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Validation of the filling factor index to study the filling process of the sEMG signal in the quadriceps

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ABSTRACT

Introduction: The EMG filling factor is an index to quantify the degree to which an EMG signal has been filled. Here, we tested the validity of such index to analyse the EMG filling process as contraction force was slowly increased.

Methods: Surface EMG signals were recorded from the *quadriceps* muscles of healthy subjects as force was gradually increased from 0 to 40% MVC. The sEMG filling process was analyzed by measuring the EMG filling factor (calculated from the non-central moments of the rectified sEMG).

Results: (1) As force was gradually increased, one or two prominent abrupt jumps in sEMG amplitude appeared between 0 and 10% of MVC force in all the *vastus lateralis* and *medialis*.

(2) The jumps in amplitude were originated when a few large-amplitude MUPs, clearly standing out from previous activity, appeared in the sEMG signal.

(3) Every time an abrupt jump in sEMG amplitude occurred, a new stage of sEMG filling was initiated.

(4) The sEMG was almost completely filled at 2–12% MVC.

(5) The filling factor decreased significantly upon the occurrence of an sEMG amplitude jump, and increased as additional MUPs were added to the sEMG signal.

(6) The filling factor curve was highly repeatable across repetitions.

Conclusions: It has been validated that the filling factor is a useful, reliable tool to analyse the sEMG filling process. As force was gradually increased in the vastus muscles, the sEMG filling process occurred in one or two stages due to the presence of abrupt jumps in sEMG amplitude.

1. Introduction

The surface electromyogram (sEMG) signal is the algebraic summation of trains of extracellular potentials generated by active motor units. As the force of an isometric contraction is gradually increased, the number of active motor unit increases, larger units are progressively recruited (Henneman et al. 1965), and already recruited units increase their firing rate (Milner-Brown et al. 1973; Desmedt and Godaux 1978). Therefore, the overall amplitude of the sEMG signal increases with increasing force (Disselhorst-Klug et al., 2009; Ranaldi et al., 2022). However, several questions related to this increase remain unresolved: (1) Does the increase in sEMG amplitude occur gradually and smoothly or does it occur in steps? (2) Is the sEMG activity at low force levels composed of a few large-amplitude spikes separated by baseline ("pulsatile" activity) or is it formed by many small-amplitude MUP spikes, hardly standing out from noise ("continuous" activity)? (3) What is the force level at which the sEMG signal is completely filled up? Despite recent advances in sEMG techniques, there is yet no complete understanding of the way in which the sEMG signal progressively builds-up (fills) as the level of muscle activation increases. Knowledge on the process of filling of the sEMG signal may provide useful information to detect motor unit (MU) loss and reinnervation (Nandedkar et al., 2020),

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and is also valuable for prosthesis control (Farina et al., 2010) and to separate motions (Nazarpour et al., 2007).

The process by which the sEMG signal is filled up with motor unit potentials (MUPs) as contraction force increases is reflected on changes in the probability density function (PDF) of the sEMG amplitudes (Fig. 1). In this respect, it has been observed that, at low contraction forces, the sEMG PDF approximates to a Laplacian due to the little overlapping between MUPs of different motor units, whereas, as force increases, overlapping also increases and the PDF converges to a Gaussian (Nazarpour et al., 2007, 2013; Ayachi et al., 2014). In a recent study of our group we presented a new index, the EMG filling factor to quantify the degree to which an EMG signal has been filled (Navallas et al., 2023). The filling factor is grounded on an analytical derivation of the EMG PDF based on the MUP waveforms and firing rates, and thus this index informs about the shape of the sEMG PDF distribution, and also serves as a measure of the overlapping between MUPs (Fig. 1). In our preliminary experiments, we verified that the filling factor increases its value as the sEMG progressively fills up with MUPs (and MUP overlapping increases), and the PDF shape evolves towards a Gaussian (Navallas et al., 2023). To illustrate this process, three sEMG signals selected at different degrees of filling are shown in Fig. 1. Whereas these preliminary observations are promising, it is yet to be established how the filling factor changes with gradually increasing force for a large population of subjects: Does the increase in the filling factor occur gradually and smoothly, or does it occur in steps? Is it similar for all subjects? Additionally, it would be highly valuable to monitor the force signal during the filling process to obtain reference information on the force level at which the sEMG signal is filled up (Nandedkar et al., 1986; Nandedkar and Sanders, 1990).

A condition necessary to study the filling process of the sEMG signal is that the sEMG activity recorded at low force levels must be "pulsatile", i.e., formed by a few large-amplitude MUPs, clearly standing out from the background noise, and with little overlap between them (see Fig. 2a). Only if this initial "pulsatile condition" is satisfied, the successively recruited MUPs will progressively fill up the baseline, thus allowing the assessment of the EMG filling process. In support of this idea, Bostock and coworkers demonstrated that the form factor (an index closely related to the filling factor) varies rather little when the sEMG activity recorded at low contraction levels is "continuous", i.e., formed by many small-amplitude MUPs which overlap extensively (see Fig. 2b) (Bostock et al., 2019). In this respect, Nandedkar and colleagues argued that, in healthy individuals, the sEMG activity at low contraction levels is generally "continuous" (Nandedkar et al., 2020). However, in Nandedkar's previous studies, sEMG signals were recorded at a few fixed levels of voluntary force (i.e., force was not increased progressively from zero force), which could have prevented the occurrence of pulsatile sEMG activity (Nandedkar et al., 2010, 2020). Therefore, it appears necessary to elucidate if, when force is increased slowly and gradually from zero force, the sEMG activity at low contraction levels is pulsatile or continuous.

The objective of the present study was to validate that the filling factor is a useful and reliable tool to analyse the EMG filling process during gradual development of muscle force in the *quadriceps* muscles.

The validation included an analysis of the changes in the filling factor as force was gradually increased from 0 to 40% MVC, and also an assessment of the test–retest reliability of the filling factor curve during such contraction. The new analysis tool, the filling factor, has the potential to characterize the EMG filling process, as well as the ability to detect possible abrupt jumps in sEMG amplitude. The filling factor analysis will also provide an estimation of the force level at which sEMG activity develops into a full interference pattern. Since some neurogenic process may alter the EMG filling process (Nandedkar et al., 2020), the filling factor analysis may have potential to detect scenarios of motor unit loss.

2. Methods

2.1. Participants

Thirty-three male participants aged between 20 and 28 years (mean ± SD: 23 ± 2 years) volunteered to participate in this study. Their average height and body mass were 182 ± 5 cm and 74 ± 4 kg,

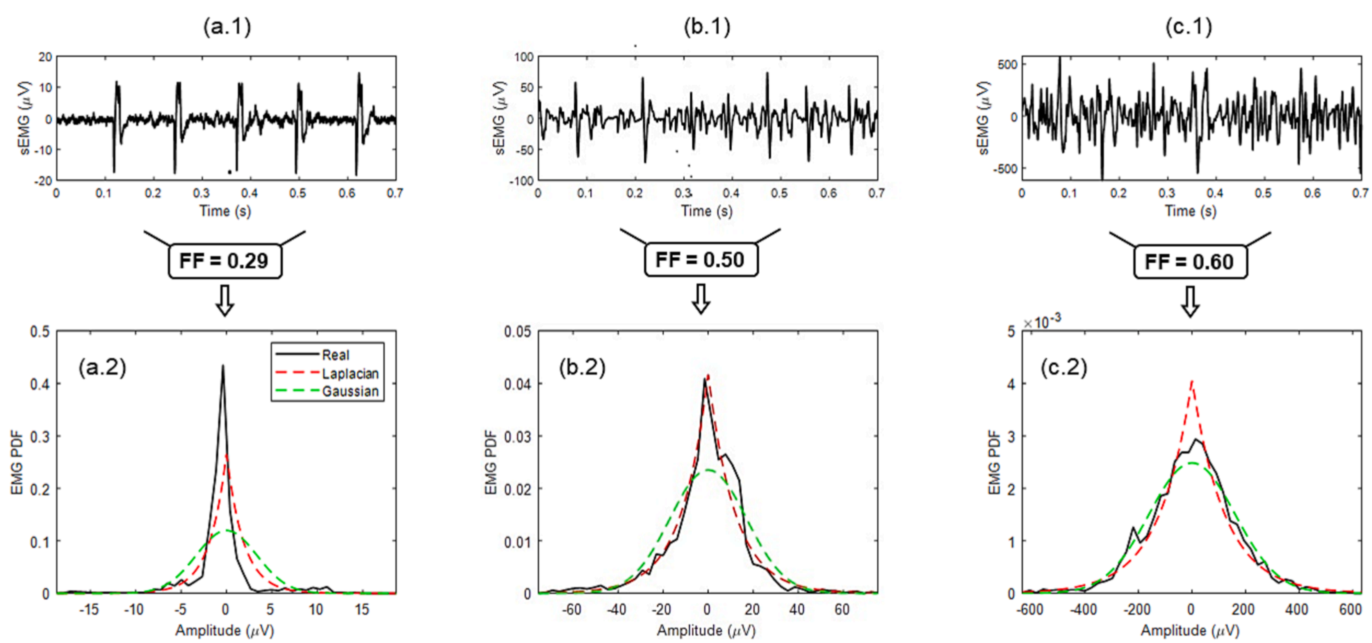

Fig. 1. First row - Three representative sEMG traces of 0.7 s duration recorded at different degrees of sEMG filling in one participant. Second row - The sEMG PDFs calculated from each of the sEMG traces of the first row (solid lines) together with the reference Laplacian (red dashed line) and Gaussian (green dashed line) distributions. The filling factor (FF) takes a low value (0.29 in plot a.1) when the sEMG signal is formed by a few MUP spikes (the corresponding PDF is close to a semi-degenerate, plot a.2). The filling factor increases (0.50 in plot b.1) as additional MUPs come into play (PDF distribution close to the Laplacian, plot b.2). The filling factor gradually approaches the value of 0.60 (c.1) as the sEMG signal becomes filled, and the sEMG PDF approaches a Gaussian distribution (c.2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

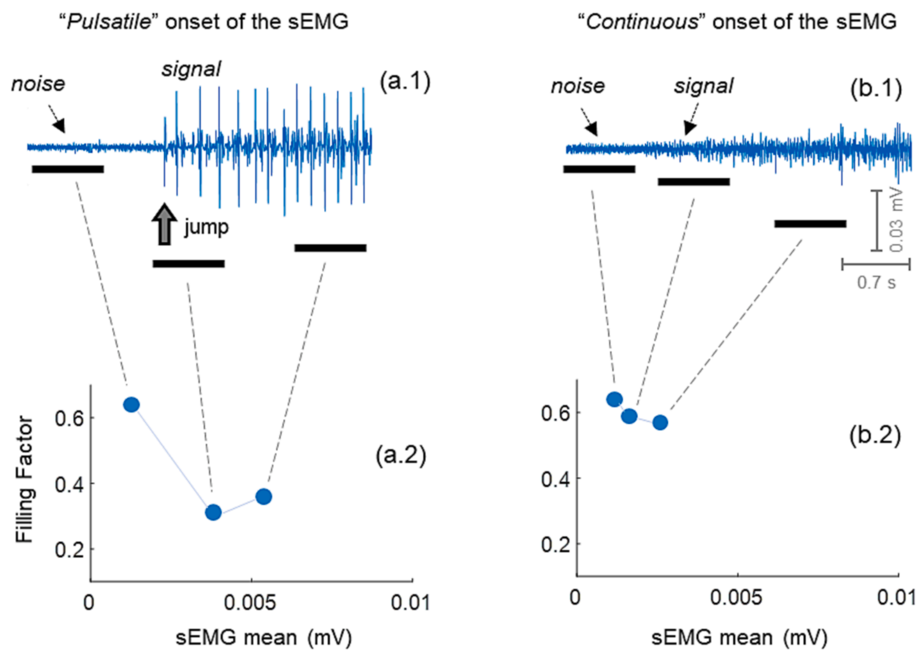

Fig. 2. Representative examples of two types of sEMG activity, "pulsatile" (a.1) and "continuous" (b.1), at the beginning of the contraction, together with their corresponding filling factor values (a.2 and b.2). When the initial sEMG is pulsatile (a.1), a prominent abrupt "jump" in sEMG amplitude occurred at the border between noise and the sEMG onset (grey arrow). This jump in amplitude made the filling factor decrease from ~ 0.63 to ~ 0.3.

respectively. Before the experimental session, all participants provided written informed consent. None of the participants reported current or recent (at least 6 months prior to the study) neuromuscular disorders. The experiments were conducted following the guidelines of the Declaration of Helsinki and were approved by the Ethics Committee Board of the Public University of Navarra, Spain (PI-010/21).

2.2. Experimental setup and force recording

Experiments were carried out on the quadriceps muscle and consisted on gradually increasing the isometric knee extension force, while participants received visual feedback of the exerted force. To do this, participants were seated comfortably on a custom-built chair with a knee angle of 90° and a trunk-thigh angle of 100°.Possible movements of the upper body were minimized by securing subjects to the seat of the chair at the shoulders and hips with an adjustable four-point restraint system. Quadriceps force was recorded during the voluntary isometric contractions using a strain gauge (STS, SWJ, China, sensitivity 2 mV/V and 0.0017 V/N, linear range: 0–2452 N) that was attached to the chair and securely strapped to the ankle with a custom made mould. The force signal (knee extension) was sampled at 1000 Hz using an analog-to-digital conversion system (MP150; BIOPAC, Goleta, CA, USA).

2.3. Localization of the innervation zone and the muscle fibers' direction

The innervation zone and muscle fibers' direction were determined in each muscle using a dry linear array of 16 electrodes (5 mm inter-electrode distance) during gentle isometric contractions. The sensor was connected to a multichannel amplifier (OT Bioelettronica, Torino; bandwidth 10–500 Hz). EMG signals were acquired under single-differential (bipolar) mode. The position of the innervation zone corresponded to the channel of the array showing phase reversal or minimum amplitude (Farina et al., 2002). Muscle fibers direction was determined by choosing the orientation of the array that yielded the clearest propagation of action potentials between the innervation zone and tendon regions (Farina et al., 2002).

2.4. Electromyographic recordings

Surface EMG potentials were recorded simultaneously from the *vastus lateralis* (VL), *vastus medialis* (VM), and *rectus femoris* (RF) muscles. EMG potentials were registered using self-adhesive surface electrodes (Ag/AgCl, Kendall Meditrace 100), with a circular shape (recording diameter 10 mm). In each muscle, a pair of electrodes were placed in bipolar configuration (distance between electrodes, 20 mm), with the proximal electrode of the pair located over the innervation zone, as shown in Rodriguez-Falces and Place (2017). In the VL and VM muscles, the two electrodes were placed along a line parallel to the orientation of the muscle fibers, whereas, in the RF, the electrodes were positioned lengthwise over the muscle belly, as performed previously (Rodriguez-Falces and Place, 2017). The "ground" electrode was placed over the patellar tendon. Before electrode placement, the skin was adequately prepared (shaving, rubbed with sandpaper) to reduce the impedance at the skin-electrode interface. Surface EMG signals were amplified (gain: 500 V/V, bandwidth: 10 to 1000 Hz) and digitized (sampling frequency of 5 kHz) using an analog-to-digital conversion system (MP150; BIOPAC, Goleta, CA) with 12 bits of resolution. The quality of the EMG recording was acceptable if noise level (peak-to-peak) remained below 5–6 μV throughout the experimental sessions (Tankisi et al., 2020).

2.5. Experimental protocol

Participants attended the laboratory on two occasions. The two sessions were 2 days apart and were conducted at the same time of the day for each subject. In each experimental session, the experiments started with the subjects performing 3 maximum voluntary contractions (MVC) of knee extension. The trials were separated by 3 min of rest. The highest MVC value was used as a reference for the determination of the force level corresponding to 40% MVC (Martinez-Valdes et al., 2016). Five minutes of rest were provided after the MVC measurement.

Then, subjects performed an isometric knee extension "ramp" contraction of 60 s duration, during which force was increased linearly from 0 up to 40% of MVC force. The desired force trajectory was displayed on a computer screen in front of the participant, along with the force exerted by the participant. The rate of force increase during the ramp contraction was intentionally slow (60 s to reach 40% MVC) so that the process of EMG filling could be thoroughly studied, especially during the first part of the contraction (15 s to reach 10% MVC). Several reasons advised the election of the 40% MVC force for the upper force limit: (1) It ensured that the sEMG interference pattern was completely formed (Bril and Fuglsang-Frederiksen, 1984); (2) The level of fatigue during the first half of the contraction (30 s to reach 20% MVC), when most of the EMG filling process took place, was expected to be negligible (Smith et al, 2007; Rodriguez-Falces and Place, 2021).

The 60-s ramp contraction was repeated three times, with a resting interval of 10 min between contractions. At the end of each resting period, one brief (4 s) MVC was performed, and the MVC peak force was measured and compared across repetitions. The MVC peak force did not differ between MVCs (one-way ANOVA, $P = 0.45$), indicating that the resting period was long enough to ensure full recovery.

2.6. Theory of the EMG filling factor

The filling factor is an index to quantify the change in the sEMG PDF shape as an EMG signal is being filled with the contributions from newly recruited MUs. The filling factor is calculated from the first two non-central moments of the rectified sEMG signal, which are estimated as follows:

$$m_1 = \frac{1}{N}\sum_{n=0}^{N-1} |x[n]\,|$$

$$m_2 = \frac{1}{N}\sum_{n=0}^{N-1} |x[n]\,|^2$$

where x[n] is the sampled sEMG signal and N is the number of samples in each segment of recording. Note that m_1 represents the rectified sEMG mean (averaged rectified value, ARV), whereas m_2 is the square of the sEMG root mean square (RMS).

Then, the EMG filling factor (FF) is calculated as the ratio between m_1^2 and m_2 as follows (Navallas et al., 2023), or, in other words, the filling factor is the ratio between the squared ARV and the RMS:

$$FF = \frac{{m_1}^2}{m_2}$$

Note that the filling factor is the inverse of the square of the form factor, as introduced by Bostock et al. (2019) to interpret MUNIX results, and as utilized by Nandedkar et al. (2020) to detect motor unit loss.

2.7. Analysis of the sEMG filling process with the filling factor

The process of sEMG filling was analyzed by calculating the filling factor of successive non-overlapping segments of the sEMG signal. The duration of the segments was 0.7 s, similar to that utilized by Navallas et al. (2023). Then, the filling factor values were plotted (and studied) against the rectified sEMG mean (i.e., the above-defined m_1), as done previously by other authors (Nandedkar et al., 2020; Navallas et al., 2023). For various reasons the filling factor was studied against the rectified sEMG mean, instead of as a function of force. First, force monitoring adds complexity to the experimental set-up in the clinical practice. Second, the sEMG mean parallels the change in force (up to a level, when amplitude cancellation remains low), and it is customary to use this EMG parameter as a surrogate of force (Nandedkar et al., 2004). Nonetheless, in the present study force was recorded simultaneously with sEMG signal to have an indication of the level of effort at which the sEMG is filled (see below).

An objective of the present investigation was to ascertain whether the increase in sEMG amplitude occur gradually or in steps. To elucidate this point, we searched for possible abrupt jumps in the amplitude of the sEMG signal. To identify and objectivize the presence of an amplitude jump, two criteria must be satisfied. (1) An amplitude criterion. The maximal peak-to-peak amplitudes for 5 sequential EMG segments of 200 ms duration, before and after the "visually-identified" jump occurred, were calculated. Then, the average of the 5 maximal peak-to-peak amplitudes after the jump must exceed in more than 100% the average amplitude before the jump.

(2) A filling factor criterion. The filling factor value of a 0.7-s sEMG segment immediately after the jump must be below 0.45.

Another objective of the study was to provide an estimation of the force level at which the sEMG signal is completely filled up. This will correspond to the moment the filling factor reached the value of Gaussian distribution, that is, 0.63 (Navallas et al., 2023). However, the value of 0.63 was not taken as a reference since this value was reached very slowly and asymptotically (Navallas et al., 2023), and thus, it would be inaccurate to make inferences on the force at which sEMG is filled up based on the 0.63 level. Instead, the filling factor value of 0.55, half-way between the Laplacian (0.50) and Gaussian (0.63) levels, was utilized as a reference threshold.

Apart from the filling factor, the following 3 variables were measured: (1) the number of jumps in sEMG amplitude during a 60-s ramp contraction; (2) the percentage of MVC force at which the jump occurred; and (3) the percentage of MVC force at which the filling factor reached 0.55. For the sake of clarity, each of these variables were averaged for the three repetitions made during the experiments. For simplicity, section 3.2 of results only utilized data from the first experimental session, whereas section 3.3 of results (intra and inter-session reliability) utilized data from both the first and second sessions.

Another goal of the study was to determine if the sEMG activity at low contraction forces was "pulsatile" or "continuous", as defined by Nandedkar et al. (2020) (for examples, see Fig. 2). Specifically, an initially pulsatile sEMG activity is composed of a few large-amplitude spikes, clearly standing out from the previous background noise (Fig. 2a), whereas an initially continuous sEMG activity contains many small-amplitude MUP spikes, hardly standing out from noise (Fig. 2b). When the initial sEMG activity was pulsatile, we considered that a "jump" in sEMG amplitude occurred at the boundary between noise and the sEMG onset (grey arrow in Fig. 2a), since both the amplitude criterion (sEMG amplitude doubles noise amplitude) and filling factor criterion (filling factor after the jump decreases below 0.45) were fulfilled. In contrast, when the initial sEMG activity was continuous, no jump in sEMG amplitude was observed at the onset of the contraction, as neither of the criteria were met. For clarity in the presentation of the results, we considered two possible jump scenarios: (1) a scenario showing a jump at a very low force level [0–1% MVC], which corresponded to what we called "initially pulsatile sEMG activity"; (2) a scenario showing a jump at a low force level [2–10% MVC], which corresponded to what we called "initially continuous sEMG activity".

Finally, to evaluate how well participants tracked the target force during the ramp contraction, the mean absolute error of the differences between target and actual force values were calculated, as well as the percentage mean absolute error of these differences, according to the definitions provided in Taraji et al., (2017).

2.8. Statistics

All the results are expressed as mean and standard deviation (SD) unless differently stated. Before comparisons, all variables were tested for normality using the Shapiro-Wilk test. Statistical significance was set at $p < 0.05$. All variables (number of jumps in sEMG amplitude, average percentage of MVC force at which the jumps occurred, and average percentage of MVC force at which the filling factor reached 0.55) were analyzed for each muscle (VM, VL, and RF) independently. To determine the level of reliability of these variables, the intra-class correlation coefficient (ICC) was used. ICC scores between 0.8 and 1 were interpreted as "excellent", 0.6–0.8 as "good", and < 0.6 as "poor" (Bartko, 1966). Additionally, a one-way repeated measure analysis of variance

(ANOVA) was performed to determine if there were significant differences within a testing session or between the two sessions. No significant main effects were observed in the repeated measures ANOVA, and thus there was no need to perform post-hoc tests.

3. Results

3.1. Representative examples of the sEMG filling process

Fig. 3 shows a representative example of an sEMG signal (plot b) recorded in one participant as force was gradually increased from 0 to 40% (plot a). The filling factor values extracted from the sEMG signal are shown in plot c. Before starting the contraction, pure (gaussian) noise, known to have a filling factor value of 0.63, was registered. It can be seen that the filling process of the sEMG occurred in 2 sequential stages. The first stage started when, as the participant slowly increased force, a few large-amplitude MUP spikes, clearly standing out from noise, appeared at 1.0 s, (in plot b, see the black arrow and the inset with FF = 0.34). The onset of these new "large-amplitude" MUPs provoked an abrupt fall in the filling factor to ~ 0.34 (see the black arrow in plot c). Afterwards, the participant continued increasing the force gradually, and additional MUPs came into play, contributing to fill the EMG during the time interval [1.0 s, 12 s]. During this first period of EMG filling, the filling factor increased from ~ 0.34 to ~ 0.58 (plot c).

The second stage of filling started when, as the participant continued increasing the force, a few MUP spikes with an amplitude notably greater than the previous sEMG activity appeared at 12 s (in plot b, see the grey arrow and the inset with FF = 0.36). The onset of these large-amplitude MUPs caused a decrease in the filling factor to ~ 0.37 (see the grey arrow in plot c). Thereafter, as force continued increasing, additional MUPs came into play, contributing to a second stage of filling (plot b) and to increase of the filling factor from ~ 0.37 to ~ 0.63 (plot c). During this second period of EMG filling, the filling factor reached the value of 0.55 at 11.3% MVC, and the plateau at about 20% MVC. The forces corresponding to the first and second abrupt jumps in sEMG amplitude were 0.5% and 7.8% of MVC force, respectively (plot a).

It could be argued that the occurrence of abrupt jumps in sEMG amplitude with increasing force is a peculiarity found in a few subjects for which the initial sEMG activity is "pulsatile", that is, a few large-amplitude spikes clearly standing out from noise, as that shown in Fig. 4. However, the abrupt jumps in amplitude were observed in all participants tested, irrespective of the fact that the sEMG activity at the beginning of the contraction was "pulsatile" or "continuous". An example of a "continuous" sEMG activity at the onset of the contraction is shown in Fig. 4. It can be seen that, during the first seconds of the contraction, there were already many small-amplitude MUP spikes, hardly standing out from noise, contributing to the sEMG (in plot b, see the inset with FF = 0.60). Notwithstanding this initial "continuous" sEMG activity, a few large-amplitude MUP spikes, with an amplitude significantly greater than the previous sEMG activity, appeared at 13 s, thus causing an abrupt jump in sEMG amplitude (in plot b, see the grey arrow and the inset with FF = 0.30). The onset of these new large-amplitude MUPs made the filling factor decrease abruptly to ~ 0.30 (in plot c, see the grey arrow). Thereafter, as force continued increasing, additional MUPs were recruited, thus progressively filling the EMG signal (in plot b, see the inset with FF = 0.54), which increased the filling factor (plot c). Note that this filling process was fast, with the filling factor reaching the value of 0.55 at 10.2% MVC, and the plateau at about 20% MVC. The abrupt jump in sEMG amplitude occurred at 8.1% of MVC force (plot a).

Fig. 5 shows the sEMG signals from the VL, VM, and RF muscles recorded simultaneously in one participant as force was gradually increased from 0 up to 40% MVC (first column), together with the corresponding filling factor values (second column). Distinct abrupt jumps in sEMG amplitude appeared in the VL and VM muscles (plots a.1 and b.1), but not in the RF, where the sEMG amplitude increased gradually and smoothly (plot c.1). The time instants at which the jumps occurred in the VL (6 s and 17 s) did not coincide with that of the VM (10 s). Regarding the filling factor analysis, each time an abrupt jump in sEMG amplitude occurred (only in the VL and VM), a clear and abrupt decrease in the filling factor appeared (see the black, grey, and white arrows in plots a.2 and b.2). In the RF, the initial sEMG activity was "continuous", and no abrupt jumps in sEMG amplitude occurred during the 60-s contraction: thus, as MUPs were successively recruited, the filling factor increased within a narrow range, from 0.53 to ~ 0.63 (c.2).

3.2. Group analysis of the sEMG filling process

The target force during the ramp contraction was tracked easily by all participants. The average mean absolute error of the differences between target and actual force values across all segments was 7.7 N, and the average percentage mean absolute error was 3.7%.

In all the VL and VM muscles examined (100%), at least one prominent abrupt jump in sEMG amplitude was observed, whereas, in the RF, jumps in amplitude were found only in a few subjects (9%, 3 of 33). A jump at a very low force level [0–1% MVC] (i.e., initially pulsatile sEMG activity) was found for a large percentage of subjects in the VL (78%, 26 of 33) and VM (89%) muscles analyzed (Table 1), whereas only 3% of subjects in the RF (1 of 33) exhibited a jump at a very low force level [0–1% MVC]. One jump in amplitude was observed in 48% (VL) and 60% (VM) of the subjects, whereas two jumps were recognized in 52% (VL) and 40% (VM) of the participants.

The histogram of Fig. 6(a) and (b) shows the distribution of the percentage of MVC force at which the jumps occurred during the first experimental session. The average force at which the jumps at a very low force level [0–1% MVC] occurred was 0.35%, 0.37%, and 0.25% of MVC force (for the VL, VM and RF, respectively, Table 1). The average force at which the jumps at a low force level [2–10% MVC] occurred was 4.1%, 4.7%, and 4.2% of MVC force (for the VL, VM and RF, respectively, Table 1). The average force at which the second jump in amplitude occurred (for those subjects for whom two jumps where observed) was 5.0% and 5.2% (for the VL and VM, respectively).

The average force at which the filling factor reached the 0.55 level was 5.6% and 5.3% of MVC force (for the VL and VM, respectively, Table 1), with a range very similar for both muscles [1.5%, 12.5%]. When these data are represented with a histogram [Fig. 6(c) and (d)], a bimodal distribution can be observed due to the subjects with two jumps in sEMG amplitude: in the first stage of sEMG filling the filling factor reached 0.55 at ~ 3% MVC, whereas in the second filling process the 0.55 level was reached at ~ 9% MVC.

3.3. Intra-session reliability and inter-session reliability

Excellent reliability was found between the three repetitions (Fig. 7, Table 2) for the three measured variables: (1) number of jumps in sEMG amplitude, (2) average percentage of MVC force at which the jumps occurred, and (3) average percentage of MVC force at which the filling factor = 0.55. None of these variables differed significantly between repetitions ($p = 0.23$, 0.28, and 0.31, respectively). Good to excellent reliability was found between the first and second experimental sessions for the measured variables (Fig. 7, Table 2). None of the three measured variables differed significantly between sessions ($p = 0.28$, 0.33, and 0.25, respectively). No significant main effects were observed in the repeated measures ANOVA for the intra and inter-session reliability analysis.

4. Discussion

The main findings of the present study were the following:

(1) When force was increased slowly and gradually from zero, one or two pronounced abrupt jumps in sEMG amplitude appeared

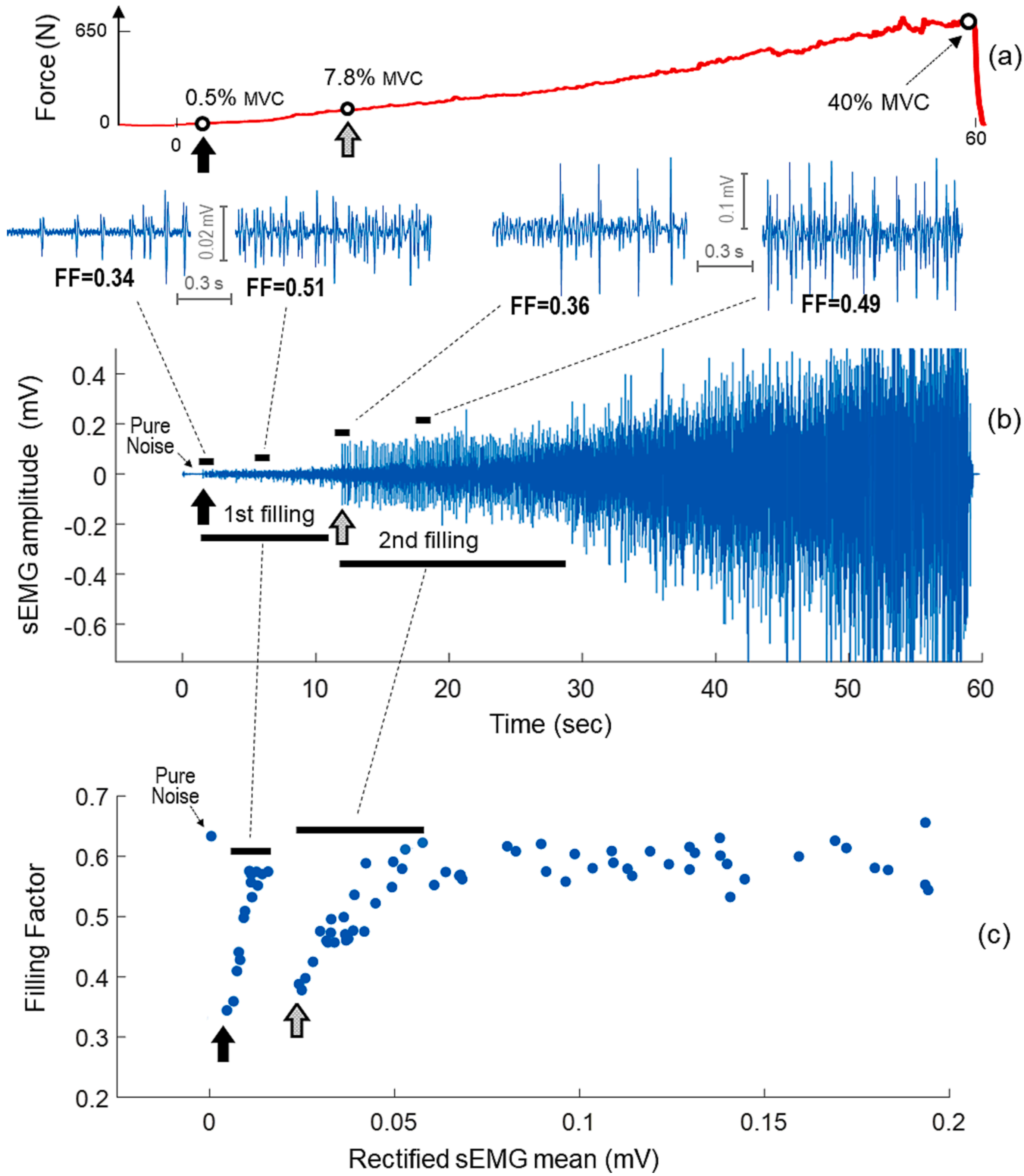

Fig. 3. Representative example of the process of sEMG filling in the *vastus lateralis* of one subject (b) as force was gradually increased from 0 to 40% MVC (a). The filling factor (FF) values calculated from the sEMG are shown in (c). Note that two abrupt jumps in sEMG amplitude occurred at 1 s and 12 s (black and grey arrows in plot b, respectively). The jumps in amplitude were originated when a few large-amplitude MUPs, clearly standing out from the previous sEMG activity, appeared in the sEMG signal (in plot b, see the insets with FF = 0.34 and 0.37). The onset of these "large-amplitude" MUP spikes made the filling factor decrease abruptly (black and grey arrows in plot c, respectively). After one jump in amplitude occurred, additional MUPs were successively recruited, contributing to fill the sEMG in this stage (b) and to increase the filling factor (c).

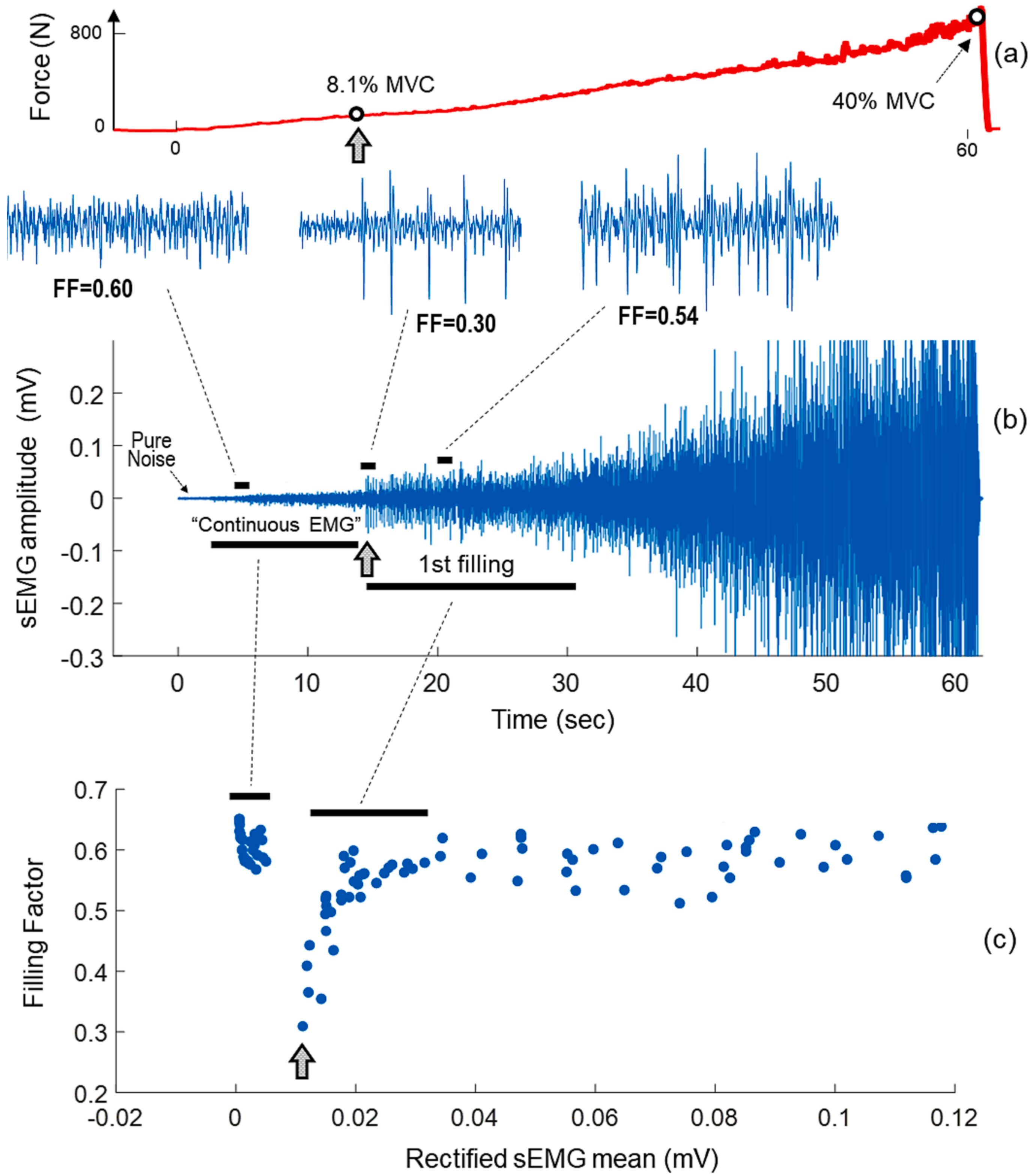

Fig. 4. Example of an sEMG filling process in the *vastus medialis* of one subject (b), while force was linearly increased from 0 to 40% MVC (a). The filling factor (FF) values calculated from the sEMG are shown in (c). The sEMG signal during the first 13 s seconds of the contraction contained many small-amplitude MUPs with great overlap between them (see the inset with FF = 0.60 in plot b). One abrupt jump in sEMG amplitude occurred at 13 s (grey arrow in plot b), which was caused by the appearance of a few large-amplitude spikes with amplitudes markedly greater than the previous sEMG activity (inset with FF = 0.30 in plot b). The onset of these large-amplitude spikes caused the filling factor to decrease abruptly (grey arrow in c).

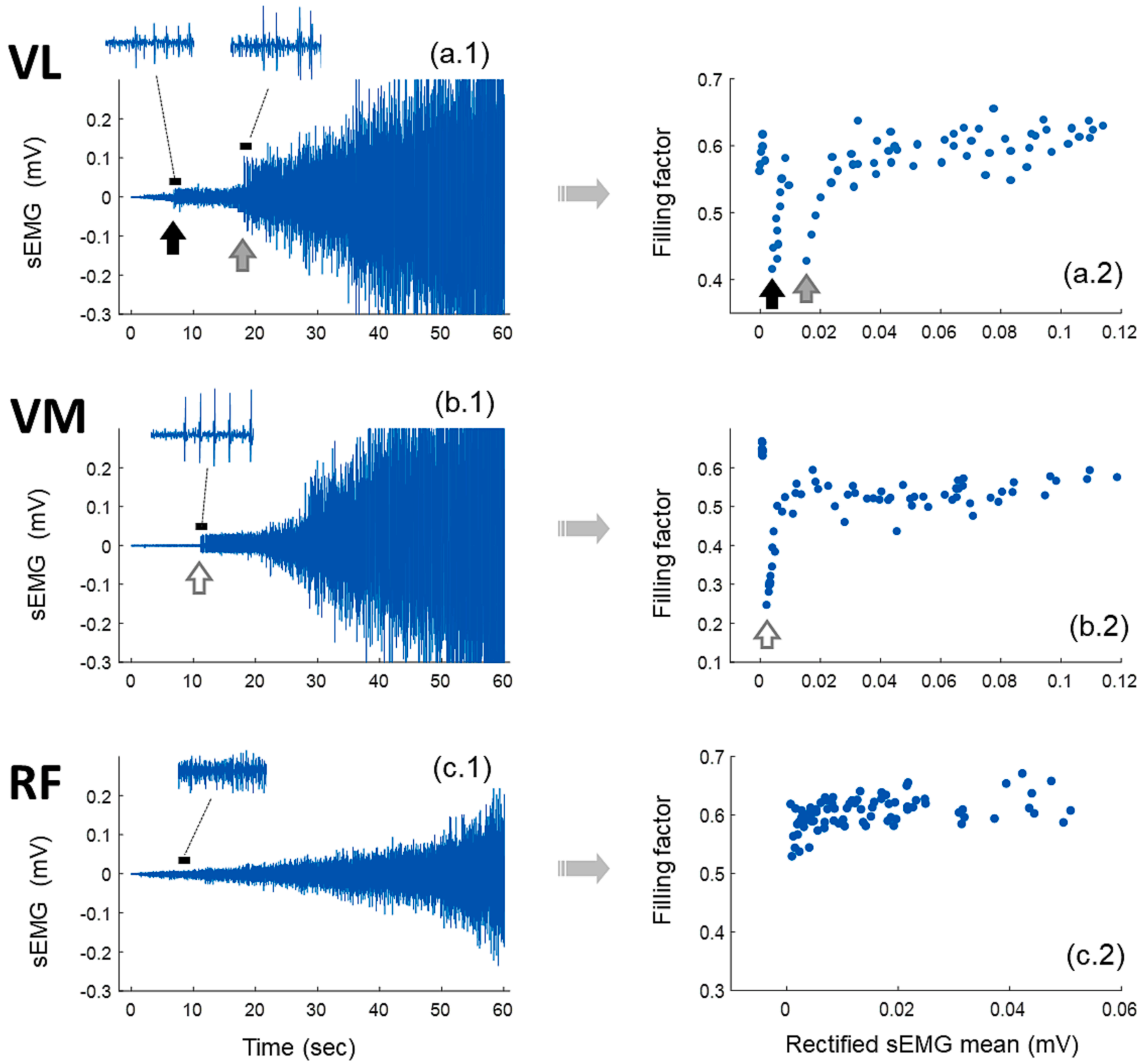

Fig. 5. Examples of the sEMG filling process in the *vastus lateralis (VL), vastus medialis (VM)* and *rectus femoris (RF)* of one subject (first column), as force was gradually increased from 0 to 40% MVC. The associated filling factor (FF) values are shown in the second column. Marked abrupt jumps in sEMG amplitude occurred in the VL and VM muscles (arrows in plots a.1 and b.1), but not in the RF (plot c.1). The time instants at which the jumps occurred in the VL (6 s and 17 s) did not coincide with that of the VM (10 s). Each time an abrupt jump in sEMG amplitude occurred, an abrupt drop in the filling factor occurred (arrows in plots a.2 and b.2).

between 0.2 and ~ 10% of MVC force in all the *vastus lateralis* and *medialis* muscles examined.

(2) The jumps in amplitude were originated when a few large-amplitude MUPs, clearly standing out from the previous sEMG activity or from the background noise, appeared in the sEMG signal.
(3) Every time an abrupt jump in sEMG amplitude occurred, a new stage of sEMG filling was initiated, in which additional MUPs were successively recruited, contributing to fill the sEMG in this stage.
(4) The filling factor decreased significantly upon the occurrence of an sEMG amplitude jump, and this index increased progressively as additional MUPs were added to the sEMG signal.
(5) The filling factor curve was highly repeatable across repetitions.

4.1. Description of the jumps encountered during sEMG filling process

We observed that, as force was increased slowly and gradually in the *vastus lateralis* and *medialis*, the increase in sEMG amplitude did not occur gradually and smoothly, as proposed by some authors (Disselhorst-Klug et al., 2009), but rather, prominent abrupt jumps in sEMG amplitude appeared between 0.2 and 10% of MVC force.

The jumps in sEMG amplitude were clearly identified in all the *vastus lateralis* and *medialis* muscles analyzed. Thus, it seems clear that the presence of jumps is not a peculiarity found in a few subjects, but a regular observation that might be related to either the spatial organization of motor units within some muscles, or to anatomical specificities of these muscles (i.e., thickness of the subcutaneous layers, arrangement of muscle fibers), or even to a “predesigned” motor unit recruitment

Table 1
Top – Percentage of subjects in which the initial activity was pulsatile vs continuous in the *vastus lateralis*, *vastus medialis* and *rectus femoris*. Bottom - Average percentage of the MVC force at which the jumps occurred and of the MVC force at which the filling factor (FF) reached the value of 0.55.

	Vastus lateralis	*Vastus medialis*	*Rectus femoris*
% of subjects with a jump at a very low force level [0–1% MVC] (i.e., initially pulsatile sEMG activity)	78%	89%	3%
% of subjects with a jump at a low force level [2–10% MVC] (i.e., initially continuous sEMG activity)	22%	11%	97%
Average % of MVC force at which the jump at a very low force level [0–1% MVC] occurred	0.35 (0.1)	0.37 (0.1)	0.25 (0.1)
Average % of MVC force at which the jump at a very low force level [2–10% MVC] occurred	4.1 (1.6)	4.7 (1.7)	4.2 (1.2)
Average % of MVC force at which FF reached 0.55	5.3 (2.3)	5.1 (2.1)	5.8 (1.8)

strategy (for details, see subsection "4.5 Possible interpretations").

We found that, in many subjects (78% in VL and 89% in VM), a jump in sEMG amplitude occurred at the very beginning of the contraction (at 0–1% of MVC force): this jump occurred because the sEMG activity started with the appearance of a few large-amplitude spikes clearly standing out from noise. This result differs from the assumption of Nandedkar that, in healthy individuals, the sEMG signal at low contraction levels normally contains many small-amplitude MUPs with a great overlap between them (Nandedkar et al., 2020).

In each participant, the pattern of sEMG filling observed (i.e., number and time of occurrence of the jumps) was almost identical in different repetitions, with the jumps appearing at the same relative force in each repetition. This high repeteability clearly indicates that, whatever the mechanism responsible for the jumps, it is not entirely governed by random variables, but rather, it appears to be predesigned.

It should be mentioned that the participants encountered no difficulty tracking the target force and that this force was accurately tracked in all cases. This indicates that the occurrence of jumps in sEMG amplitude was not due to possible abrupt jumps in the force series.

4.2. The importance of the filling factor to correctly interpret the sEMG filling process

The filling factor is particularly useful to analyse the filling process of the sEMG signal (Navallas et al., 2023). Here, we observed that the filling factor increased so long as additional MUPs were added to the sEMG signal, but this increase was not unlimited. In fact, the increasing curve could be divided into two separate regions: a first region (low forces) in which the filling factor curve increased rapidly with additional recruitment, and a second region where the curve flattens as the overlapping between MUPs increases, and the filling factor asymptotically approaches the value of 0.63 (value of Gaussian distribution). In addition, the filling factor is useful to detect jumps in sEMG amplitude: namely, an abrupt drop in the filling factor unequivocally indicates the

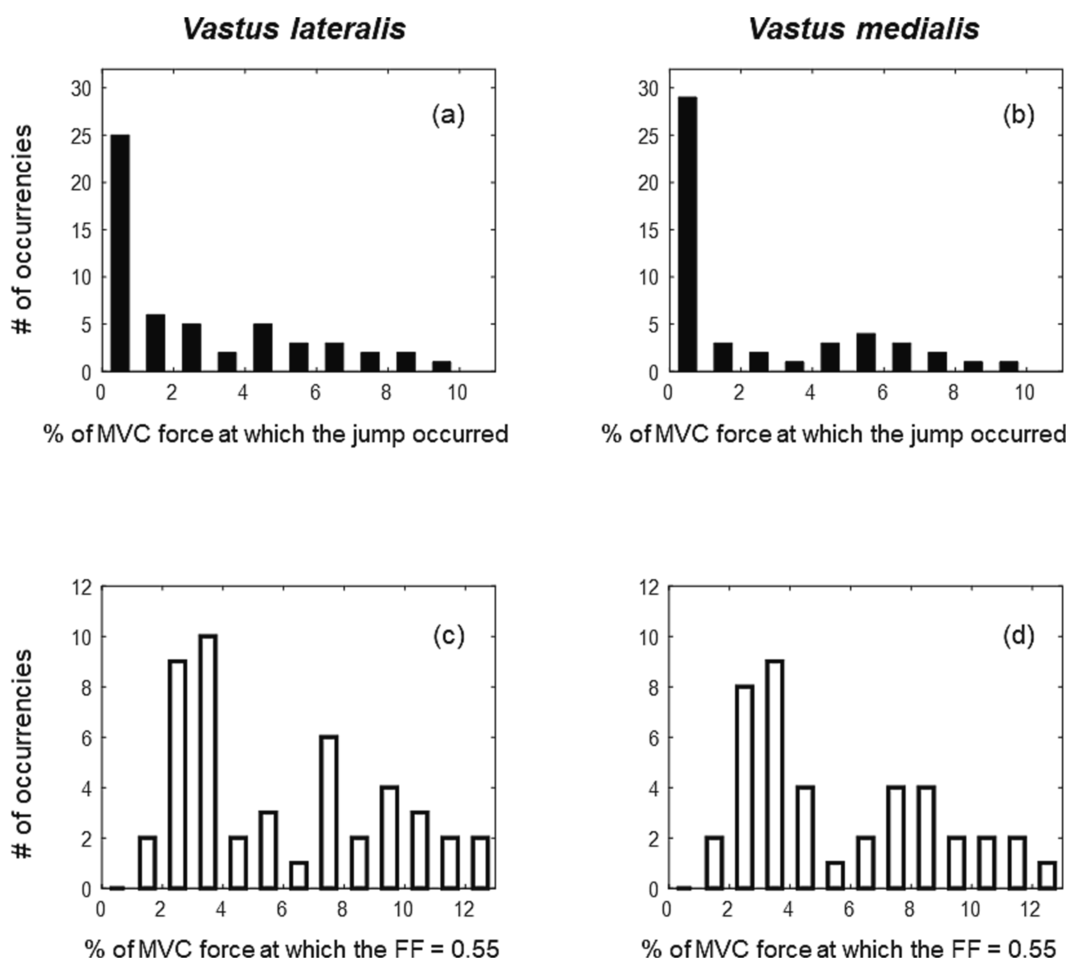

Fig. 6. (a)-(b) Frequency distribution of the percentage of the MVC force at which the jumps in sEMG amplitude occurred in the *vastus lateralis* and *vastus medialis* for the whole study group (n = 33). (c)-(d) Frequency distribution of the percentage of the MVC force at which the filling factor reached the value of 0.55 in the *vastus lateralis* and *vastus medialis* for the whole study group (n = 33). Data were extracted from all repetitions of the first session.

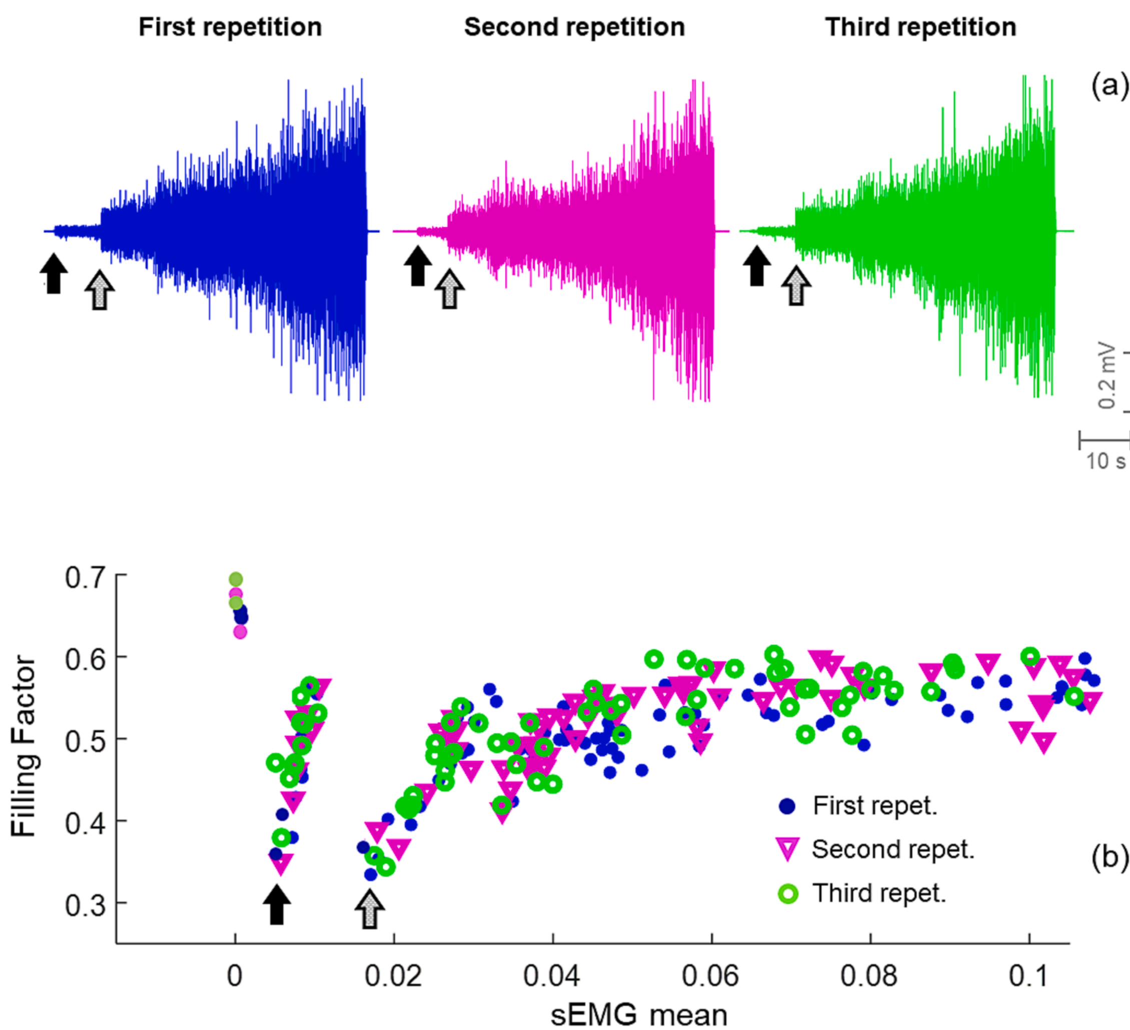

Fig. 7. (a) Example of 3 repetitions of a 60-s ramp contraction in the *vastus lateralis* of one participant. The black and grey arrows indicate the abrupt jumps in sEMG amplitude in each repetition. (b) Filling factor values associated to the three 60-s contractions. The black and grey arrows correspond to the instants when abrupt jumps in sEMG amplitude occurred.

Table 2
Intra-session reliability and inter-session reliability for the 3 measured variables in each muscle. Averaged intra-session reliability values, from the 2 experimental sessions, are presented for each variable.

Variable	Intra-session reliability study		
	ICC (Vastus lateralis)	ICC (Vastus medialis)	ICC (Rectus femoris)
Number of jumps in sEMG amplitude	0.95	0.91	0.88
Average % of MVC force at which the jumps occurred	0.92	0.89	0.85
Average % of MVC force at which FF = 0.55	0.87	0.85	0.83
Number of jumps in sEMG amplitude	0.87	0.94	0.82
Average % of MVC force at which the jumps occurred	0.84	0.82	0.79
Average % of MVC force at which FF = 0.55	0.83	0.78	0.78

appearance of a few large-amplitude spikes standing out from the previous sEMG activity.

It is important to emphasize that the filling factor carries specific information about the degree of sEMG filling that cannot be obtained from more classical parameters such as the RMS, more focused in the amplitude characteristics of the sEMG. For example, in the examples of Figs. 3 and 4 it can be seen that, as force was gradually increased, the filling factor reached the plateau at about 20% MVC, while the amplitude of the sEMG continued increasing until the end of the contraction (40% MVC). This means that the sEMG signal was completely filled-up at 20% MVC, despite the fact that additional motor unit recruitment continued for higher forces. Moreover, our filling factor analysis indicate that, in the *vastus* muscles, a high degree of EMG filling (filling factor = 0.55) was reached at very low contraction forces, namely at ~ 5.3% MVC, which means that the sEMG filling process occurred very fast in these muscles.

4.3. Why an abrupt jump in sEMG amplitude causes an abrupt decrease in the filling factor

To understand the cause for the abrupt drops in the filling factor, one should first consider the two "opposite" scenarios of sEMG activity. The first scenario would be one with little overlapping between MUPs (i.e., a few large-amplitude MUP spikes present in the signal, and thus "pulsatile" sEMG activity), in which the PDF is close to a semi-degenerate, and the filling factor within the range 0.25–0.40. The opposite scenario would be one with great overlapping between MUPs (i.e., a "continuous" sEMG activity), in which the PDF is close to a Gaussian, and the filling factor within the range 0.55–0.63. When a few MUP spikes with an amplitude significantly greater than the previous sEMG activity appears in the signal, then in statistical terms this previous sEMG activity now becomes "noise" of small amplitude. This way, the appearance of new large-amplitude spikes causes an abrupt return to a scenario with minimal overlapping between MUPs: in other words, the sEMG activity switches from "continuous" to "pulsatile" in an abrupt manner. It is like pressing the RESET button that shifts the shape of sEMG PDF distribution, from Gaussian to semi-degenerate, thus causing an abrupt fall in the filling factor, as shown in Figs. 3, 4 and 5. Specifically, the jumps in sEMG amplitude produced abrupt decreases in the filling factor from [0.55–0.63] to [0.25–0.45].

4.4. The importance of the occurrence of jumps in the sEMG amplitude

Information about the sEMG filling process can only be obtained in scenarios in which additional recruitment of MUPs allows the filling factor to increase within a wide range, say, from ~ 0.3 to ~ 0.63. This is possible when the sEMG activity at the beginning of the contraction is "pulsatile", and the initial filling factor values are low, say 0.3–0.4 (as in Fig. 3). However, in many instances we found that the initial sEMG activity was "continuous", with filling factor values within 0.55–0.63 (as in the 0–13 s period of Fig. 4): thus, in these cases the filling factor had no room to increase with additional recruitment of MUPs (Bostock et al., 2019). It is precisely in these cases when the importance of the abrupt jumps in sEMG amplitude comes in. Indeed, the abrupt appearance of a few large-amplitude spikes causes the filling factor to "reset" to a low value (0.3–0.4): thus, after this sudden drop, the successive recruitment of MUPs would make the filling factor increase within a wide range [0.3, 0.63].

4.5. Possible interpretations to explain the abrupt jumps in sEMG amplitude

The occurrence of abrupt jumps in sEMG amplitude may be due to different families of factors. The first family of factors is a methodological one: the selectivity of the surface EMG recordings, which comprises a variety of factors including the electrode size, inter-electrode distance, volume conductor properties, and the electrode-muscle fiber geometrical configuration. In this respect, the more selective the electrode configuration (small electrodes, small inter-electrode distance), the more likely the occurrence of abrupt jumps in sEMG amplitude. This is because a high selectivity of the recording causes the MUP amplitude to decrease more steeply with the MU-to-electrode distance (Roeleveld et al., 1997). The implication is that those motor units located superficially in the muscle (and close to the electrode) would generate MUPs much larger in amplitude compared to units located deeper in the muscle.

The second family refers to motor unit properties (such as distribution of the innervation ratio, muscle fiber types, and recruitment range). In this respect, an abrupt jump in amplitude may occur if a few motor units significantly larger in size than the previously recruited units came into play at 2–10% MVC. This could certainly happen as it has been shown that motor unit sizes (muscle fiber diameters and innervation ratio) are distributed continuously (and not separated in clusters) within the motor unit pool (Del Vecchio et al., 2018). More specifically, fiber diameter increases linearly with force, but a high dispersion in fiber diameter was observed around this linear regression line (see Fig. 4 of the cited paper of Del Vecchio). This means that a large motor unit can be activated before all small motor units have been recruited.

The third family of factors refers to the fiber-to-electrode distance and the spatial distribution of the motor units throughout the muscle cross-section. From this perspective, the occurrence of an abrupt jump in sEMG amplitude could be explained if a motor unit significantly closer to the electrode as compared to the previously recruited ones was called in at 2–10% MVC. This is certainly possible since, as force increases and more motor units are activated, it is more likely that a newly recruited unit is located at close proximity from the electrode. At this point, the phenomenon of "regionalization" in the vastus lateralis (i.e., that small motor units are located deep in the muscle, whilst large units lie in more superficial regions) should be considered (Knight and Kamen, 2005). Indeed, a possible explanation would be that, for very low contraction forces, motor unit recruitment starts at the deeper regions of the muscle, where small motor units are located, and, as force increases, recruitment advances to more superficial regions, where larger units are a majority.

The fact that the jumps in sEMG amplitude were found in all the *vastus lateralis* and *medialis* (100%), but only in a few *rectus femoris* (9%), suggests that the jumps may be related to the anatomical specificities of the muscle. One likely explanation for the absence of amplitude jumps in the *rectus femoris* is that the subcutaneous layer in this muscle is markedly thicker (~9 mm) as compared to that of the *vastus* muscles (~5 mm), as found by Caresio et al. (2015). Actually, the explanation would be based on the exponential decline of MUP amplitude with MU-to-electrode distance (Roeleveld et al., 1997). Thus, in those muscles with a "thick" subcutaneous layer, such as the *rectus femoris*, there would be only moderate differences in MUP amplitude between the motor units located at superficial and deep regions of the muscle: this scenario makes the appearance of amplitude jumps less likely. In contrast, in muscles with "thin" subcutaneous layers, the motor units located superficially in the muscle would generate MUPs with much greater amplitude than those located at deeper regions, thus favoring the appearance of jumps in sEMG amplitude.

4.6. Clinical applications and future research

In the present study, we have shown that the filling process of the sEMG signal in the *vastus* muscles can be characterized by assessing the changes in the filling factor as motor units are successively recruited. The resulting sEMG filling curves extracted from healthy subjects should be quantified and parametrized to create a database of "normality" patterns with which to compare the filling curves from neurogenic patients. We expect that, in patients with collateral reinnervation or loss of motor units, the sEMG filling curves would be altered compared to the filling curves from healthy individuals. Future research must be conducted to determine those muscles in which the filling factor analysis is most appropriate to characterize the sEMG filling process. Additionally, it remains to be evaluated how different conditions (sex, age, diseases, detection conditions) influence the filling factor analysis.

4.7. Limitations of the study

This study presents several limitations. First, only the muscles of the quadriceps group were assessed, and it would be necessary to extend the filling factor analysis to other muscles. Second, the subcutaneous layer thickness was not measured, while it may be a key aspect in the occurrence of the sEMG amplitude jumps. For future research it would interesting to assess the possible relation between the fat layer thickness and the appearance of sEMG jumps. Moreover, the present work could not evaluate the effect of motor unit properties (such as distribution of the innervation ratio, muscle fiber types, and recruitment range) on the occurrence of jumps. The influence of these properties could be investigated using sEMG simulation models. These sEMG models would also be helpful to ascertain the effects of the spatial distribution of motor unit types throughout the muscle cross-section.

4.8. Conclusions

It has been found that, as force was increased slowly in the *vastus lateralis* and *medialis*, sEMG amplitude did not increase gradually and smoothly, but rather, one or two prominent abrupt jumps in amplitude appeared between 0 and 10% of MVC force. The jumps in amplitude were originated when a few large-amplitude MUPs, clearly standing out from the previous activity, appeared in the sEMG signal. Every time an abrupt jump in sEMG amplitude occurred, a new stage of sEMG filling was initiated, in which additional MUPs were successively recruited, contributing to fill the sEMG in this stage. No jumps in amplitude were observed in the *rectus femoris*. The filling process of the sEMG signal was fast, being almost complete at 2–12% MVC.

The filling factor, an index based on the PDF distribution of the sEMG signal, decreased significantly every time an abrupt jump in sEMG amplitude occurred, and this index increased progressively as additional MUPs are successively incorporated to the sEMG signal.

The results of this study demonstrate that the filling factor curve is highly repeatable in healthy participants' quadriceps muscles. The study has validated that the filling factor is a potentially useful, reliable tool to analyse the sEMG filling process.

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Financial Disclosure

I declare that the authors have no financial interests.

Novelty and significance

+ For the first time, it has been shown that, as force is gradually increased in the vastus muscles, one or two prominent abrupt jumps in sEMG amplitude appears between 0 and 10% of MVC force.

+ The filling factor is a useful, reliable tool to analyse the sEMG filling process.

+ The sEMG signal is almost completely filled at very low forces (2–12% MVC).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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