Electromechanical delay revisited using very high frame rate ultrasound

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[1]*Université de Nantes, Laboratoire "Motricité, Interactions, Performance," Nantes; and* [2]*Université de Grenoble, Laboratoire de Géophysique Interne et Tectonophysique, Centre National de la Recherche Scientifique, Grenoble, France*

Nordez A, Gallot T, Catheline S, Guével A, Cornu C, Hug F. Electromechanical delay revisited using very high frame rate ultrasound. *J Appl Physiol* 106: 1970–1975, 2009. First published April 9, 2009; doi:10.1152/japplphysiol.00221.2009.—Electromechanical delay (EMD) represents the time lag between muscle activation and muscle force production and is used to assess muscle function in healthy and pathological subjects. There is no experimental methodology to quantify the actual contribution of each series elastic component structures that together contribute to the EMD. We designed the present study to determine, using very high frame rate ultrasound (4 kHz), the onset of muscle fascicles and tendon motion induced by electrical stimulation. Nine subjects underwent two bouts composed of five electrically evoked contractions with the echographic probe maintained over *1*) the gastrocnemius medialis muscle belly (muscle trials) and *2*) the myotendinous junction of the gastrocnemius medialis muscle (tendon trials). EMD was 11.63 ± 1.51 and 11.67 ± 1.27 ms for muscle trials and tendon trials, respectively. Significant difference ($P < 0.001$) was found between the onset of muscle fascicles motion (6.05 ± 0.64 ms) and the onset of myotendinous junction motion (8.42 ± 1.63 ms). The noninvasive methodology used in the present study enabled us to determine the relative contribution of the passive part of the series elastic component ($47.5 \pm 6.0\%$ of EMD) and each of the two main structures of this component (aponeurosis and tendon, representing $20.3 \pm 10.7\%$ and $27.6 \pm 11.4\%$ of EMD, respectively). The relative contributions of the synaptic transmission, the excitation-contraction coupling, and the active part of the series elastic component could not be directly quantified with our results. However, they suggest a minor role of the active part of the series elastic component that needs to be confirmed by further experiments.

muscle; tendon; ultrasonography

ELECTROMECHANICAL DELAY (EMD) represents the time lag between muscle activation and muscle force production (3). It has been shown that EMD is modified during a fatiguing task (32), in response to a training program (14), after ligament reconstruction (19), and in case of neuropathies (13) or myopathies (31). EMD has been considered to be influenced by several structures and mechanisms such as (3) *1*) the propagation of the action potential and the excitation-contraction coupling processes (E-C coupling) and *2*) the muscle force transmission along the series elastic component [SEC; first introduced by Hill (15)]. Experiments have shown significant changes in EMD by experimental manipulation of the tension in the SEC (27, 29, 40). These results are considered by some to provide support for the hypothesis that the time required to stretch the SEC would be the primary determinant of the EMD (29); however, they provide only indirect evidence. In addition, all the structures of the SEC, classically composed of an active part (located in myofibrils) and a passive part (mainly aponeurosis and tendon) (39), could contribute differently to EMD. To date, there is no experimental methodology in humans to quantify the actual contribution of each SEC structure in the EMD.

A more complete characterization of the EMD, including detection of the onset of muscle fascicles and tendon motion, would give more information about the mechanisms and structures (E-C coupling, aponeurosis, tendon, muscle) involved in muscle force transmission efficiency. Real-time motion of muscle fascicles and tendons can be assessed using ultrasonography (for review, see Ref. 9). Since the sampling rates of traditional echographic devices are limited to ~50–100 Hz, the temporal resolution is very limited, and thus these devices cannot be used to study very short events such as EMD (values ranged from ~8 to 80 ms; Ref. 14). A few studies have determined the onset of muscle (34, 38) or tendon (4) motion using high frame rate ultrasound. However, despite a higher sampling rate than conventional echographic devices (200–333 Hz), the temporal resolution remains insufficient. For instance, the frame rate used by Chen et al. (4) induced a temporal resolution of 5 ms, representing ~27% of the measured delay for the vastus medialis muscle. Therefore, these devices cannot be used to accurately determine the onset of tissue motions and to monitor changes in EMD (28). A solution to this drawback exists. The lastest generation of echographic devices gives access to two-dimensional radio frequency (RF) images at a few thousand Hertz (up to 5 kHz) using a modified imaging sequence. In 2006, Deffieux and coworkers published a proof of concept paper demonstrating the feasability of using such very high frame rate ultrasound to track muscle contraction in vivo (7). They recently consolidated these findings showing real-time muscle displacements in response to myoelectrical stimulation (8). However, the latter study was conducted in three subjects and did not focus on the determination of muscle and tendon motion onset.

We designed the present study to determine, using very high frame rate ultrasound, the onset of muscle fascicles and tendon motion evoked by electrical stimulation. The original information gained by this study could contribute to a better understanding of the EMD and muscle force transmission processes.

MATERIALS AND METHODS

Subjects

Nine sedentary healthy men (age: 26.8 ± 5.1 yr; height: 177.8 ± 6.7 cm; weight: 71.7 ± 8.1 kg) volunteered to participate in this study. They were given detailed information about the purpose of the study and methods used and gave written consent. This study was conducted according to the Helsinki Statement (last modified in 2004) and has been approved by the local ethics committee.

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Instrumentation

Ergometer. A home made ergometer was used to measure the force produced by the plantar flexors (Fig. 1). Subjects were required to lie prone and fully extend their right leg. Their right foot was secured in a rigid cycling shoe fixed on an adjustable system and placed on a force sensor near the metatarsal joint (ZF200kg, sensibility: 3 mV/V, Scaime, Annemasse, France). Rigid cycling shoe was chosen to avoid possible dynamics in coupling between the shoe and the force sensor. The ankle was set at 10° in plantar flexion (0° represents the foot perpendicular to the shank). The force signal was digitized at a sampling rate of 5 kHz (MP36, Biopac) and stored on a computer.

Ultrasonography. A very high frame rate ultrasound device (64 channels, Lecoeur Electronique, Chuelles, France) was piloted by Matlab software (The Mathworks). Raw ultrasonic echoes at a 3.5-MHz central frequency backscattered by tissue heterogeneities were sampled at 40 MHz. These RF images acquired at a 4-kHz rate were stored in a computer. Due to a partial failure of the device, only 40 of the 64 consecutive channels supplied by the medical array were available and used for further analysis. RF images were then segmented into 1.5-mm windows. Then, correlation algorithms between windows of different RF images could give the displacement field consecutive to the contraction (37).

Electromyography. Bipolar surface electromyographic (EMG) signals were recorded from the soleus, gastrocnemius lateralis, and gastrocnemius medialis (GM) muscles. For each muscle, a pair of surface Ag/AgCl electrodes (EL 503, Biopac) was attached to the skin with a 2-cm interelectrode distance. The electrodes were placed longitudinally with respect to the underlying muscle fiber arrangement and located according to the recommendations by SENIAM (surface EMG for noninvasive assessment of muscles). For the GM muscle, electrodes were placed ~1 cm distally of the stimulation electrodes (see below). Reference electrodes were placed over the lateral and medial malleolus. Before electrode application, the skin was shaved and cleaned with alcohol to minimize impedance. EMG signals were amplified (×1,000) and digitized (bandwidth of 0–2,000 Hz) at a

sampling rate of 5 kHz (MP36, Biopac), and data were stored on a computer.

Electrical stimulation. Contraction of the GM muscle was elicited by means of percutaneous electrical stimulation. Electrical stimulation was used because it can activate only a target muscle (21, 22, 27). A constant current stimulator (Digitimer DS7A, Digitimer, Letchworth Garden City, UK) delivered single electrical pulses (pulse duration = 200 μs) through two electrodes (2 × 1.5 cm, Compex, Annecy-le-vieux, France) placed on the motor point and proximal portion of GM muscle (27). To find the motor point, the electrode was moved to obtain the strongest twitch with the lowest electrical stimulation. To determine the stimulation intensity, the output current was incrementally increased (incremental step = 1 mA, from 400 V) until a maximum tolerable current output that did not elicit M wave on the two other muscles of the triceps surae (i.e., soleus and gastrocnemius lateralis) was achieved (mean maximum current ouput used: 125 ± 11 mA).

Synchronization. Muscle stimulations were started using a trigger delivered by the ultrafast echographic device with a 50.00-ms delay to have a sufficient baseline to dectect the onset of tissue motion. A preliminary experiment was performed to check that the beginning of the echographic acquisition occurred exactly 50.00 ms before the onset of muscle stimulation. The trigger of the stimulation, force, and GM EMG signals were recorded using the same device (MP36, Biopac), discarding possible desynchronization between them. No detectable delay was found between the trigger of the stimulation and the stimulation artifact recorded by the GM electrodes.

Protocol

For each subject, two bouts (named muscle trials and tendon trials) composed of five electrically evoked contractions with a 3-min rest between each were performed. During the muscle trials, the echographic probe was maintained parallel to the bone, 1 cm medial to the EMG electrodes of the GM muscle. During the tendon trials, the echographic probe was maintained on the previously localized myotendinous junction of the GM muscle as described in many studies (27). These two bouts were performed in a randomized order.

Processing

The data processing was performed using standardized Matlab scripts (The Mathworks) and is depicted in Fig. 2. Ultrasonic raw data (i.e., RF signals) were processed as described by Deffieux et al. (7, 8). First, these raw data were used to create echographic images with a submilimetric resolution by applying a conventional beam formation, i.e., applying a time-delay operation to compensate for the travel time differences. These echographic images were used to determine the region of interest (ROI) for each contraction (i.e., between the two aponeurosis for the GM muscle for muscle trials and on the GM myotendinous junction for tendon trials; see Fig. 2). Then, a processing similar to Doppler was used to measure the tissue motion: the echographic images are segmented into 1.5-mm windows. Using one-dimensional cross correlation of windows of consecutive echographic images, the displacements along the ultrasound beam axis (i.e., *y*-axis in Fig. 2) were estimated. Thus the tissue motion between the two consecutive images (i.e., particle velocity) was measured with a micrometric precision (30).

Then, absolute particle velocities were averaged using previously determined ROI, and these averaged signals were used to detect the onset of motion (Fig. 2). Briefly, the derivative of each of these averaged signals was computed. Then, the onset of motion was defined as the first point with a negative derivative in the reverse direction of time (10). Visual inspection was performed to check the onset detection for each signal. The same method was used to automatically detect the onset of the force production (Fig. 2). We defined the EMD as the time lag between the onset of the electrical stimulation and the onset of force production. We also determined the
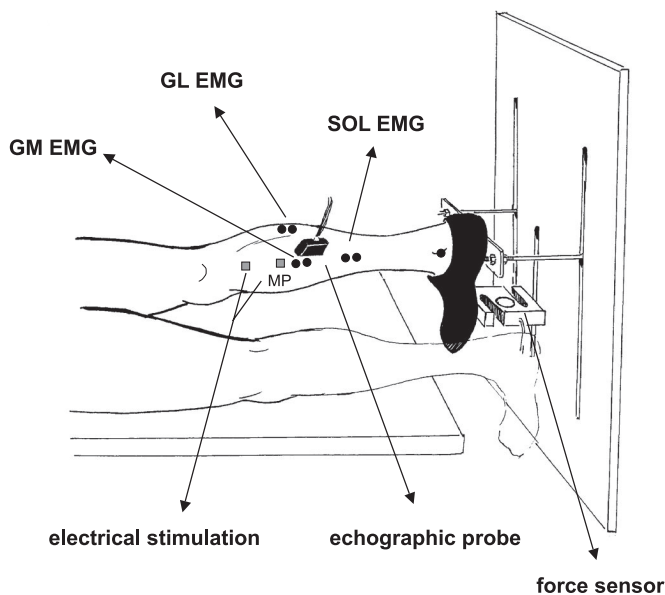

Fig. 1. Experimental setup. Percutaneous electrical stimulation was delivered on the motor point (MP). Force and electromyographic (EMG) activity of the gastrocnemius lateralis (GL), gastrocnemius medialis (GM), and soleus (SOL) muscles were recorded. Each subject underwent two bouts composed of five electrically evoked contractions with the echographic probe maintained over *1*) the GM muscle belly (as depicted in the figure) and *2*) the myotendinous junction of the GM muscle.
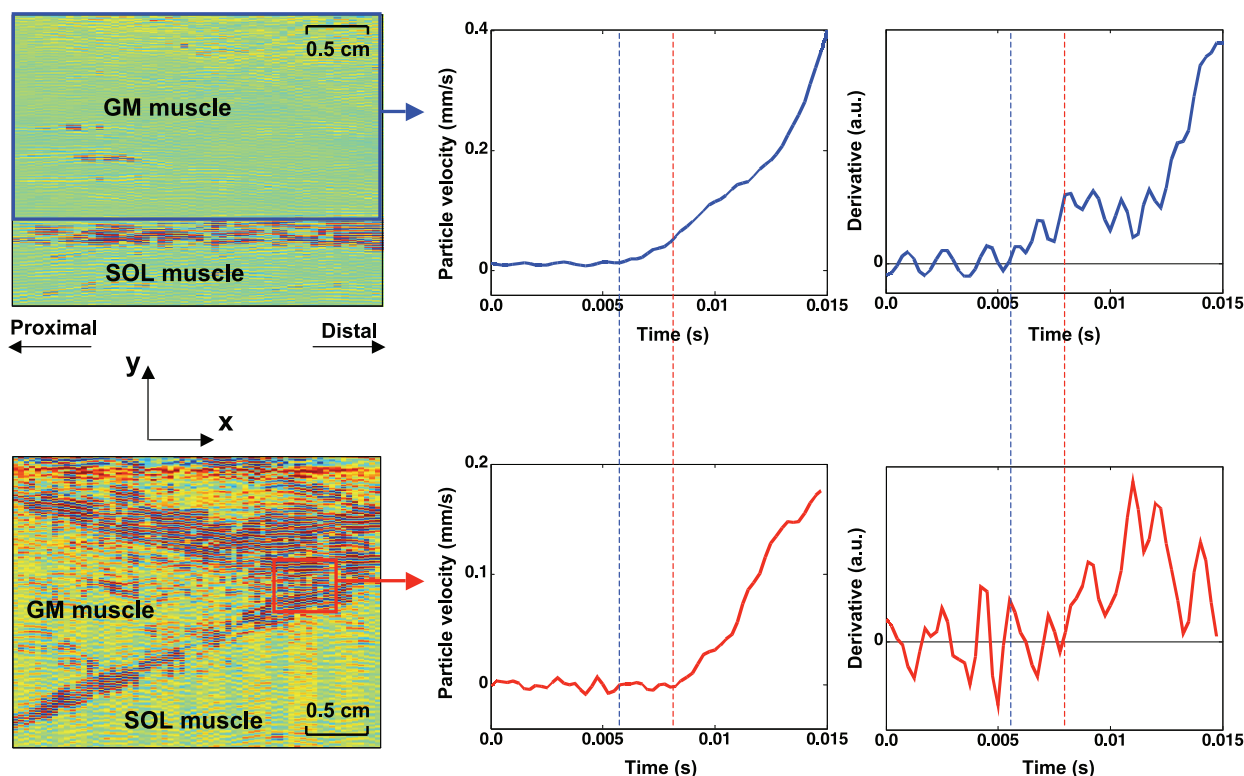
Fig. 2. Determination of the onset of muscle fascicles and tendon motion. Typical examples of the determination of tissue motion onset for muscle trials with the echographic probe placed on the muscle (*top* graphs) and for tendon trials with the probe placed on the musculo-tendinous junction (*bottom* graphs). The *left* graphs represent the echographic images obtained after the beam formation of raw radio frequency signals. The *middle* graphs represent the particle velocity obtained using the cross-correlation algorithm averaged on the regions of interest (blue and red squares for muscle and myotendinous junction, respectively). The *right* graphs represent the derivative of the particle velocity. The onset was automatically detected as the first point with a negative derivative in the reverse direction of time (vertical lines). Note that all displacements were calculated in the ultrasound beam axis (*y*-axis in this figure).

delay between the onset of electrical stimulation and the onset of muscle fascicles motion (Dm, for muscle trials) and between the onset of electrical stimulation and the onset of tendon motion (Dt, for tendon trials).

Statistical Analysis

Data distributions consistently passed the Shapiro-Wilk normality test (Prism 4.01, Graphpad Software, San Diego, CA). Values are therefore reported as means ± SD. To determine the repeatability of all our measurements, the standard error in measurement (SE) and the coefficient of variation (CV) were calculated for the five repeats within each bout. Finally, the Dm (EMD values of muscle trials) and Dt (EMD values of tendon trials) were compared using analysis of variance for repeated measures with orthogonal contrasts as the post hoc test (Statistix, Tallahassee, FL). Statistical significance was established at $P < 0.05$.

RESULTS

Due to a partial failure of the ultrasound device, two trials of two subjects for the tendon trials were not considered for analysis. The SE and CV values calculated for the five electrically evoked contractions of the muscle trials were 0.66 ms and 11.6%, respectively, for Dm and 0.54 ms and 5.0%, respectively, for the EMD. For the tendon trials, SE and CV were 0.88 ms and 10.5%, respectively, for Dt and 0.71 ms and 6.3%, respectively, for the EMD. Averaged results across the five contractions for each of the nine subjects are provided in Table 1. Statistical analysis showed significant differences

($P < 0.001$) between Dm (6.05 ± 0.64 ms) and Dt (8.42 ± 1.63 ms), between Dm and EMD (11.63 ± 1.51 ms) for muscle trials, and between Dt and EMD (11.67 ± 1.27 ms) for tendon trials. No significant difference was found between the EMD measured in muscle trials and tendon trials.

DISCUSSION

In the present study, very high frame rate (4 kHz) ultrasound was used to analyze the EMD in the GM muscle. The high temporal resolution (i.e., ± 0.125 ms) offered by this technique allowed us to determine in vivo, for the first time, the delay between electrical stimulation and the onset of muscle fascicles and tendon motion. Since these delays are repeatable, they can be used for a more complete characterization of the EMD and thus can give more information about the relative contribution of structures and mechanisms involved in EMD.

Methodological Considerations

Raw RF signals were used to calculate displacements (i.e., tissue velocity) with micrometric precision (30), but only in the ultrasound beam axis (i.e., *y*-axis in Fig. 2). Fortunately, since muscle tissues can be considered as an incompressible material (12), muscle volume remains constant during contraction (20), and thus the longitudinal displacements of the muscle are directly linked to the perpendicular ones measured in the present study (8). In addition, the myotendinous junction dis-

Table 1. *Onset times for muscle trials and tendon trials*

Muscle trials	EMD, ms	Dm, ms	Dm, %EMD
Subject 1	13.08±0.54	7.00±0.87	53.6±7.0
Subject 2	9.08±0.36	5.30±0.27	58.4±3.8
Subject 3	10.40±0.20	6.00±0.50	57.7±5.4
Subject 4	11.32±0.54	5.25±0.68	46.6±7.6
Subject 5	12.96±0.36	6.25±0.31	48.3±2.7
Subject 6	11.96±0.68	6.34±0.45	53.2±5.0
Subject 7	12.15±1.10	6.94±1.84	58.2±19.1
Subject 8	13.56±0.45	5.60±0.28	41.4±3.5
Subject 9	10.20±0.78	5.80±0.89	56.7±6.0
Mean	11.63	6.05	52.5
SD	1.51	0.64	5.9

Tendon trials	EMD, ms	Dt, ms	Dt, %EMD
Subject 1	13.80±0.86	11.16±1.37	80.7±5.3
Subject 2	9.64±0.51	7.9±0.89	82.1±10.0
Subject 3	11.24±0.38	6.45±0.41	57.4±4.4
Subject 4	11.12±0.36	8.75±0.92	78.9±10.6
Subject 5	11.68±0.69	7.24±0.71	62.4±9.6
Subject 6	11.72±0.78	9.35±0.65	81.5±5.9
Subject 7	12.27±0.81	10.5±1.09	85.8±10.0
Subject 8	12.96±0.98	7.35±0.60	56.9±6.1
Subject 9	10.35±0.82	7.12±0.18	69.2±2.7
Mean	11.65	8.42	71.8
SD	1.27	1.63	15.4

Values are means ± SD. Onset times for muscle trials (echographic probe maintained over the muscle belly) and tendon trials (echographic proble maintained over the myotenndineous junction). EMD, electromechanical delay; Dm, onset of muscle fascicles motion; Dt, onset of myotendinous junction motion.

placements always have a component in the ultrasound beam axis as shown in *movie 1* (see supplemental material available online at the *Journal of Applied Physiology* website) and as depicted by Maganaris and Paul (23). Consequently, the latencies of muscle/myotendinous junction thickening measured in the present study and longitudinal displacement are synchronized (18). As demonstrated by Muraoka et al. (27), the ankle joint angle used in the present study (i.e., 10° in plantar flexion) induced a residual tendon strain at rest (tendon is not slack), highly suggesting that the onset of myotendinous junction is directly linked to tendon elongation. However, our method does not permit exclusion of "rigid body motion" of the whole structure from the calculated displacements.

In accordance with various works studying the EMD (17, 27), we chose to use electrical muscle stimulation to evoke muscle contraction because it permits the activation of only a target muscle (4, 22, 27), as displayed in the present study by the absence of M wave on gastrocnemius lateralis and soleus muscles (see METHODS). In addition, it enabled us to better standardize the contraction and thus induce low variability between the trials (17), as demonstrated by the good repeatability of our results. The EMD values determined in the present study (11.63 ± 1.51 and 11.65 ± 1.27 ms for muscle and tendon trials, respectively) are in agreement with the relatively large range of values (7.90–18.77 ms) reported by other studies that used electrical stimulation of GM muscle (14, 17, 25, 27). This relatively large range of EMD values could be partially explained by the methodology used to determine the onset of force production. Most of the previous studies used an arbitrary threshold to determine the onset of force production (14, 17, 25, 27). Since a high threshold may result in a greater

delay, this threshold value is an important factor that affects EMD (6). To minimize this artificial delay linked to an arbitrary threshold, we chose to use, for all signals, a method based on the derivative (10) (Fig. 2). Pilot experiments have shown that this detection method is more precise than using an arbitrary threshold value. The high range of values reported in literature focusing on EMD could also be explained by the site of stimulation (nerve vs. muscle) and the reference used for the EMD calculation. Although some studies determined the reference as the onset of the stimulation artifact (4, 27) (i.e., EMD calculated as the time lag between this reference and the onset of force production), others determined the reference as the onset of the M wave (14, 17, 40). The action potentials propagate along the muscle fibers at ~4 m/s (physiological range between 3 and 5 m/s) (26). In our study, the distance between the motor point and the myotendinous junction was ~10 cm, inducing a total propagation time of the action potentials of ~25 ms. Therefore, it can reasonably be assumed that the onset of myotendinous junction displacements observed in our study (i.e., 8.42 ± 1.63 ms) is due to the onset of the force produced by the first recruited motor units (near the motor point) rather than by muscle fibers localized under the EMG electrodes. Consequently, it seems more appropriate to consider the EMD as the time lag between the onset of the stimulation artifact and the onset of force production, as done in the present study.

Physiological Significance

The onset of muscle contraction has been extensively studied in isolated frog muscles in response to electrical stimulation (5, 11, 16, 35, 36). The time interval that elapses between the instant of application of an electrical stimulus to the muscle fiber and the instant at which contraction starts is termed the "latent period" (35). However, as early observed by Rauh (1922; cited by Ref. 35), a minute precontractile elongation of the muscle occurs during the second phase of this latent period. This phenomenon, called latency relaxation, causes the tension to fall before it begins to rise (i.e., before the contraction). Interestingly, focusing on the initial mechanomyographic signals in response to myoelectrical stimulation, some human studies (2, 18, 33) reported an initial negative wave and interpreted it as the transverse latency relaxation. Barry (2) showed that this negative wave is more visible when the probe is placed over the motor point. This phenomenon is in accordance with the initial negative particle velocity of the muscle fascicles that we found in some proximal channels of the echographic probe as shown in Fig. 3. Since we determined the onset of fascicle motion from absolute particle velocity averaged on ROI (and thus independently of the particle velocity sign; Fig. 3), Dm should correspond to the onset of the latency relaxation rather than to the latent period.

Our original results show that the onset of muscle fascicles motion (Dm) is reached at 6.05 ± 0.64 ms after the myoelectrical stimulation, representing ~52.5 ± 5.9% of the EMD (Table 1; Fig. 4). Certainly due to the lack of accurate technique to detect the onset of muscle fascicle motions in vivo, very few studies have been conducted in humans. Using surface mechanomyography, an indirect measurement, Hufshmidt (18) reported a time lag between the onset of myoelectrical stimulation and the onset of muscle motion (named electro-mechanic
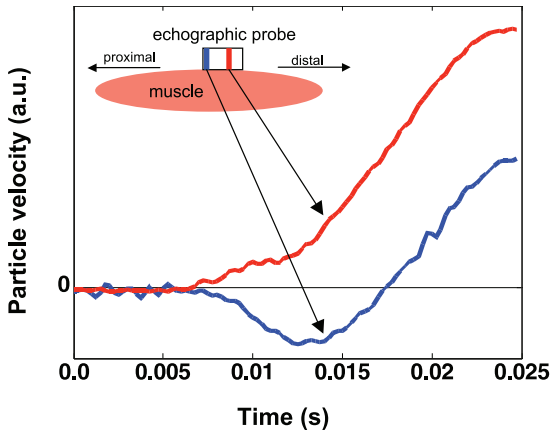
Fig. 3. Spatial changes in particle velocity. A typical example of spatial changes in particle velocity obtained during the muscle trials with the probe placed on the muscle belly for the most proximal (in blue) and the most distal (in red) channels. Because no spatial difference in the onset of fascicle motion (determined from the absolute signals) was found, onset of fascicle motion was determined from the averaged absolute signal (see Fig. 2). The initial negative particle velocity of the muscle fascicles that we found in some proximal channels (in blue) of the echographic probe could be explained by the latency relaxation phenomenon as mentioned in DISCUSSION.

latency) close to the Dm value reported in the present study. In a proof of concept article, Deffieux et al. (8) recently used very high frame rate ultrasound to measure Dm in the biceps brachialis muscle. Despite the fact that this study was conducted in only three subjects, the Dm values obtained were similar to values reported in the present work (i.e., ~7.1 ms). Considering the mechanisms and structures classically cited in literature (3, 4), Dm could be mainly attributed to the synaptic transmission, propagation of the action potential, E-C coupling, and force transmission along the active part of the SEC (located in myofibrils). Assuming a muscle fiber conduction velocity of 4 m/s (26), the propagation of the action potential along the distance corresponding to the 3-cm echographic probe would be ~7 ms. Since no spatial difference in Dm was found (Fig. 3), it can be assumed that the onset of motion for all muscle fascicles is due to the first recruited muscle fibers. Consequently, the propagation of the action potential should not affect the Dm and the EMD, contrary to what is suggested

in various papers (14, 17, 27). Thus the Dm could be mainly attributed to the synaptic transmission, the E-C coupling, and the force transmission along the active part of the SEC. To date, to our knowledge, these different contributions to the EMD cannot be separated in vivo. Assuming a relative high velocity of muscle force transmission in the muscle (~30 m/s), as suggested by Morimoto and Takemori (24), we could hypothesize that the force transmission via the active part of the SEC does not represent the major part of Dm, emphasizing the probable important contributions of the synaptic transmission and the E-C coupling. Dm would then be interpreted as an index of these physiological processes, as suggested by some studies (18, 33).

Since the remaining $47.5 \pm 6.0\%$ of the EMD could be mainly attributed to the muscle force propagation along the passive part of the SEC (i.e., aponeurosis and tendon) (Fig. 4), our original results confirm previous indirect observations suggesting that a large part of the EMD is due to the stretch of the SEC (27, 29, 40). In addition, the onset of myotendinous junction motion determined in the present study indicates that the delay due to the muscle force transmission along the aponeurosis (Dt-Dm = 2.37 ± 1.30 ms) and tendon (EMD-Dt = 3.22 ± 1.41 ms) represents $20.3 \pm 10.7\%$ and $27.6 \pm 11.4\%$ of the EMD, respectively. Interestingly, these results highlight an intersubject variability of muscle force transmission velocity along tendon and aponeurosis that could be linked to intersubject variability of tendon and aponeurosis mechanical properties (1). Note that the the delay attributed to the muscle force transmission along the tendon might be slightly affected by dynamic in the couplings between *1*) the shoe and the force sensor and *2*) the foot and the shoe. As mentioned in METHODS, rigid cycling shoe has been used to avoid possible dynamics in the coupling between the shoe and the force sensor (checked by preexperiments).

In conclusion, the noninvasive methodology used in the present study enabled us to isolate the contribution of the passive component of the SEC ($47.5 \pm 6.0\%$ of the total EMD) and each of the two main structures of this component ($20.3 \pm 10.7\%$ for aponeurosis and $27.6 \pm 11.4\%$ for tendon). The relative contributions of the synaptic transmission, the E-C coupling, and the active part of the SEC cannot be directly quantified with our results. However, they suggest a minor role
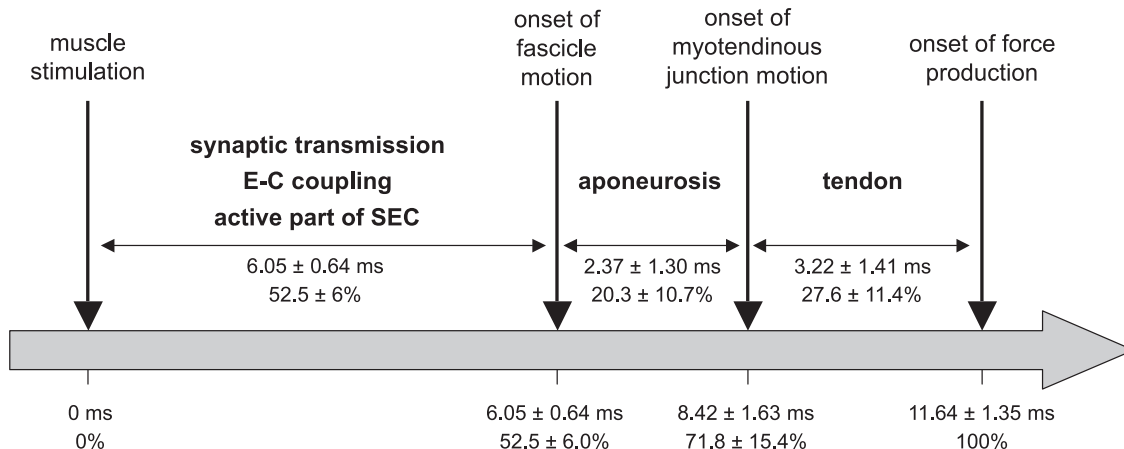

Fig. 4. Schematic representation of the time lag between the muscle stimulation and onset of fascicles motion, musculo-tendinous junction motion, and external force [electromechanical delay (EMD)].

of the active part of the SEC that needs to be confirmed by further experiments. Moreover, our results highlight an inter-subject variability of muscle force transmission velocity along tendon and aponeurosis that could be linked to intersubject variability in mechanical properties of these structures.

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