

Clinical Study

Reliability of the Electromechanical Delay Components Assessment during the Relaxation Phase

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The study aimed to assess by an electromyographic (EMG), mechanomyographic (MMG), and force-combined approach the electrochemical and mechanical components of the overall electromechanical delay during relaxation (R-EMD). Reliability of the measurements was also assessed. To this purpose, supramaximal tetanic stimulations (50 Hz) were delivered to the *gastrocnemius medialis* muscle of 17 participants. During stimulations, the EMG, MMG, and force signals were detected, and the time lag between EMG cessation and the beginning of force decay (Δt EMG-F, as temporal indicators of the electrochemical events) and from the initial force decrease to the largest negative peak of MMG signal during relaxation (Δt F-MMG, as temporal indicators of the mechanical events) was calculated, together with overall R-EMD duration (from EMG cessation to the largest MMG negative peak during relaxation). Peak force (pF), half relaxation time (HRT), and MMG peak-to-peak during the relaxation phase (R-MMG p-p) were also calculated. Test-retest reliability was assessed by Intraclass Correlation Coefficient (ICC). With a total R-EMD duration of 96.9 ± 1.9 ms, Δt EMG-F contributed for about 24% (23.4 ± 2.7 ms) while Δt F-MMG for about 76% (73.5 ± 3.2 ms). Reliability of the measurements was high for all variables. Our findings show that the main contributor to R-EMD is represented by the mechanical components (series elastic components and muscle fibres behaviour), with a high reliability level for this type of approach.

1. Introduction

Similarly to the electromechanical delay (EMD), which is generally defined as the time lag between the onset of muscle electrical activation and the onset of force production during the contraction, a delay between the cessation of EMG activity and the beginning of force decrease can be observed also during the relaxation phase [1]. While EMD has been widely investigated and has been shown to include the electrochemical and mechanical events from neuromuscular activation to force transmission to the bone [2–10], few studies approached the delay during the relaxation phase (R-EMD), which comprises (i) the cessation of the neuromuscular activation; (ii) Ca^{2+} reuptake by the sarcoplasmic reticulum and the block of the actomyosin interaction by troponin and tropomyosin; and (iii) the release of the elastic elements previously stretched during contraction and the return of the sarcomeres to their resting length [1, 11–15]. In those studies, the R-EMD ranged from 30 ms [15] to about 250 ms [12]. Several factors contributed to this large R-EMD

variability, among which (i) the endpoint considered for R-EMD calculation (from neuromuscular activation cessation to the beginning of force decay or to the end of force decay) and (ii) the criteria utilized to assess R-EMD endpoint (three standard deviations from contraction baseline or cross-correlation technique). Additionally, some authors raised the issue about the influence of the ability to suddenly and effectively relax the contracting muscle after the application of an external trigger (visual, acoustic, and mechanical) on R-EMD [12, 13, 15]. The use of an electrically-evoked supramaximal stimulation, though, may allow to minimize the central nervous system mechanisms which could inhibit muscle relaxation during voluntary contraction.

While EMD and its electrochemical and mechanical components have been already well documented [3, 4, 16, 17], the duration of the single events during the relaxation phase, from neuromuscular activation cessation to the return of the contractile elements and in series elastic components (SEC) towards their precontraction geometry, is still an open

question and its identification could gain more insights on the mechanisms involved in muscle relaxation phase.

Recently, a combined electromyographic (EMG) and mechanomyographic (MMG) approach together with the force signal has been proposed to partition the electrochemical and mechanical contribution to EMD [3, 4, 17]. A temporal indication of the electrochemical components from the propagation of the motor unit action potential at the sarcolemmal level to cross-bridges formation (i.e., the latency between the onset of EMG and the onset of MMG) and SEC elongation (i.e., the time lag between the onset of MMG and force) could be obtained.

Conversely, at the end of tetanic stimulations force decay precedes MMG signal return to baseline during the relaxation phase [18]. This MMG signal delay compared to force during relaxation has been attributed to two main mechanisms: (i) the restoration of the spatial relationships between the blood vessels and the muscle fibres due to a decrease in intramuscular pressure and (ii) the reoccupation during relaxation of the interfibre space by the interstitial fluid squeezed outside during contraction [18, 19]. Thus, two distinct R-EMD components can be calculated. The former comprises the events, that are mainly electrochemical in nature, between the cessation of neuromuscular activity and the initial force decay (Δt EMG-F; from Ca^{2+} reuptake in the sarcoplasmic reticulum to the block of the actomyosin interaction by troponin and tropomyosin and the consecutive cessation of cross-bridges activity). The latter includes the mechanical events from the onset of force decay to the negative peak of the largest MMG displacement (Δt F-MMG) and represents the time for passive return of SEC and muscle surface to their resting position.

Thus, the aim of the study was to assess by an EMG, MMG, and force-combined approach the electrochemical and mechanical components of the R-EMD at the end of an electrically-evoked tetanic stimulation. The reliability of the delays measurement during the relaxation phase was also evaluated. Hypothesis can be made that, similarly to the contraction phase, also in the relaxation phase the mechanical process would be the major contributor to the overall R-EMD.

2. Methods

2.1. Participants. After receiving a full explanation of the aim of the study and of the experimental procedures, 17 physically active male participants gave their informed consent to volunteer in the study. Their physical and anthropometric characteristics are given in Table 1. Participants were all clinically healthy with no history of previous lower limb injuries. They were asked to abstain from caffeine or similar beverages in the 24 h preceding the tests and to report to the laboratory without any form of physical exercise of heavy intensity of the lower limbs in the previous 48 h. The study was approved by the local University Ethical Committee and had been performed in accordance with the principles of the 1975 Declaration of Helsinki.

TABLE 1: Anthropometric characteristics of participants ($n = 17$). GM, *gastrocnemius medialis* muscle.

Age (years)	25.2 \pm 3.5
Body mass (kg)	74.5 \pm 15.7
Stature (m)	1.78 \pm 0.07
GM skinfold thickness (mm)	7.5 \pm 2.3
Calf circumference (cm)	38.4 \pm 2.2

2.2. Experimental Design. Participants reported to the laboratory twice. The first visit served for familiarization purpose and reliability assessment and the second visit to perform the experimental session, with at least 96 h in between. In both occasions, they were tested at about the same time of the day to minimize possible differences induced by circadian effects. The first visit consisted of two sessions of tetanic stimulations of the *gastrocnemius medialis* muscle (GM), with a 10 min pause in between. During the second visit, only one set of tetanic stimulations was administered.

2.3. Experimental Procedures and Measurements. All experiments were performed in a laboratory at constant temperature ($22 \pm 1^\circ\text{C}$). During tests, all participants were sitting on a custom-built ergometer with a resonant frequency >200 Hz. The knee of the dominant limb was fully extended, and to minimize the elongation of the *triceps surae* muscle and avoid pretensioning of the fibres, which can *per se* influence the EMD duration [6], the ankle joint was fixed at 20° in plantarflexion, and the reference position (0°) is perpendicularity on the tibia relative to the sole. The foot was attached by Velcro straps (Velcro Industries Inc., Willemstad, Netherlands Antilles) to a metal plate with a heel support, as shown in Figure 1(a).

Neuromuscular electrical stimulation under isometric condition was delivered to GM muscle in monopolar technique by an electrical stimulator (mod. St-Pro Multichannel Programmable Neuromuscular Stimulator, LiSin, Turin, Italy). The stimulation return electrode (anode: 130×100 mm) was positioned at the third distal of the leg, whereas the stimulation electrode (cathode: 90×40 mm) was placed over the most proximal motor point of the GM muscle [20]. A set of brief 2 Hz stimulations of increasing amplitude was administered to determine the maximum compound motor unit stimulus (M-wave). Once detecting the stimulus that elicited the maximal peak-to-peak M-wave, a resting period of 5 min was allowed. Hence, a set of three tetanic stimulations consisting of a train of pulses (wave shape: biphasic; pulse duration: $304 \mu\text{s}$; stimulation frequency: 50 Hz; current amplitude: 110% of the maximum M-wave; duration: 3 s) was delivered. Care was taken not to induce pain or high level of discomfort to the participants while supramaximally stimulated and not to elicit any activation of *tibialis anterior* muscle. Participants were instructed not to interfere with the GM electrical stimulation by avoiding simultaneous voluntary contractions of the plantar flexor muscles of the same leg. After the first set of tetanic stimulations, participants were allowed to rest for 10 min while sitting on the ergometer.

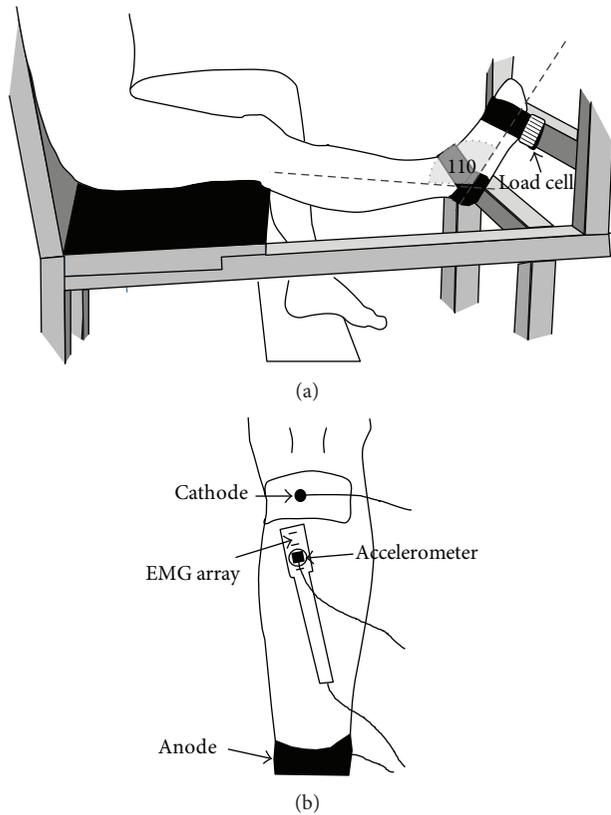


FIGURE 1: Schematic representation of the experimental setup. (a) Positioning of the participant on the ergometer. (b) Positioning of the stimulation and EMG electrodes and of the accelerometer for MMG detection on the investigated leg. For stimulation, the cathode was positioned on the motor point of the *gastrocnemius medialis* muscle, whereas the anode was positioned posteriorly above the third distal of the leg.

Then, the same set of stimulations was delivered for a second time.

Skin temperature was measured close to the EMG-MMG probe by an infrared thermometer with a laser beam pointer (mod. 826-T2, Testo, Lenzkirch, Germany), before and after stimulations. As proposed previously [21], the following equation was utilized to calculate muscle temperature: muscle temperature = $1.02 * \text{skin temperature} + 0.89$.

2.4. Signal Acquisition. Probe positioning and electrode positioning on the investigated muscle are shown in Figure 1(b). The stimulation current and the surface EMG and MMG signals from the GM muscle were acquired by a multichannel amplifier (mod. EMG-USB, Ot Bioelettronica, Turin, Italy; input impedance: $>90 \text{ M}\Omega$; CMRR: $>96 \text{ dB}$; EMG and MMG bandwidth: 10–750 and 0.7–100 Hz, resp.; gain: $\times 1$, $\times 1000$ and $\times 2$, resp.) with a sampling rate of 10240 Hz. Force signal was recorded by a calibrated load cell (mod. SM-1000 N, Interface, UK) operating linearly between 0 and 1000 N, amplified (gain: $\times 200$) by a 16 bits A/D converter (mod. UM150, Biopac System, CA, USA) and driven to the auxiliary input of the EMG amplifier. EMG signal was detected by a linear array of

4 electrodes (Ot Bioelettronica, Turin, Italy; $45 \text{ mm} \times 20 \text{ mm}$; electrode length 2 mm; interelectrode distance 10 mm) fixed to the skin by dual-adhesive foam filled with conductive gel (Cogel, Comedal, Trento, Italy). The EMG-MMG probe was positioned on the muscle belly in the area of maximal displacement during contraction. The EMG electrodes' array was oriented with the major axis parallel to the muscle fibres direction and with the EMG electrodes positioned perpendicularly to the major axes of muscle fibres, in accordance with the European recommendations about surface EMG [22]. The skin area under the electrodes was cleaned carefully with ethyl alcohol and gently abraded with fine sand paper and a special conductive cream (Nuprep, Weaver and Co., Aurora, CO, USA) to achieve an interelectrode impedance below 2000Ω . The third electrode of the EMG array was removed and replaced by a monodirectional accelerometer (mod. ADXL103, Analog Devices, Norwood, MA, USA; device weight: $<1.0 \text{ g}$; sensitivity: 1000 mV/g ; measure range: $\pm 1.7 \text{ g}$) placed over the muscle belly for MMG detection. One additional EMG array was positioned on the *tibialis anterior* muscle to verify the absence of any electrical activity of the antagonist during GM stimulation.

2.5. Data Analysis. Data analysis was performed by a custom-built routine using a commercially available software (Labview 7.1, National Instruments, Austin, TX, USA). In Figure 2 the four signals (stimulation current, EMG, force, and MMG) from a representative participant and the vertical lines for delays computation are given.

The end of the electrical activity coincided with the last negative peak of the EMG signal. For force signal relaxation onset, a condition of three standard deviations from the 200 ms preceding the end of the stimulation for three consecutive points was required [6]. To define the onset of MMG signal return to baseline after contraction, the maximum negative peak of MMG deflection was considered because of the shape of the MMG signal between the end of stimulation and the R-MMG p-p.

The overall time delay between the end of EMG signal and the onset of MMG return to baseline (R-EMD) and its components (time delay between EMG and force, $\Delta t \text{ EMG-F}$, and between force and MMG, $\Delta t \text{ F-MMG}$) were calculated offline by an expert operator.

After identifying the peak force (pF) as the highest level of force achieved, during relaxation, only the transient phase between 95% and 5% of the pF was considered for calculations. The half-relaxation time (HRT, i.e., the time taken by the force to halve the 95% of pF during the relaxation phase) and the maximum MMG amplitude of the relaxation phase (R-MMG p-p) were successively determined.

2.6. Statistical Analysis. Raw data were analysed using a statistical software package (IBM SPSS Statistics v. 19, Armonk, NY, USA). To check the normal distribution of the sampling a Kolmogorov-Smirnov test was applied. A sample size of 17 participants was selected to ensure a statistical power higher than 0.80. A two-way analysis of variance (ANOVA)

TABLE 2: Test-retest reliability of R-EMD components, MMG, and force parameters.

	Session 1 ($m \pm SD$)	Session 2 ($m \pm SD$)	ICC	SEM%	P
Δt EMG-F (ms)	23.1 ± 2.9	23.8 ± 2.8	0.864	4.5	0.551
Δt F-MMG (ms)	73.5 ± 12.0	73.7 ± 16.0	0.834	7.8	0.901
R-EMD (ms)	96.8 ± 9.4	97.6 ± 10.2	0.795	4.5	0.456
R-MMG p-p ($m \cdot s^{-2}$)	12.9 ± 1.5	13.4 ± 1.8	0.766	6.0	0.621
pF (N)	637 ± 64	641 ± 66	0.997	<1.0	0.683
HRT (ms)	67.8 ± 2.3	66.3 ± 2	0.818	<1.0	0.396

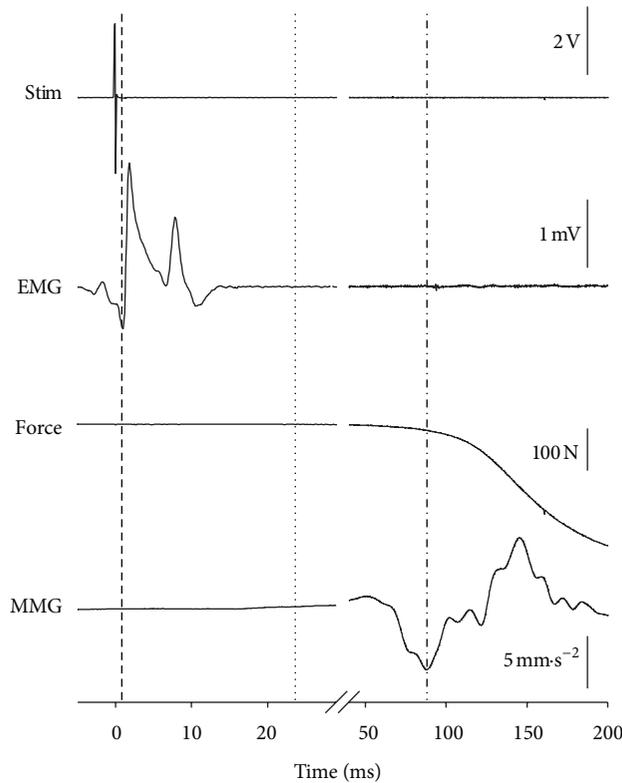


FIGURE 2: Stimulation current (Stim), EMG, force, and MMG signals in a representative participant. Dashed line detects the last negative peak of the EMG signal, dotted line detects the decrease of force signal, and dashed and dotted line detects maximum negative peak of the MMG during the relaxation phase.

for repeated measures was used on pF, HRT, R-MMG p-p, and muscle temperature values to determine possible differences among the three tetanic stimulations between sessions and within each session. The location of possible differences was assessed by a Holm-Sidak post hoc test. Test-retest reliability was assessed by a two-way, mixed model, Intraclass Correlation Coefficient (ICC) approach, and the standard error of measurements as a percentage (SEM%, as an index of test precision). ICC values were considered as very high if >0.90 , high if between 0.70 and 0.89, and moderate if between 0.50 and 0.69 [23]. The level of significance was set at $\alpha < 0.05$. Unless otherwise stated, the results are expressed as mean \pm standard error.

3. Results

3.1. Reliability. In Table 2 the ICC, SEM%, and P values during visit 1 for the investigated variables are given. According to our categories (see Section 2.6), variables in session 1 and 2 presented a high test-retest reliability. No significant differences between the two sessions were found for any parameter ($P > 0.05$).

3.2. MMG, Force Parameters, and Muscle Temperature. ANOVA did not disclose significant differences in MMG and force parameters and muscle temperature among the three tetanic stimulations during visit 2. The results of the three

TABLE 3: Duration of the different components of R-EMD and of the overall R-EMD and the relative contribution of the single components.

	Duration (ms)	Contribution (%)
Δt EMG-F	23.4 ± 2.7	24.1 ± 2.3
Δt MMG-F	73.5 ± 3.2	75.9 ± 1.7
R-EMD	96.9 ± 1.9	

tetanic stimulations were therefore pooled together for further analysis. The average values of R-MMG p-p, pF, and HRT were $13.2 \pm 1.5 \text{ m} \cdot \text{s}^{-2}$, $640 \pm 63 \text{ N}$, and $67 \pm 2 \text{ ms}$, respectively. Muscle temperature was $32.2 \pm 0.2^\circ \text{C}$.

3.3. Delays. Average values of R-EMD and its components are shown in Table 3. The different components weighted on the total R-EMD 24.5% and 75.5% for Δt EMG-F and Δt F-MMG, respectively.

4. Discussion

The novel finding of the present study was that the electrochemical and mechanical contribution to overall R-EMD could be quantitatively assessed. R-EMD major contributor, indeed, was the mechanical component (Δt F-MMG = 76%), while the electrochemical component (Δt EMG-F) was only 24%. Moreover, measurement reliability of R-EMD and its components was high.

Similar values of muscle temperature between session 1 and 2 were retrieved, indicating that the present findings were not influenced by this variable, which was reported to affect some electrochemical and mechanical events contributing to muscle relaxation [24].

4.1. R-EMD and Its Components. The total R-EMD duration was about 97 ms, a time lag included in the large range of R-EMD mean values reported in the literature (from about 250 ms to about 30 ms). After partitioning R-EMD into an electrochemical (Δt EMG-F) and a mechanical (Δt F-MMG) component, the major contributor to R-EMD was found to be the mechanical processes.

To the best of our knowledge only one study calculated the latency between EMG cessation and the beginning of force decrease [14], founding an average value of 89 ms. In that study, though, the latency was called R-EMD, while in the present study the same latency represents mainly the electrochemical component of the overall R-EMD. Thus, the correct comparison that should be done is between R-EMD in Vos study and Δt EMG-F (23 ms) in the present study. This difference may reside in contraction modality (electrically elicited versus voluntary contraction), in theoretical approach (different off-phase onset detection method), and in the investigated muscles (knee extensors versus plantar flexors). Moreover, a previous study reported shorter latencies during electrically evoked contractions compared to voluntary contractions due to different motor unit recruitment strategies [5]. This could be the case also during the relaxation phase, during which

motor units are derecruited differently under voluntary with respect to electrically elicited contractions, thus explaining, at least in part, the discrepancy.

The Δt F-MMG represents the mechanical component of R-EMD, that is, the time lag between the initial force decay and the larger acceleration occurring at the muscle level during the return to its initial length. In the present study this latency lasted about 74 ms (76% of the total R-EMD). In accordance with Jaskólska et al. [25] and Tesi et al. [26], within this period, the force signal showed a first slow decay as a consequence of passive relaxation of SEC, after being stretched during contraction. This first slow component of muscle relaxation was mainly attributed to the transition of cross-bridges from a force-generating to a nonforce-generating states [26]. Then, force reduction slope became steeper mainly because of the rapid change in muscle fibres length [25, 26], which produced a higher negative acceleration of the muscle belly (negative peak of R-MMG). The different behaviour of SEC and muscle fibres during muscle relaxation might also support the mechanical model proposed by Orizio et al. [18, 27] in which SEC are split into two distinct elastic elements (K_1 , tendon and K_2 , muscle fibres), being K_2 more compliant and with a nonlinear elasticity. The presence of K_2 is also supported by the greater compliance of muscle fibres compared to tendons, as indicated by Ettema [28]. During the contraction phase, this model may help to explain how muscle fibres shorten in parallel with muscle geometry changes, even in presence of low levels of force at the tendon when the extension limit of K_2 has not been reached yet. Beyond this limit, K_2 behaves as a rigid tension transmitter from the contractile elements to K_1 and larger amounts of tension are transmitted to the tendon with lower muscle geometry changes. During the relaxation phase, the first slow phase of force decay can be attributed to the passive return of tendon (K_1 , in the mechanical model proposed by Orizio, with lower compliance than K_2) toward its precontraction length, with no or negligible changes in sarcomeres length (K_2), which could present a low compliance level in this phase, in consideration of the their state. Thereafter, the force decay becomes steeper due to the sudden modifications in sarcomeres length as a result of the change in the their state and number [26]. These changes could potentially make muscle fibres more compliant. It should be taken also in consideration that sarcomere compliance is influenced by its length [29]. Indeed, sarcomeres with an initial length on the descending limb of the force-length relationship are stiffer than when their initial length is on the ascending limb. Thus, in the present study, the sarcomere length at the end of contraction could have influenced both the slow and the fast phase of force decay. Our findings showed that this mechanical latency is the major contributor to R-EMD. To the best of our knowledge this is the first study in which R-EMD components were calculated; thus comparisons with other investigations cannot be made.

4.2. Reliability. The high reliability level found for R-EMD (ICC = 0.795) is in line with that reported by Blanpied

and Oksendahl [11] (ICC = 0.840), indicating that this methodological approach provides repeatable results during the relaxation phase. Reliability results were high also for R-EMD components (ICC = 0.864 and 0.834 for Δt EMG-F and Δt F-MMG, resp.). Also SEM values were considerably low for all components, suggesting a good level of consistency of the measurements. However, this is the first study to assess the reliability of the different components contributing to the overall R-EMD, thus making any comparison with previous studies impossible.

4.3. Critique of the Method. Under a methodological point of view, MMG signal in the present study was detected by an accelerometer. On one hand, this transducer provides a signal that gives a direct time mark of the highest acceleration provoked by the muscle geometry changes during the relaxation phase. On the other hand, the accelerometer signal gives the highest MMG dynamics within the HRT. This aspect suggests that this method can provide effective information about the first part of the force decay after stimulation train cessation, where the highest level of acceleration is reached. Conversely, during the second part of the relaxation process, this transducer has a limited sensibility that might underestimate the lower level of acceleration reached by muscle relaxation. However, while a displacement transducer, as a laser or a condenser microphone, is suitable for the precise identification of an MMG system reflecting only muscle longitudinal mechanical characteristics, an accelerometer could be suitable for the identification of an MMG system reflecting both the longitudinal muscle mechanical characteristics (contractile component) and the transverse mechanical viscoelastic characteristics of the muscle, subcutaneous tissue, and skin [30].

5. Conclusions

The simultaneous recording of EMG, MMG, and force signals allowed us to discriminate the time lag of electrochemical and mechanical events involved in the overall muscle electromechanical delay during the relaxation phase. The main contributor to R-EMD was represented by the mechanical components, which accounted for about 76% of the total R-EMD. Under a practical point of view, the approach utilized in the present study in detecting the different R-EMD components could provide reliable information about the overall duration of the electrochemical and mechanical events involved during relaxation in all those activities requiring the ability to relax quickly the muscle after contraction, both in rehabilitation and in physical training. The present study could be also considered as a first step in a clinical field. Thus, further studies focused on the interday and interoperator reliability analysis for detection of possible differences induced by pathologies or other factors (fatigue, training, or rehabilitative programs) are required.

Conflict of Interests

The authors declare that they have no conflict of interests.

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