phase [28]. With regard to the M-wave second phase, in the pennate architecture, is mainly formed by action potentials terminating on the superficial aponeurosis, which can be spread out several centimeters across the muscle [40]. In *biceps brachii*, however, the distal tendon flattens into a strap-like internal aponeurosis whose length is roughly 30% of the *biceps* length [30]. Moreover, because of the different arrangement of muscle fibers, muscles with pennate and fusiform architecture may operate at a different relative length after a contraction, thereby leading to dissimilar changes in the EMG activity. Considering the impact of muscle/tendon geometry on M-wave characteristics, it appears necessary to investigate whether the contrasting potentiation of the first and second M-wave phases is also observed in a muscle with a fusiform architecture.

The muscle-specific discrepancies in the potentiation of the first and second M-wave phases have been attributed to the different electrical formation of these phases [37, 38]. Specifically, the first

phase essentially results from the propagation of the intracellular action potentials along the fiber membrane [23], and thus potentiation of this phase would reflect transient changes in the sarcolemmal excitability after a contraction. Our previous research showed that the M-wave first phase increases after MVCs of long (≥30s) duration for the quadriceps muscle [37], but such increase was not observed in the tibialis anterior [35]. The possible contribution of muscle architecture to such a discrepancy can only be quantified by extending the investigation to other muscles with different fiber-skin arrangement. In other words, we need to examine other muscles to clarify the behavior of the M-wave first phase after long maximal contractions.

The second phase of the M wave represents the extinction of intracellular action potentials at the tendon [11], and thus it would be highly sensitive to changes in muscle architectural features [34]. Our previous studies show that the amplitude of this phase was increased after MVCs with durations ranging from 1s to 1min, thus indicating that the potentiation of this phase cannot be explained solely by changes in membrane properties: rather changes in muscle architectural features might be involved [35, 37]. It is known that muscle and tendon stiffness change during a conditioning contraction and that such changes may persist for a few seconds/minutes afterwards [15, 16, 20, 21, 31]. Because in the pennate and fusiform architectures, muscle fibers are oriented differently with respect to the muscle force-generating axis, the possible alterations in muscle and tendon stiffness after a conditioning contraction may differ in these two fiber arrangements. The present study, therefore, would allow us to gain insights into the role/involvement of muscle architecture in the potentiation of the second phase.

The objective of the present study was to examine separately the potentiation of the first and second phases of the M wave in the *biceps brachii* after conditioning maximal contractions of different durations. Based on our previous experiments on the *tibialis anterior* and *quadriceps*, we hypothesized that: (1) the M-wave first phase would not be enlarged after short (≤10s) MVCs, but it could be enlarged after longer (≥30s) MVCs; (2) the M-wave second phase would be enlarged after all MVCs, regardless of their duration. The study was designed to unravel the mechanisms underlying the potentiation of the M-wave first and second phases, with a view to establish whether the first, the second, or both phases of can be used reliably to detect changes in sarcolemmal membrane excitability.

Addressing these issues may provide potentially relevant insights for clinicians who use the M wave as the reference signal in the so-called “exercise test” for periodic paralysis examinations. In this test, it would be valuable to determine whether the first or the second phase of the M-wave (or both) is

decreased after exercise. The study would also be of interest for sports scientists as the results may clarify whether impaired membrane excitability is manifested by an increase or decrease of the M-wave first phase in the widely studied *biceps brachii* muscle. Additionally, the present findings would be of importance to more fundamental physiologists as they may advance knowledge on the mechanisms underlying M-wave potentiation.

MATERIAL AND METHODS

Participants

Eight participants (two women) aged between 23 and 39 years (mean ± SD: 31.4 ± 5.4 years) volunteered to participate in this study. Their average height and body mass were 175.6 ± 4.9 cm and 72.2 ± 5.3 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 months prior to 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.

Experimental setup

Experiments were performed on the *biceps brachii* muscle and consisted of isometric elbow flexion contractions. Participants were comfortably seated with the arm flexed at 120° and the forearm vertical and supinated, while the hand held an adjustable handle. The shoulder was 90° abducted. Elbow flexion torque was measured using a custom-built ergometer equipped with two torque independent torquemeters (mod. TR11, CCT Transducers, Torino, Italy), one on each side of the arm.

Identification of the muscle fibers' direction and innervation zone

The procedures to identify the muscle fibers' direction and the innervation zone in the *biceps brachii* were similar to those described by Farina et al. (2002) [8]. Surface EMG signals were recorded with a dry linear array of 16 electrodes (5×1 mm, 5 mm inter-electrode distance), which was connected to a multichannel amplifier (EMG-USB, OT Bioelettronica, Torino; bandwidth 10–750 Hz). EMG signals were detected in single differential configuration. The direction of the muscle fibers was identified by choosing the orientation of the array leading to the minimal variation in the shape of action potentials [8]. A line parallel to the orientation of muscle fibers was marked on the skin with a waterproof felt-tip pen. The main innervation zone was defined by the central position between two consecutive channels (pairs of electrodes) providing bipolar EMGs with clear phase opposition and from which propagation could be well apreciated.

Electromyographic recordings

Surface EMG signals were recorded by a 2-dimensional adhesive grid (SPES Medica, Genova, Italy) of 13×5 equally spaced electrodes (3 mm diameter, 8 mm inter-electrode distance in both directions) with one missing electrode at the upper right corner [Fig. 1(a)]. The grid was placed between the

proximal and distal tendons of the *biceps brachii*, with the electrode columns (each formed by 13 electrodes) oriented along the direction of the muscle fibers. The grid was located so that its 7th row was lined up with the innervation zone of the *biceps brachii* short head [Fig. 1(a)]. The four most medial columns of the grid were positioned over the *biceps brachii* short head, whereas the most lateral column of electrodes was lying above the long head. Ultrasound imaging (Echo Blaster 128, Telemed Ltd., Vilnius, Lithuania) was used to identify the junction between the biceps short and long heads.

The region of the skin where the array was located was abraded with abrasive paste (Meditec-Every, Parma, Italy). To ensure proper electrode-skin contact, conductive paste (TEN 20 Conductive Paste; Weaver, Aurora, Colorado) was spread with a spatula into the cavities of a bi-adhesive foam used to secure the array to the skin. Monopolar surface EMGs were amplified by a factor 100, filtered in the 10-750Hz band, sampled at 2048 Hz, and A/D converted with a resolution of 12 bits (multichannel surface EMG amplifier, EMG-USB, OT Bioelettronica, Torino, Italy).

— FIGURE 1 about here —

Brachial plexus stimulation

The brachial plexus was stimulated using a rectangular (size, 35×45 mm) self-adhesive electrode (cathode) positioned on the supraclavicular fossa, whereas the anode (size, 35×45 mm) was placed on the acromion [39]. Single rectangular pulse stimuli of 0.1 ms duration were delivered by a high-voltage constant current stimulator (Model DS7AH; Digitimer, Hertfordshire, UK). The maximal stimulus intensity was determined by gradually increasing the stimulation intensity until a plateau in the M-wave amplitude was observed. This level of intensity was then further increased by 20% to ensure that the stimulation remained supramaximal throughout the experimental session [36].

Experimental protocol

The experimental protocol consisted of applying transcutaneous electrical stimulation to the brachial plexus before and at various times after isometric MVCs of different durations (see Fig. 2). Specifically, the participants were asked to perform, in random order, conditioning MVCs of 1, 3, 6, and 10s. Subsequently, participants also performed a 30-s and a 60-s MVC (in this order) to avoid a possible effect of these long contractions on the signals 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 to investigate the influence of a long-lasting MVC on M-wave characteristics. The protocol comprised 6 sequences, each corresponding to a different MVC duration. Each sequence started with three electrical stimulations (control responses), separated by 5-s intervals, evoked before the conditioning contraction with the muscle at rest. Thereafter, the conditioning contraction (MVC) was performed and, subsequently, single electrical stimuli were delivered to the brachial plexus at 1s, 5s, 10s, 15s, 30s, 1 min, 2 min, 5 min, and 10 min after the contraction.

A 10-min recovery period was left between sequences. 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 in M-wave peak amplitude exceeded 5%, then the resting period was then prolonged by 5 min. In addition, the MVC peak force was measured at the beginning of each contraction and compared across sequences. The MVC peak force did not differ between MVCs (one-way ANOVA, $P = 0.47$), indicating that the resting period was long enough to ensure full recovery.

Data analysis

M-wave data were recorded with a Matlab script (version R2012b; The Math-Works, Natick, MA, USA), written specifically for the real-time visualization and acquisition of EMGs. 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 analyzed using ad-hoc Matlab scripts.

For each M-wave, the amplitude, duration, and area of the first ($Ampli_{FIRST}$, Dur_{FIRST}, and $Area_{FIRST}$) and second ($Ampli_{SECOND}$, Dur_{SECOND}, and $Area_{SECOND}$) phases were measured [see Fig. 1 (b)]. For the calculation of Dur_{FIRST}, the starting point was determined by a deviation greater than 2 standard deviations (SDs) of the baseline noise from the baseline, whereas the ending point corresponded to the baseline-crossing point. The Dur_{SECOND} parameter was computed as the time interval between the previous crossing point and the end of the M-wave, which was determined by the same deviation (2 SDs of baseline noise) from the baseline. The area variables were calculated as the integral of the absolute value of the M wave over the above-defined phases. Total area ($Area_{TOTAL}$) was the sum of the areas of the first and second phases. Peak-to-peak amplitude ($Ampli_{PP}$) was the sum of $Ampli_{FIRST}$ and $Ampli_{SECOND}$. Peak-to-peak duration (Dur_{PP}) was defined as the time interval between the first and

second peaks of the M wave. All these variables were measured for the monopolar M wave recorded over the innervation zone, as done previously [35, 37]. To improve signal-to-noise ratio, the analyzed M wave was the average of the M waves recorded by the channels over the innervation zone [see Fig. 1(a)]. In each of the sequences of the experimental protocol, the three responses evoked before the conditioning contraction were averaged, and the result was used as the control M wave. All M-wave characteristics recorded after the conditioning contractions were expressed as percentage of those measured in the control M wave.

Statistics

Kolmogorov-Smirnov tests confirmed that each of the M-wave characteristics analyzed in the current study was normally distributed ($P>0.05$). Differences in the control values of the M-wave characteristics across the MVC-duration sequences were examined using a one-way ANOVA. M-wave characteristics 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. For each MVC-duration sequence, the Pearson's correlation coefficient was computed to determine the relationship between the following pairs of variables during the recovery: $Ampli_{FIRST}$ and Dur_{FIRST}, $Ampli_{SECOND}$ and Dur_{SECOND}, and $Ampli_{PP}$ and Dur_{PP}. Significance was set at $p<0.05$. Data were presented as mean ± standard deviation in the text and tables and as mean ± standard error in the figures.

RESULTS

Representative M waves after conditioning MVCs of different durations

Figure 2 provides representative examples of M waves recorded in one participant before (control) and after conditioning MVCs of 1s (a), 10s (b), and 60s (c). It can be seen that the amplitude of the M-wave first and second phases changed in an opposite manner after both the 1-s MVC [Fig. 2(a1)] and 10-s MVC [Fig. 2(b1)]: specifically, $Ampli_{FIRST}$ decreased, while $Ampli_{SECOND}$ augmented, immediately (1s) after these brief contraction. These changes in $Ampli_{FIRST}$ and $Ampli_{SECOND}$ disappeared very rapidly (10s). Conversely, after the 60-s MVC [Fig. 2(c1)] both $Ampli_{FIRST}$ and $Ampli_{SECOND}$ increased. However, whereas $Ampli_{SECOND}$ returned very rapidly (5s) to control values, $Ampli_{FIRST}$ remained enlarged even 5 min after the cessation of the 60-s MVC.

To better appreciate the changes in M-wave duration, in the plots on the right of Fig. 2 we show, superimposed, various M waves evoked at different times after the MVCs [Figs. 2(a2), (b2), (c2)]. In Figs. 2(a2) and (b2), the M wave evoked immediately (1s) after the brief (≤10s) MVC had shorter duration than the control M wave. In contrast, immediately (1s) after the 60s-MVC, the M wave had longer duration than the pre-contraction M wave [Fig. 2(c2)]. Moreover, changes in duration induced by the short MVCs almost vanished within the first 5s following the MVC. However, after the 60-s MVC, the broadening of the M wave remained for longer than 1 min.

— FIGURE 2 about here —

Amplitude characteristics of the M wave

Figures 3 and 4 shows group data of the amplitude and area for the M-wave first (first column) and second phases (second column), and for the whole M wave (third column), during the recovery after conditioning MVCs of different durations. In Fig. 3(a) it can be seen that $Ampli_{FIRST}$ decreased immediately (1s) after short (≤10s) MVCs ($P<0.05$, Table 1), while it increased significantly right after the long (≥30s) MVCs ($P<0.05$, Table 1). After the brief (≤10s) MVCs, $Ampli_{FIRST}$ returned very rapidly (within 15s) to control values. In contrast, after the long (≥30s) MVCs, $Ampli_{FIRST}$ remained elevated for at least 5 min before returning to the control level. Unlike $Ampli_{FIRST}$, $Ampli_{SECOND}$ [Fig. 3(b)] increased immediately (1s) after all MVCs tested regardless of their duration ($P<0.05$, Table 1), and then decreased rapidly to control values (5s). Noteworthy, $Ampli_{SECOND}$ was observed to remain below control values for up to 30s after the MVCs ≥6s ($P<0.05$). Of note, $Ampli_{PP}$ did not change after

the short (≤10s) MVCs, whereas it increased immediately (1s) after the longer MVCs (30s and 60s, $P<0.05$).

Contrary to the amplitude, the area of the first and second M-wave phases showed a similar time course during the recovery [Figs. 3(d) and (e)]. Specifically, both $Area_{FIRST}$ and $Area_{SECOND}$ decreased immediately (1s) after the short (≤10s) MVCs ($P<0.05$, Table 1), whereas these parameters increased significantly right after the long (≥30s) MVCs ($P<0.05$, Table 1). After the brief (≤10s) MVCs, the greatest drop in $Area_{FIRST}$ and $Area_{SECOND}$ occurred 1s after exercise, but this decrease only lasted for 15s, before normalizing. In contrast, after the long (≥30s) MVCs, both $Area_{FIRST}$ and $Area_{SECOND}$ remained elevated for 5 min ($P<0.05$, Table 1). The recovery of $Area_{TOTAL}$ [Fig. 3(f)] was similar in all aspects to that of $Area_{FIRST}$ and $Area_{SECOND}$.

— FIGURE 3 about here —

— TABLE 1 about here —

— FIGURE 4 about here —

Duration characteristics of the M wave

The time course of Dur_{FIRST} and Dur_{SECOND} showed important similarities (Fig. 5). First, both Dur_{FIRST} and Dur_{SECOND} decreased immediately (1s) after the short (≤10s) MVCs ($P<0.05$, Table 1), while they increased significantly right after the 60-s MVC ($P<0.05$, Table 1). Moreover, after the brief (≤10s) MVCs, the greatest decline in both Dur_{FIRST} and Dur_{SECOND} was reached 1s after exercise, but this decrease only lasted for 15s. In contrast, after the long (≥30s) MVCs, both Dur_{FIRST} and Dur_{SECOND} remained elevated for 5 min ($P<0.05$, Table 1). However, the time courses of Dur_{FIRST} and Dur_{SECOND} exhibited an important difference: whereas Dur_{FIRST} increased only between 1s and 15s after exercise [Fig. 5(a)], Dur_{SECOND} increased uninterruptedly until about 2 min into the recovery [Fig. 5(b)]. Of note, the time course of recovery of Dur_{FIRST} was similar to that of $Ampli_{FIRST}$ and $Area_{FIRST}$.

— FIGURE 5 about here —

Correlation between the time course of changes in M-wave characteristics during recovery

Table 2 shows the correlation coefficients between the time course of changes of various pairs of M-wave variables ($Ampli_{FIRST}$ and Dur_{FIRST}; $Ampli_{SECOND}$ and Dur_{SECOND}; $Ampli_{FIRST}$ and $Ampli_{SECOND}$; $Ampli_{PP}$ and Dur_{PP}) after each MVC duration. The correlation between $Ampli_{FIRST}$ and Dur_{FIRST} was significant for both short (≤10s) and long (≥30s) MVCs, indicating that the temporal variation of $Ampli_{FIRST}$ during recovery was closely related to that of Dur_{FIRST} irrespective of the contraction duration. $Ampli_{SECOND}$ vs Dur_{SECOND} were significantly (negatively) correlated only for the short (≤10s) MVCs; thus, a dissociation existed between the time courses of $Ampli_{SECOND}$ and Dur_{SECOND} after long fatiguing contractions. Similarly, $Ampli_{FIRST}$ and $Ampli_{SECOND}$ only showed a significant (negative) correlation after the short MVCs. Finally, the correlation between $Ampli_{PP}$ vs Dur_{PP} reached statistical significance only for the long (≥30s) MVCs; hence, the time course of changes in $Ampli_{PP}$ was not related to that Dur_{PP} after short contractions.

— TABLE 2 about here —

The average force at the end of the 30-s and 60-s MVCs declined respectively by 27.5 ± 12.5% and 45.7 ± 15.9% of the force recorded at the onset of the MVCs (unfatigued state). Stimulation intensities used for supramaximal stimulation of the brachial plexus were 63 ± 0.13 mA.

DISCUSSION

The present study was conducted to investigate separately the potentiation of the first and second phases of the M wave in the *biceps brachii* muscle. The main findings of the study were: (1) $Ampli_{FIRST}$ increased after MVCs of long (≥30s) duration, while it decreased after MVCs of short (≤10s) duration; (2) The increase in $Ampli_{FIRST}$ after the long MVCs lasted for 5 min, whilst the decrease in $Ampli_{FIRST}$ after the short MVCs vanished rapidly, within 15s; (3) The amplitude of the second phase increased immediately (1s) after all MVCs tested, regardless of their duration, and then returned rapidly (10s) to control levels; (4) Unexpectedly, the amplitude of the second phase decreased below control values between 15s and 1 min after the MVCs lasting ≥6s.

Amplitude of the M-wave first phase

We found that $Ampli_{FIRST}$ increased only after MVCs of duration ≥30s, that is, after contractions that caused a significant decline in MVC force. A similar result was observed in the *quadriceps* muscle, where $Ampli_{FIRST}$ increased only after the 30-s and 60-s MVCs [37]. Thus, the presence of fatigue appeared to be a prerequisite for the enlargement of the first phase [38]. The present results provide several pieces of evidence supporting this view. First, the increase in $Ampli_{FIRST}$ occurred in parallel with a widening of the M-wave first phase, i.e., a sign of a slowing of impulse conduction (see the correlation between $Ampli_{FIRST}$ and Dur_{FIRST}, Table 2). Second, the degree of increase in $Ampli_{FIRST}$ was greater after the 60-s MVC (which produced a force loss of ~46%) than after the 30-s MVC (force loss of ~27 %), indicating that the mechanisms underlying this enlargement were augmented with greater decreases in MVC force. Moreover, $Ampli_{FIRST}$ remained elevated for longer than 5 min after the fatiguing contraction, indicating that augmentation of this phase occurs only while the effects of fatigue persist in the muscle.

Another remarkable finding of the study was that $Ampli_{FIRST}$ decreased immediately (1s) after short (≤10s) MVCs. Interestingly, this decline only lasted for a few seconds (~15s). Thus, not only the sign, but also the time course of recovery of $Ampli_{FIRST}$ was totally different after short and long contractions. Moreover, fatigue was not a prerequisite for the depression of the first phase as $Ampli_{FIRST}$ was already decreased after an MVC as short as 1s. It is therefore clear that the mechanisms responsible for the decrease in the M-wave first phase after a brief contraction are necessarily different from those underlying the augmentation of this phase after a prolonged maximal effort.

Differential changes in the amplitude of the first and second phases after short contractions

We found that $Ampli_{FIRST}$ and $Ampli_{SECOND}$ changed in opposite directions ($Ampli_{FIRST}$ decreased, while $Ampli_{SECOND}$ increased) immediately after short (≤10s) MVCs. Whereas a divergent behavior of the first and second M-wave phases has already been observed in the *tibialis anterior* [35] and *quadriceps* [37], in these studies $Ampli_{FIRST}$ remained unchanged. Thus, the present study is the first to show that a short MVC induces a depression of the M-wave first phase, while an enlargement of the second one. The contrasting behavior of $Ampli_{FIRST}$ and $Ampli_{SECOND}$ has remained unnoticed by the majority of previous investigations in which the M wave was considered as a whole unit, i.e., without analyzing separately its first and second phases [3, 18]. In these previous works, researchers only considered the gross M-wave features ($Ampli_{PP}$ and/or $Area_{TOTAL}$). In the light of the present results, such practice could have led to misinterpretation of the findings. For example, here we showed that the lack of change in $Ampli_{PP}$ after the short MVCs did not imply that $Ampli_{FIRST}$ and $Ampli_{SECOND}$ were "individually" unchanged; indeed the absence of change in $Ampli_{PP}$ was the result of the decrease in $Ampli_{FIRST}$ and the concomitant increase in $Ampli_{SECOND}$, respectively. Therefore, a separate analysis of the first and second M-wave phases is mandatory to adequately detect and characterize the contraction effects on M-wave features.

Possible mechanisms underlying the augmentation of the M-wave first phase after long contractions

As mentioned above, the present results support the idea that a decline in MVC force is a necessary condition for the augmentation of the M-wave first phase [38]. These observations, in combination with the fact that the M-wave first phase essentially results from the propagation of action potentials along the fiber membrane [38], suggest that the augmentation of the M-wave first phase would be caused by fatigue-induced changes in the sarcolemmal membrane properties. One plausible membrane-related explanation for the increase in $Ampli_{FIRST}$ is that, as a result of the rise in extracellular K^+ concentration during the long fatiguing contractions, individual transmembrane action potentials become longer and with prominent negative after-potentials [14, 24, 22, 29]. Indeed, a lengthening of the intracellular action potential has been shown to result in greater and longer extracellular potentials when recorded over the muscle surface [1, 5, 6, 7]. Several lines of evidence support an association between the broadening of the intracellular potential and the increase in $Ampli_{FIRST}$. First, we observed that an MVC of at least 30s was required to produce a detectable enlargement of $Ampli_{FIRST}$. In agreement, Hanson and Persson (1971) [13] found that at least 20s of repetitive stimulation at 10 Hz was necessary for the broadening of the intracellular potential to be recognizable. Second, our finding that the increase of $Ampli_{FIRST}$ was greater after the 60-s MVC than after the 30-s MVC is consistent with the finding of Hanson and Persson (1971) [13] that the broadening of the intracellular potential becomes more pronounced as the duration of the repetitive

stimulation protocol was made longer. Finally, we found that $Ampli_{FIRST}$ remained elevated for 5 min after the long MVC, corroborating the observation that the intracellular potential remained lengthened for about 3 min after 60s of repetitive stimulation at 10 Hz (see Fig. 1 of Hanson and Persson, 1971).

In our previous studies, we found that $Ampli_{FIRST}$ increases after long (≥30s) MVCs for the *quadriceps* muscles [37], but such increase was not seen in the *tibialis anterior* [35]. Consistent with the results on the *quadriceps* (but not with the *tibialis anterior*), we found that $Ampli_{FIRST}$ increased only after the 30-s and 60-s MVCs. This finding is extremely important for two reasons. First, it lends support to the idea that the presence of fatigue is a prerequisite to induce an enlargement of the first phase. Second, it is interesting to note that the increase in $Ampli_{FIRST}$ was observed in muscles with a predominance of type II fibers (i.e., the *quadriceps* and *biceps brachii*), whereas $Ampli_{FIRST}$ remained unchanged in a muscle with a preponderance of type I fibers, such as the tibialis anterior. Unfortunately, muscle biopsies were not performed in any of the above mentioned studies, and thus we can only speculate that fiber type composition may play a role in the augmentation of the M-wave first phase after long fatiguing contractions. Therefore, the present findings would prompt future research into the influence of fiber type composition on the potentiation of the first phase.

It is difficult to establish whether fiber pennation has a role in the potentiation of the M-wave first phase. It has been shown that different muscles with a pennate architecture (such as the *tibialis anterior* and *vastus lateralis*) exhibit different patterns of EMG activity, possibly due to differences in fibers' orientation in relation to EMG electrodes [2, 41, 42]. In fact, it has been shown that surface EMG potentials detected from the *vastus lateralis* are more similar to those detected from the *biceps brachii* than from the *tibialis anterior* [4, 32, 42].

Possible mechanisms for the changes in the amplitude and duration of the first and second phases after a short contraction

At first sight, it might be argued that the opposing changes in $Ampli_{FIRST}$ and $Ampli_{SECOND}$ observed immediately after a short (≤10s) MVC could not be caused by the same mechanism. However, we observed that the recovery of these features had a roughly similar time course: $Ampli_{FIRST}$ increased between 1s and 15s before stabilizing, whereas $Ampli_{SECOND}$ decreased in the same period before stabilizing (see the correlation between $Ampli_{FIRST}$ and $Ampli_{SECOND}$, Table 2). Moreover, not only the amplitude characteristics, but also Dur_{FIRST} and Dur_{SECOND} increased rapidly between 1s and 15s before normalizing. Thus, the parallel temporal changes in the amplitude and duration of the first and second M-wave phases may indicate that these phases could be affected by a common mechanism. If

this is the case, then such a mechanism would exert opposite effects on $Ampli_{FIRST}$ and $Ampli_{SECOND}$. With this logic, any explanation for the concurrent changes in $Ampli_{SECOND}$ and $Ampli_{SECOND}$ based on fiber membrane properties seems improbable as they would make $Ampli_{FIRST}$ and $Ampli_{SECOND}$ change in the same direction, in contrast to what we observed.

A family of factors capable of causing opposite changes in $Ampli_{FIRST}$ and $Ampli_{SECOND}$, while altering the duration of both M-wave phases, are changes in muscle architecture during/after a contraction. In particular, it might be hypothesized that the reduction in fibers' length that occurs during a contraction could persist for a few seconds (~15s) after the cessation of the contraction, before returning to normal values. On one hand, such muscle shortening would cause a better synchronization between the "terminal" phases of the constituent motor unit potentials of the M wave, thus leading to a larger and briefer M-wave second phase (increase in $Ampli_{SECOND}$, decrease in Dur_{SECOND}) [34]. On the other hand, in the evoked compound muscle potential the propagating component of some fibers inevitably overlaps with the non-propagating component of other fibers [38]. Muscle shortening enlarges the portion of overlapping, thereby increasing the phase cancellation between the propagating and non-propagating components of the different fibers, which could result in a depression of the first phase ($Ampli_{FIRST}$). In favor of the muscle shortening hypothesis is the observation that both Dur_{FIRST} and Dur_{SECOND} were transiently decreased during the first 15s after the short MVCs. Therefore, it might be speculated that the similar time course of changes in $Ampli_{FIRST}$, Dur_{FIRST}, $Ampli_{SECOND}$, and Dur_{SECOND} during the first 15s after a short contraction reflect the process by which the muscle fibers return to their normal length after a transient shortening.

Possible mechanisms for the depression of the second phase after contractions longer than 6s

A unique finding of the study, which was not observed in our previous works in the *tibialis anterior* and *quadriceps*, was that $Ampli_{SECOND}$ decreased below control values between 15s and 1 min after the MVCs with duration ≥6s. This depression in $Ampli_{SECOND}$ is unlikely to be due to fatigue mechanisms, since the extent of the decrease in $Ampli_{SECOND}$ was similar for MVCs ranging between 6 and 60s. Besides, for the same conditions, no decline was observed in $Ampli_{FIRST}$. Instead, because the M-wave second phase is highly sensitive to changes in muscle and tendon architectural features [34], the depression in $Ampli_{SECOND}$ could be related to the geometrical specificities of the *biceps brachii*, which, unlike the *tibialis anterior* and *quadriceps*, is a fusiform muscle. In particular, it is conceivable that, between 15s and 1 min after the MVC, a small transient increase in muscle stiffness, or a decrease in tendon stiffness, occurred [15, 16, 20, 21, 31], which would cause the muscle to operate at a longer

length [25, 26]. Thus, a transient muscle lengthening would increase the time dispersion between the "terminal" phases of the constituent motor unit potentials of the M wave, thus leading to a smaller and longer M-wave second phase. In support of this idea, we observed a transient increase in Dur_{SECOND} between 15s and 1 min after the MVCs with duration ≥6s [see Fig. 2(b2)]. Collectively, these findings reinforces our view that changes in the M-wave second phase after a contraction are muscle dependent, i.e., they depend on the specific architecture/fiber arrangement of each muscle.

The area and amplitude characteristics cannot be used interchangeably

It is relevant at this point to add a note on the area parameter and its use as an indicator to detect changes in sarcolemmal membrane properties. Here, we observed that both $Area_{FIRST}$ and $Area_{SECOND}$ behaved in a similar fashion: both features decreased after short contractions, while they increased after long maximal effort. However, the almost identical behavior of $Area_{FIRST}$ and $Area_{SECOND}$ does not signify that the first and second phases of the M wave reacted in the same way to a conditioning MVC; indeed, we found that the amplitude of these phases changed in opposite directions after the short contractions. These apparently contradictory findings are explained by the fact that the behaviour of M-wave area was dominated by the changes in the duration of the compound potential. Specifically, after the short MVCs, the narrowing of the M wave made both $Area_{FIRST}$ and $Area_{SECOND}$ to decrease, whereas, after the long MVCs, the broadening of the M wave increased both $Area_{FIRST}$ and $Area_{SECOND}$. From the above discussion, two conclusions emerge. First, the area feature is so sensitive to changes in the duration of the evoked potential that it might miss important information relative to the amplitude of the potential [9]. Second, the amplitude and area features cannot be used interchangeably; these features may offer different but complementary information on the changes in membrane properties [38].

Cautions and considerations for clinicians and sport science practitioners

The present findings are of importance to the fields of clinical neurophysiology, sports science, and muscle physiology for various reasons. First, our results highlight the need to reinterpret the concept of "impaired membrane excitability" as we have shown that it is an augmentation (and not a depression) of the M-wave first phase what reflects a reduction of sarcolemmal excitability. Second, the widespread explanation for M-wave potentiation proposed by Hicks and McComas [17, 18], namely the Na^+-K^+ pump-induced hyperpolarization of individual muscle fibers, should be revisited. Indeed, McComas and colleagues claimed that a short MVC (≤10s) was sufficient to enhance the Na^+-K^+ pumping. Thus, if a true enhancement of the Na^+-K^+ pumping take place, it would cause a distinct

increase in both the first and second M-wave phases: however, we found that only the second, but not the first, phase of the M wave increases in amplitude after a short MVC. Finally, it must be stressed that both the first and second M-wave phases underwent short-term changes in amplitude immediately after an MVC (lasting approximately 15s), the causes of which remain unknown. Thus, we advise not to quantify M waves within the first 15s after a contraction.

In summary, we found that the amplitude of the M-wave first phase was increased only after contractions that induced fatigue (MVCs of 30 and 60s duration). Because the augmentation of the first phase persisted for 5 min after the contraction and occurred in parallel with an increase in the duration of this phase, it was proposed that such augmentation should be caused by fatigue-induced changes in the sarcolemmal membrane. Collectively, this findings (1) reinforce the idea that the presence of fatigue is a necessary condition to induce an enlargement of the M-wave first phase and (2) suggest that the enlargement of the first phase would be greater (and occur sooner) in muscles with a predominance of type II fibers (*quadriceps* and *biceps brachii*) compared to type-I predominant muscles (*tibialis anterior*). On the other hand, the amplitude of the M-wave second phase increased immediately after (1s) all MVCs tested, regardless of their duration, and subsequently decreased rapidly (within 10s) to normal values. A unique finding of the study, which was not observed in our previous works in the *tibialis anterior* and *quadriceps*, was that the amplitude of the M-wave first phase decreased immediately after (1s) a brief (≤10s) MVC, and then returned rapidly (15s) to normal. Also not seen in our previous research, we found that the amplitude of the M-wave second phase decreased below control values between 15s and 1 min after the MVCs with duration ≥6s. In conclusion, although, in essence, M-wave potentiation in the *biceps brachii* is similar to that of other studied muscles (*tibialis anterior* and *quadriceps*), some unique peculiarities were observed, suggesting that the determinants for the changes in M-wave first and second phases after a contraction are muscle dependent.

GRANTS

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FINANCIAL DISCLOSURE

I declare that the authors have no financial interests.

CONFLICT OF INTEREST

I declare that the authors have no conflict of interest.

AUTHOR CONTRIBUTIONS

J.R-F, A.B, T.V, and N.P designed experimental study;

J.R-F, A.B, and T.V performed experiments;

J.R-F and A.B analyzed data;

J.R-F, A.B, T.V, and N.P interpreted results of experiments;

J.R-F drafted manuscript;

J.R-F, A.B, T.V, and N.P edited and revised manuscript;

J.R-F, A.B, T.V, and N.P approved final version of manuscript.

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Taian Vieira

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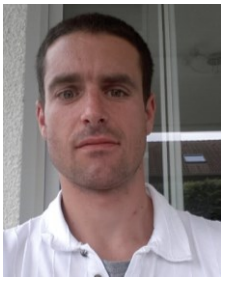
Nicolas Place



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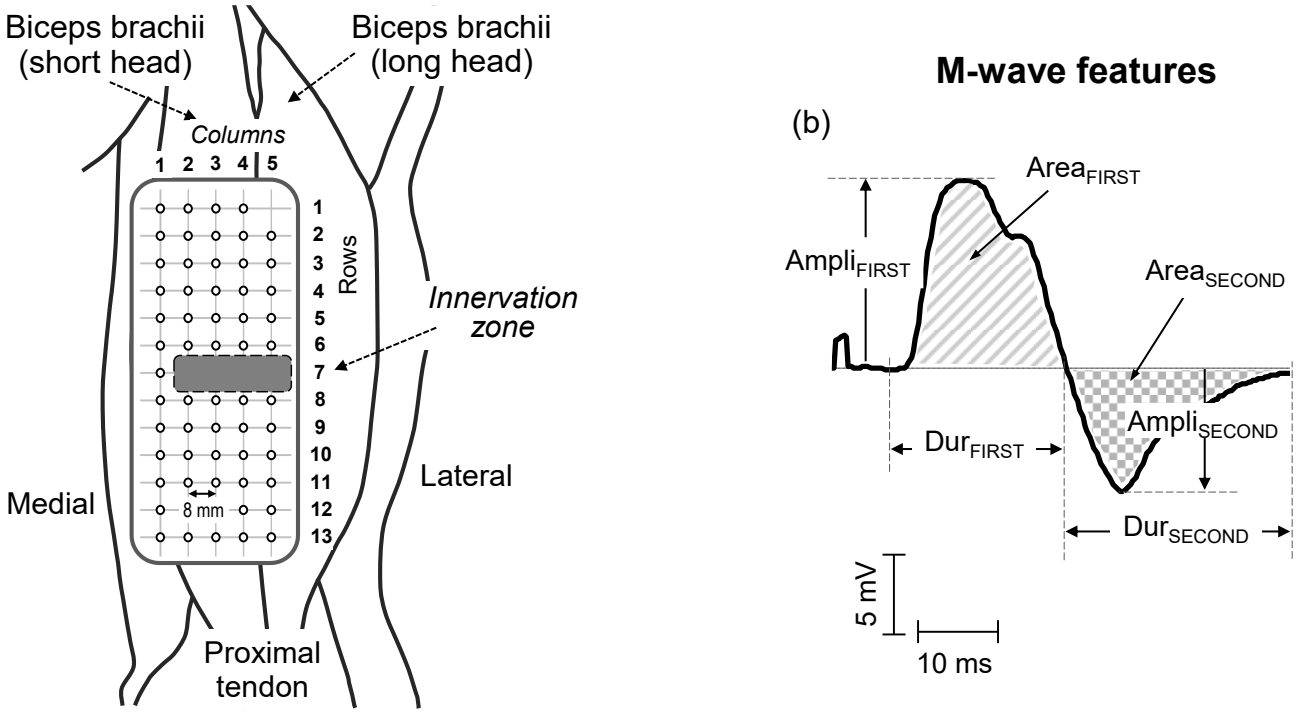
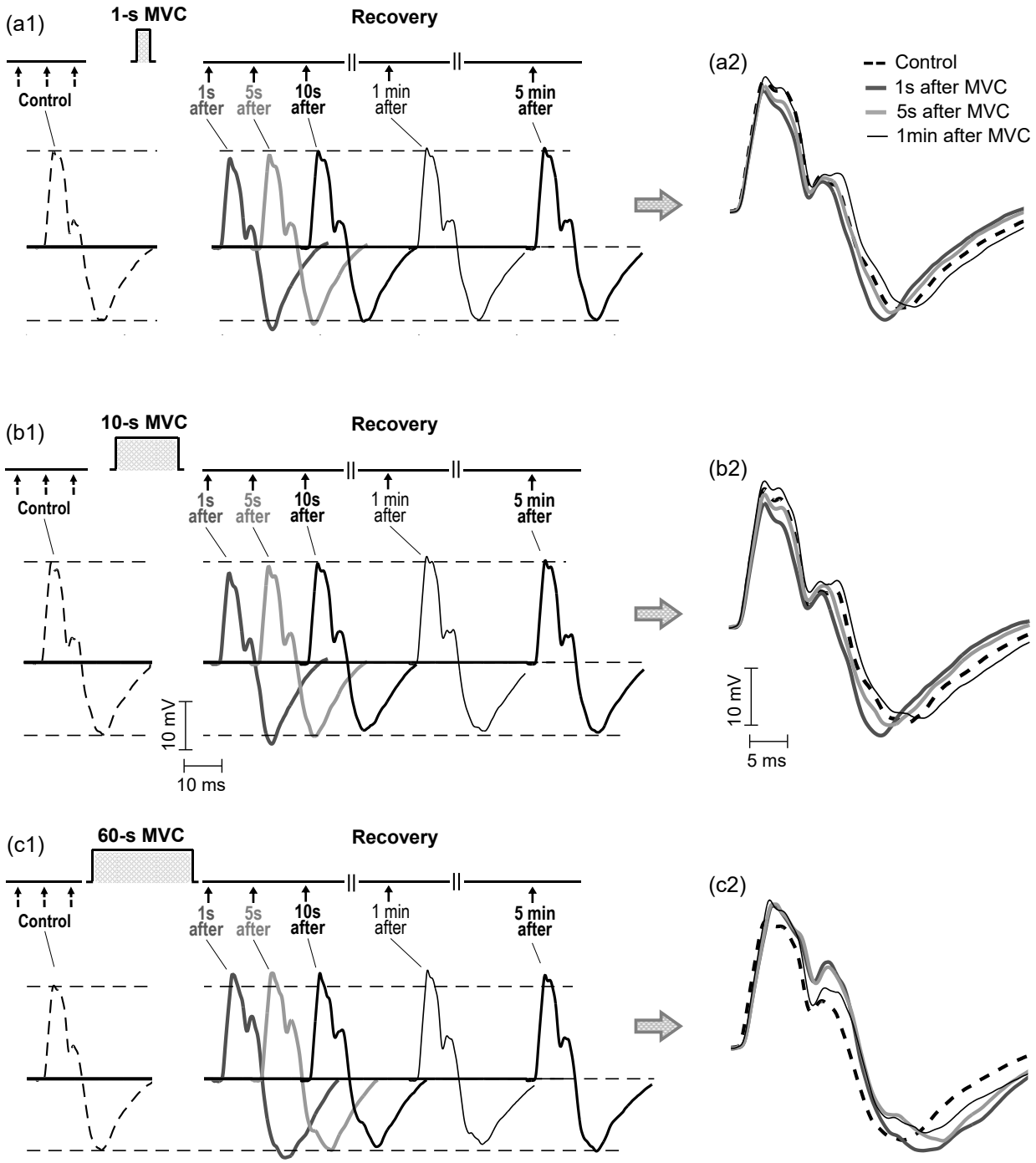
Alberto Botter

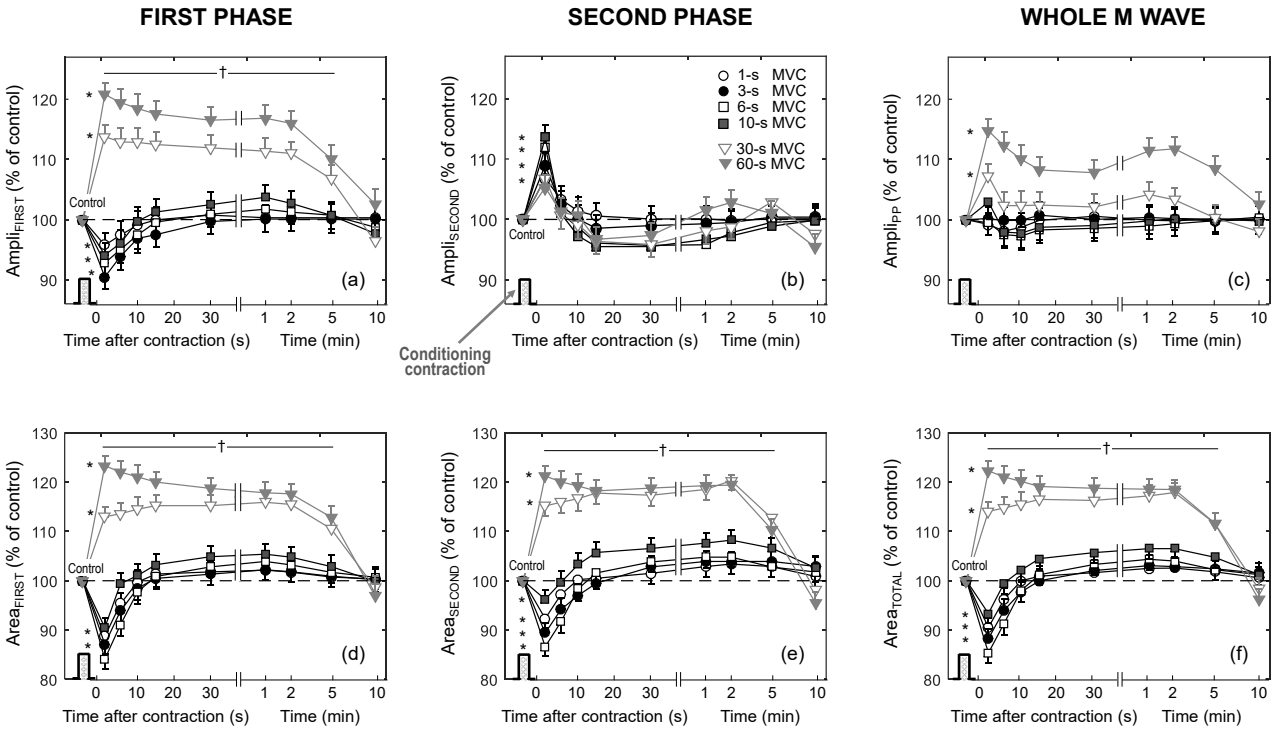
FIGURES



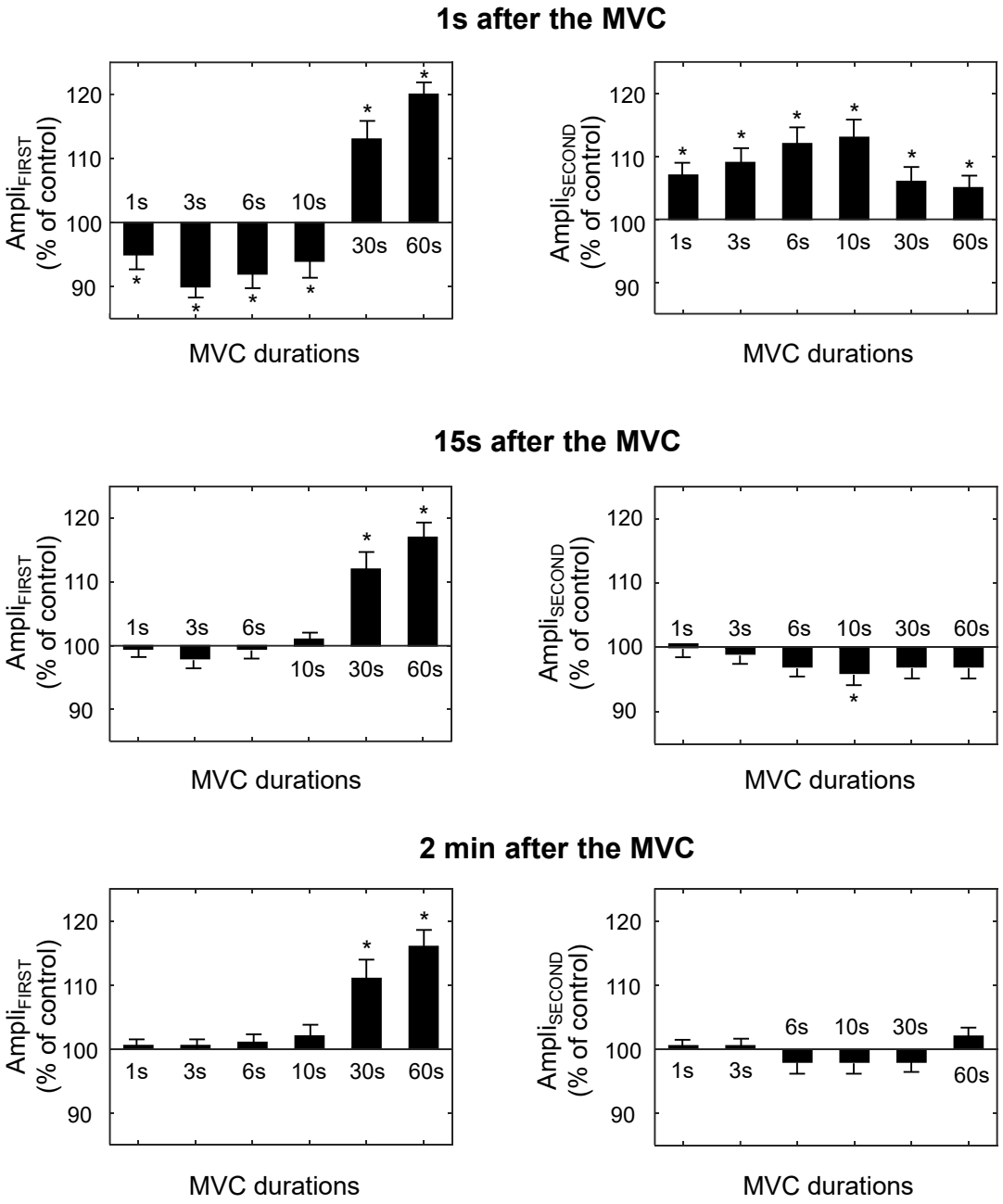
(Fig. 1)


(a1)
1-s MVC
Recovery
Control
1s after
5s after
10s after
1 min after
5 min after
(a2)
Control
1s after MVC
5s after MVC
1min after MVC
(b1)
10-s MVC
Recovery
Control
1s after
5s after
10s after
1 min after
5 min after
10 mV
10 ms
(b2)
10 mV
5 ms
(c1)
60-s MVC
Recovery
Control
1s after
5s after
10s after
1 min after
5 min after
(c2)

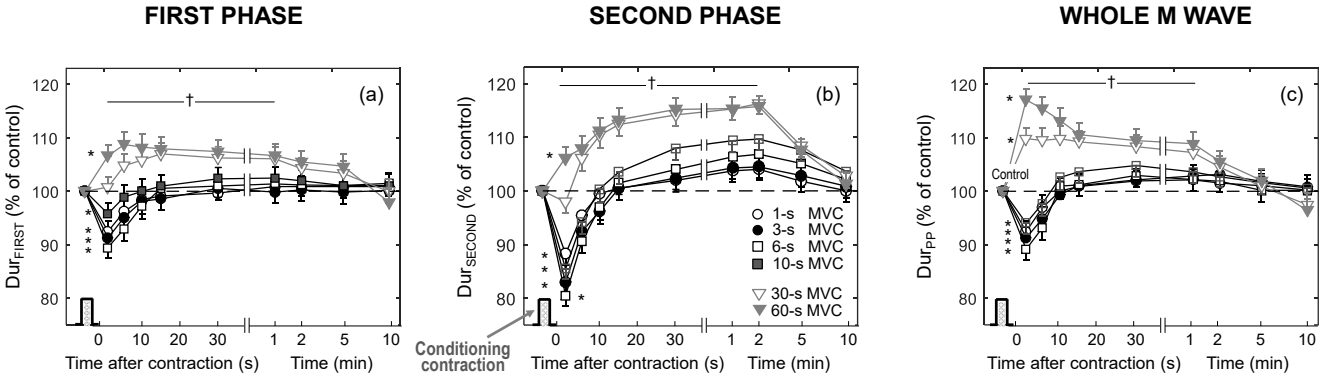
(Fig. 2)


FIRST PHASE
SECOND PHASE
WHOLE M WAVE
1-s MVC
3-s MVC
6-s MVC
10-s MVC
30-s MVC
60-s MVC
Control
Conditioning contraction
Time after contraction (s)
Time (min)
(a)
(b)
(c)
(d)
(e)
(f)

(Fig. 3)


1s after the MVC
Ampli$_{FIRST}$ (% of control)
120
110
100
90
1s
3s
6s
10s
30s
60s
MVC durations
Ampli$_{SECOND}$ (% of control)
15s after the MVC
2 min after the MVC

(Fig. 4)


FIRST PHASE
SECOND PHASE
WHOLE M WAVE
(a)
(b)
(c)
Dur_FIRST (% of control)
Dur_SECOND (% of control)
Dur_PP (% of control)
Time after contraction (s)
Time (min)
Conditioning contraction
Control
1-s MVC
3-s MVC
6-s MVC
10-s MVC
30-s MVC
60-s MVC

(Fig. 5)

			Time points during the recovery after the MVC						
MVC duration	M-wave characteristics	Control values	1s	5s	10s	30s	2 min	5 min	10 min
	$Ampli_{FIRST}$ (mV)	15.2 ± 3.8	13.6 ± 3.5*	14.3 ± 3.7*	14.7 ± 3.8	15.1 ± 3.5	15.2 ± 3.6	15.2 ± 3.7	15.2 ± 3.8
3-s MVC	$Ampli_{SECOND}$ (mV)	14.7 ± 3.8	15.8 ± 3.6*	15.0 ± 3.8	14.7 ± 3.9	14.6 ± 3.9	14.6 ± 4.1	14.7 ± 3.9	14.7 ± 3.8
	$Area_{FIRST}$ (mV·ms)	89.7 ± 21.3	76.7 ± 19.8*	82.5 ± 22.6*	86.5 ± 21.5	91.3 ± 23.2	91.7 ± 24.7	90.2 ± 20.0	89.7 ± 22.5
	$Ampli_{FIRST}$ (mV)	15.2 ± 3.4	14.3 ± 3.5*	14.6 ± 3.7*	15.1 ± 3.8	15.5 ± 3.6	15.6 ± 3.7*	15.3 ± 3.4	14.9 ± 3.7
10-s MVC	$Ampli_{SECOND}$ (mV)	14.8 ± 3.3	16.0 ± 3.3*	14.9 ± 3.6	14.7 ± 3.5	14.0 ± 3.7*	14.4 ± 3.3	14.7 ± 3.8	14.8 ± 3.8
	$Area_{FIRST}$ (mV·ms)	90.2 ± 23.3	81.0 ± 21.5*	89.8 ± 22.8	91.4 ± 23.0	94.5 ± 20.3*	94.2 ± 22.9	92.2 ± 23.4	90.7 ± 23.8
	$Ampli_{FIRST}$ (mV)	15.3 ± 3.5	17.3 ± 3.5*	17.2 ± 3.7*	17.1 ± 3.5*	16.9 ± 3.5*	16.6 ± 3.5*	16.3 ± 3.5*	14.9 ± 3.5
30-s MVC	$Ampli_{SECOND}$ (mV)	14.6 ± 3.7	15.3 ± 3.7*	14.7 ± 3.6	14.4 ± 3.6	13.9 ± 3.5*	14.6 ± 3.4	15.0 ± 3.9	14.3 ± 3.8
	$Area_{FIRST}$ (mV·ms)	88.3 ± 24.5	98.2 ± 23.7*	99.3 ± 25.5*	100.7 ± 26.3*	100.5 ± 24.9*	100.6 ± 25.0*	95.4 ± 25.2*	86.3 ± 26.9
	$Ampli_{FIRST}$ (mV)	15.4 ± 3.7	18.5± 3.8*	18.3 ± 3.6*	18.1 ± 4.1*	17.7 ± 3.9*	17.6 ± 4.0*	16.8 ± 3.7*	15.6 ± 4.2
60-s MVC	$Ampli_{SECOND}$ (mV)	14.4 ± 3.8	15.3± 3.5*	14.4 ± 3.5	14.3 ± 3.5	13.9 ± 3.6*	14.7 ± 3.5	14.4 ± 3.5	13.8 ± 3.5*
	$Area_{FIRST}$ (mV·ms)	91.6 ± 25.7	111.4 ± 26.4*	109.4 ± 28.5*	107.0 ± 24.5*	104.5 ± 26.0*	102.9 ± 27.3*	101.3 ± 26.6*	87.2 ± 24.9

(Table 1)

	$Ampli_{FIRST}$ vs Dur_{FIRST}	$Ampli_{SECOND}$ vs Dur_{SECOND}	$Ampli_{FIRST}$ vs $Ampli_{SECOND}$	$Ampli_{PP}$ vs Dur_{PP}
1-s MVC	0.47 ± 0.13 *	-0.65 ± 0.15 *	-0.53 ± 0.14 *	0.22 ± 0.03
3-s MVC	0.51 ± 0.12 *	-0.58 ± 0.14 *	-0.54 ± 0.13 *	0.24 ± 0.04
6-s MVC	0.55 ± 0.13 *	-0.64 ± 0.16 *	-0.51 ± 0.13*	0.29 ± 0.04
10-s MVC	0.61 ± 0.13 *	-0.45 ± 0.15 *	-0.52 ± 0.14 *	0.32 ± 0.05
30-s MVC	0.68 ± 0.15 *	0.19 ± 0.05	0.12 ± 0.04	0.48 ± 0.13 *
60-s MVC	0.79 ± 0.15 *	0.13 ± 0.04	0.15 ± 0.03	0.53 ± 0.14 *

(Table 2)

CAPTIONS

Fig. 1 (a) Electrode arrangements for the recording of surface EMG from the *biceps brachii*. Surface electrodes were arranged in a 13×5 grid with an inter-electrode distance of 8 mm. Columns of the grid were oriented along the direction of the muscle fibers. The 7th row of the array coincided with the innervation zone of the *biceps brachii* short head. The four most medial columns of the grid were positioned over the *biceps brachii* short head. (b) Definition of the M-wave features: amplitude, duration, and area of the first ($Ampli_{FIRST}$, Dur_{FIRST}, and $Area_{FIRST}$) and second ($Ampli_{SECOND}$, Dur_{SECOND}, and $Area_{SECOND}$) phases.

Fig. 2 Representative examples of M waves recorded in one participant before (control) and at various times after conditioning maximal voluntary contractions (MVCs) of 1s, 10s, and 60s. In the left panels (a1, b1 and c1), M waves are shown in chronological sequence to better appreciate the changes in the amplitude of the first and second phases after the contraction. Note that $Ampli_{FIRST}$ decreased, while the $Ampli_{SECOND}$ increased, after the 1-s and 10-s MVC. In contrast, both $Ampli_{FIRST}$ and $Ampli_{SECOND}$ increased after the 60s-MVC. In the right panels (a2, b2 and c2), M waves are shown superimposed to better illustrate the changes in M-wave duration. The M waves evoked after the brief (≤10s) MVC had shorter duration than the control M wave, whereas M waves elicited after the long (60s) MVC are broader than the pre-contraction M wave.

Fig. 3 Time course of recovery of the amplitude and area for the M-wave first phase ($Ampli_{FIRST}$ and $Area_{FIRST}$, respectively, panels a and d), second phase ($Ampli_{SECOND}$ and $Area_{SECOND}$, panels b and e), and for the whole M wave ($Ampli_{PP}$ and $Area_{TOTAL}$, panels c and f), after conditioning maximal voluntary contractions (MVCs) of different durations. All data are expressed in percentage of control values and reported as mean ± SE (n=8). † Significant difference between the 1-s, 3-s, 6-s, and 10-s MVCs compared with the 30-s and 60-s MVCs ($p<0.05$). * Significant difference with control ($p<0.05$).

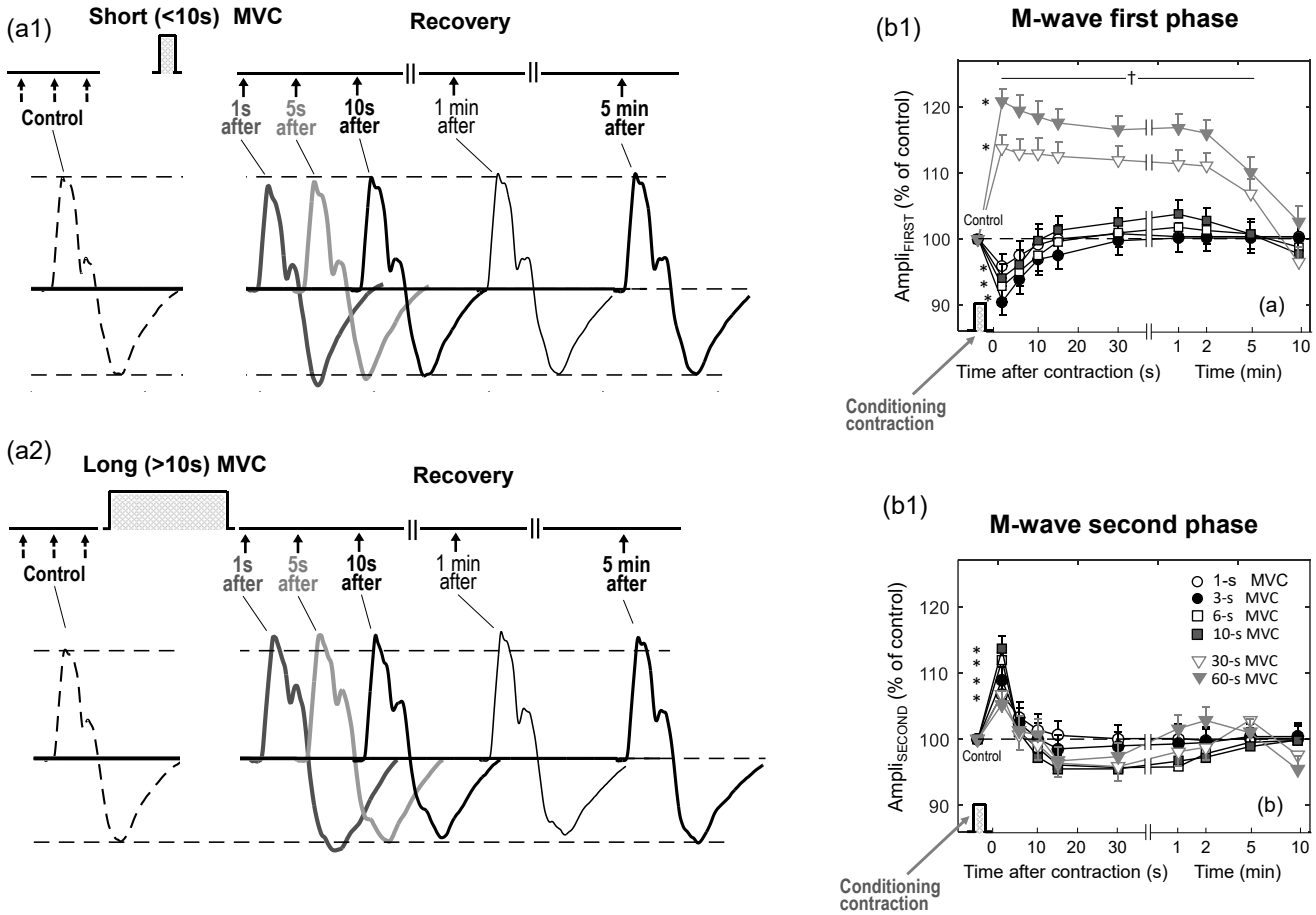
Fig. 4 Bar diagram showing the mean changes in the amplitude of the first ($Ampli_{FIRST}$) and second ($Ampli_{SECOND}$) phases of the M wave after maximal voluntary contractions (MVCs) of different durations. It can be seen that only the 30-s and 60-s MVCs induced long-term changes in $Ampli_{FIRST}$. All data are expressed in percentage of control values and reported as mean ± SE (n=8). * Significant difference with control ($p<0.05$).

Fig. 5 Time course of recovery of the duration for the M-wave first phase (Dur_{FIRST}), second phase (Dur_{SECOND}), and also for the whole M wave (Dur_{PP}) after conditioning maximal voluntary contractions (MVCs) of different durations. All data are expressed in percentage of control values and reported as mean ± SE (n=8). † Significant difference between the 1-s, 3-s, 6-s, and 10-s MVCs compared with the 30-s and 60-s MVCs ($p<0.05$). * Significant difference with control ($p<0.05$).

Table 1. Amplitude and area features of the monopolar M wave at various time points after conditioning maximal voluntary contractions (MVCs) of different durations for the *biceps brachii*. All values are expressed as mean ± SD. * Significant difference with control ($P<0.05$).

Table 2. Correlation coefficients measuring the relations between the recoveries of various pairs of M-wave variables after MVC durations of 1, 3, 6, 10, 30, and 60s. All values are expressed as mean ± SD. * Indicates statistical significance ($p<0.05$).

GRAPHICAL ABSTRACT



Left panel - Representative examples of M waves recorded in one participant before (control) and at various times after conditioning maximal voluntary contractions (MVCs) of short (a1) and long (a2) duration. Left panel - Time course of recovery of the amplitude of the first (b1) and second (b2) phases of the M wave after conditioning MVCs of different durations.