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Frequency-Induced Fatigue in Electrically Stimulated Sheep Hindlimb Muscles

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Abstract

Functional electrical stimulation (FES) is an effective technique for restoring motor function in patients with paralysis. The early onset of muscle fatigue remains a major drawback, limiting its widespread clinical adoption. It is hypothesized that the high frequencies used in FES may be the primary factor determining muscle fatigue onset. Yet few studies have assessed the dependence of muscle fatigue on stimulation frequency. In particular, there is a need for a systematic evaluation across a continuous range of frequencies. Muscle fatigue dependence on stimulation frequency was assessed in anesthetized sheep, with the aim of modeling human musculature with physiological fidelity in the absence of potentially interfering reflexes. Following surgical muscle exposure, symmetrical 250+250 μ s biphasic pulse trains were delivered via hook wire intramuscular monopolar electrodes to either the tibialis cranialis or the extensor digitorum lateralis muscle, and isometric contraction forces were recorded. Eleven frequencies were assayed from 5 Hz to 100 Hz, with rest periods of over 10 minutes between trials. The extracted parameters included peak force, time to peak force, time to fatigue (defined as a 25% force drop), and the slope of force decline at fatigue. Additionally, muscle contraction ripple was assessed. Both muscles exhibited increasing fatigue with frequency, revealing three distinct frequency ranges. Fatigue rate was very slow below 15–20 Hz, gradually increased between ~20–50 Hz, and rised sharply above 50–75 Hz reaching fatigue in a few seconds. Remarkably, fatigue rate only started to increase substantially at 10–20 Hz. Force fusion increased with stimulation frequency, with both muscles showing fused contractions from approximately 12 Hz. The minimal fatigue observed at frequencies corresponding to natural motor unit firing rates suggests that the high frequencies used in FES are a key driver of fatigue.

Keywords: Functional electrical stimulation, Isometric force, Muscle fatigue, Neuromuscular stimulation, Neuroprostheses.

1. Introduction

Functional electrical stimulation (FES) is a technique aimed at restoring motor functions by artificially triggering action

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3 potentials to induce muscle contractions [1,2]. This is
4 accomplished by delivering short electrical pulses through
5 stimulating electrodes, which may be applied directly to
6 nerves [3] or muscles [4], or superficially on the skin [5]. By
7 targeting the peripheral nerves or the intramuscular nerve
8 branches that innervate the muscles, FES enables the
9 activation of paralyzed muscles to produce purposeful limb
10 movements [6,7]. Hence, FES has the potential to be an
11 effective tool for restoring motor functions in patients who
12 have suffered a CNS injury or other neurological conditions
13 and are unable to generate or transmit motor commands to
14 their muscles [8,9]. In fact, FES is already used to restore
15 bladder and bowel function, respiratory function, and upper
16 and lower extremity function [1,10–12]. Unfortunately,
17 several challenges hinder its widespread adoption, a key
18 limiting factor among them the early onset of muscle fatigue
19 [11,13–23]. Muscle fatigue occurs much earlier with electrical
20 stimulation than under voluntary contraction. This early onset
21 of fatigue is a major limitation that hinders the full potential
22 of FES. For instance, while FES can enable a person with
23 complete spinal cord injury (SCI) to stand, the posture can
24 only be sustained briefly [24].

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31 Three main hypotheses, or contributing factors, have been
32 proposed to explain the onset of early fatigue. The first
33 involves the altered histology of paralyzed muscles, the
34 second relates to the way motor units are recruited, and the
35 third attributes early fatigue to the high frequencies used
36 during electrical stimulation.

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39 Paralyzed muscle exhibits distinct histological differences
40 compared to healthy muscle [1]. When an SCI occurs, muscles
41 that become paralyzed due to their loss of connection with
42 their upper motor neurons lose their normal mosaic pattern of
43 Type I and Type II muscular fibers, and they become mostly
44 composed of Type II fibers [25,26]. The metabolism of those
45 fibers is mostly glycolytic, which makes the muscle tissue
46 prone to early muscular fatigue [1,26]. Remarkably, it has
47 been proven that with electrical stimulation, fast fatigable
48 muscle fibers (type II) can be converted into Type I muscular
49 fibers [1]. This, along with the fact that rapid fatigue also
50 occurs in healthy, non-paralyzed muscles during electrical
51 stimulation, suggests that altered muscle histology is not the
52 primary cause of stimulation-induced fatigue during FES [21].

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57 The second hypothesis attributes early fatigue to differences

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between motor unit recruitment during electrical stimulation and natural muscle contractions. During physiological contractions, Henneman's size principle states that the CNS recruits smaller, fatigue-resistant (Type I) muscle fibers first to produce weak forces, followed by larger, fast-fatiguing (Type II) fibers as stronger forces are needed [13,17]. Electrical stimulation, however, bypasses this sequence by readily recruiting larger motor units due to their larger axons which are more excitable than thinner axons, potentially leading to earlier fatigue [1,27].

Nevertheless, because electrical stimulation is applied through electrode setups that create a non-uniform recruitment field, it is expected that not only large (fast, fatigable) fibers but also a substantial portion of small (slow, fatigue-resistant) fibers are recruited. Therefore, a significant amount of force should persist even after fast motor units become fatigued. However, experimental observations show that electrical stimulation at moderate frequencies can completely exhaust the muscle within seconds to a very few minutes [28]. This suggests that inverse motor unit recruitment alone cannot fully explain fatigue during FES and must be accompanied by other contributing factors.

The last of the three hypotheses attributes early fatigue to the high stimulation frequencies used in FES. In FES, relatively high stimulation frequencies, typically around 20–30Hz, are used to produce strong and fused muscle contractions.

High-frequency stimulation is commonly reported in the literature to cause rapid fatigue [10]. Among the various stimulation parameters that can be adjusted in electrical stimulation to modulate the resulting contraction, such as pulse width, current amplitude, and frequency, frequency appears to have the most significant impact on fatigue. On the other hand, low-frequency stimulation produces lower, unfused forces but delays the onset of fatigue [1,10,22,28].

The tendency of high-frequency electrical stimulation to induce fatigue is likely related to the fact that muscle fibers are not naturally adapted to sustain such rapid activation rates [29]. In natural muscle contractions, multiple independent motor units (MUs) are activated asynchronously, resulting in a smooth, fused overall contraction. In contrast, electrical stimulation applied to whole nerves activates a fixed set of motor units synchronously and requires higher frequencies,

known as fusion frequencies, than those observed during natural motor unit activation to produce a fused contraction [22,28]. Based on this understanding, as an alternative to conventional electrical stimulation, interleaved electrical stimulation has been proposed to address fatigue [17,18,30–32]. Interleaved stimulation is intended to combine the fatigue-resistance of low-frequency stimulation with the smooth, fused contractions characteristic of high-frequency stimulation. It uses multiple electrodes to activate independent motor unit groups within a muscle or among agonist muscles with phase-shifted, low-frequency stimuli [28]. This approach reduces fatigue for each motor unit group while maintaining a high overall frequency to ensure smooth muscle contractions.

Remarkably, despite numerous studies describing the impact of stimulation frequency on muscle fatigue, there is a noticeable absence of comprehensive investigations into the relationship between stimulation frequency and fatigue onset. Existing studies examine only a limited set of frequencies [21,33], limiting the ability to generalize fatigue trends across the commonly used frequency range of 10–100Hz. The lack of sufficient data points hinders robust predictions of fatigue dynamics in FES applications. Addressing this gap could yield valuable insights into fatigue mechanisms and improve the effectiveness of FES strategies. In particular, establishing this relationship is crucial for optimizing FES protocols to maintain muscle performance and minimize fatigue in rehabilitative and therapeutic applications. In light of this, we decided to conduct a study specifically designed to investigate this issue.

Although muscle fatigue induced by electrical stimulation can be assessed non-invasively in human participants, and has indeed been evaluated for limited sets of frequencies [20,33], this approach compromises the ability to reliably determine the relationship between stimulation frequency and fatigue. First, non-invasive stimulation using surface electrodes lacks consistency and muscle selectivity. That is, surface stimulation cannot reliably target a specific muscle or muscle group, nor ensure consistent activation of the same motor units throughout a trial. In addition, surface stimulation easily activates cutaneous nociceptors, potentially eliciting reflex responses that may interfere with the assessment of muscle fatigue. Alternatively, percutaneous electrodes could be employed in a minimally-invasive setup for ensuring selectivity and consistency; however, interference of reflex

responses because of muscle proprioceptors would still be a concern. And conducting such trials in anesthetized human volunteers would be ethically questionable due to the associated risks.

Therefore, the optimal conditions for studying the relationship between stimulation frequency and fatigue require, first, performing electrical stimulation assays under anesthesia, as it minimizes reflex responses, such as nociceptive reflexes, and prevents any voluntary movements in the targeted area [15]. Second, to ensure consistency, it is essential to confirm that only the target muscle is being activated [10]. This necessitates the muscles to be exposed, allowing for visual verification that no adjacent muscles are inadvertently stimulated. These requirements make it impractical to conduct such experimental protocols in human participants. For these reasons, *in vivo* animal studies provide a more suitable experimental framework aligned with the objectives of this study, as they offer a highly controlled environment that minimizes the influence of confounding factors on the results.

The aim of this *in vivo* study presented here was to investigate the effect of stimulation frequency on the development of muscular fatigue in two distinct ovine calf muscles. Pulse trains at frequencies from 5 Hz to 100 Hz were applied to stimulate the muscles, and fatigue was evaluated during isometric contractions.

2. Methods

2.1. Animal preparation

The *in vivo* procedure was approved by the ethics committee. Three adult female sheep, aged between 3 and 6 years old, were used in three experimental sessions, with one animal per session. Sheep were selected for this study due to their anatomical and physiological similarities to humans, including comparable body size and weight [34]. Their size also facilitates the identification of individual muscles and allows for more selective stimulation, which can be more challenging in smaller animals. Although larger animals offer these advantages for the study, their higher costs, specifically for handling and experimentation, restricted the sample size and prevented statistical analyses.

The procedures were performed under general anesthesia. The sheep were anesthetized by administering intravenously (IV) ketamine (3-5 mg/kg), xylazine (0.02-0.2 mg/kg), and butorphanol (0.05-0.2 mg/kg) into the jugular vein in the neck.

The lateral hip region, along with the posterior limbs, underwent hair clipping and depilation with depilatory cream. A longitudinal incision was made along the tibia, exposing the lateral muscles of that region while maintaining the fascia. The first session served as a pilot and data from it are not reported here.

2.2. Measurement setup

Two muscles were targeted for stimulation, one in each session following the pilot: the tibialis cranialis, responsible for dorsiflexion of the foot, and the extensor digitorum lateralis, a finger extensor [35]. These muscles were selected due to their accessibility and their ability to generate distinctive movements (Fig. 1), ensuring that the generated force could be effectively measured. Although other muscles with different fiber-type compositions were available within the same limb segment, only the tibialis cranialis and extensor digitorum lateralis allowed precise electrode placement without risking damage to adjacent structures, while providing an optimal balance between safety and the ability to elicit large, observable limb movements upon stimulation



Fig. 1. Lateral view of the posterior limb muscles in the sheep, highlighting the tibialis cranialis and extensor digitorum lateralis, which were the targets of this study.

The limb was positioned on an acrylic baseplate, with regularly spaced mounting holes, that was secured to the operating table. To immobilize the limb, custom 3D-printed PLA brackets and fastening straps were used to fix it to the baseplate. Force measurements were performed via a load cell (Celtron STC-10K S-beam), positioned and aligned with the movement elicited by the stimulation. The load cell was connected to the limb segment through a custom 3D-printed contact plate (Fig. 2).

Two different fixation and force measurement arrangements were used. To measure dorsiflexion force during tibialis cranialis stimulation, the ankle and tibial segment of the limb were secured to the acrylic baseplate, and the load cell was positioned against the dorsal surface of the foot. For finger extension force measurement during extensor digitorum lateralis stimulation, the ankle and the foot were fixed to the acrylic baseplate, and the load cell was positioned against the dorsal surface of the hoof. The load cell was positioned to maintain a compressive force between 1 N and 2 N, as verified by baseline force measurements.

The load cell was connected to a custom-developed instrumentation amplifier, and its output signal was acquired at 1,000 samples per second using a data acquisition board (National Instruments NI USB-6211). Data collection and control were performed via USB using custom-developed routines in MATLAB 2023b.

Electrical stimulation waveforms were generated using a commercial function generator (BK Precision 4064) and converted from voltage to current waveforms via a custom-developed voltage-to-current converter. The settings of the function generator were controlled via USB by custom developed MATLAB routines synchronized with the force acquisition routines.

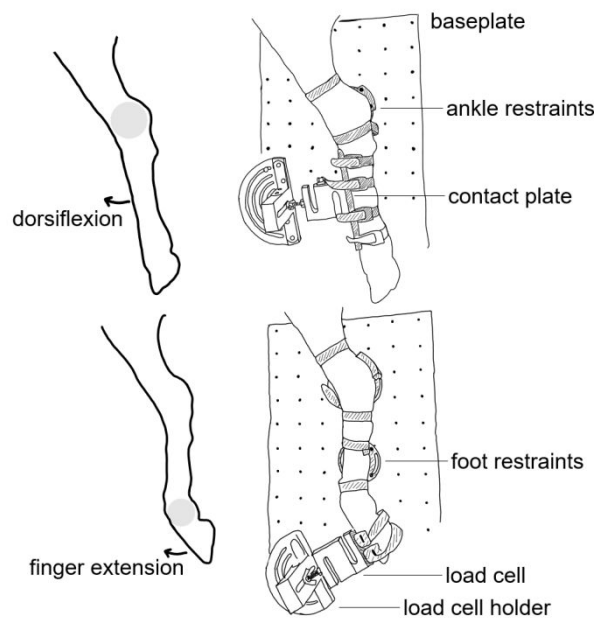


Fig. 2. Schematic of the experimental setup for measuring isometric forces generated by stimulation of the tibialis cranialis (top) and the extensor digitorum lateralis (bottom).

2.3. Electrical stimulation assays

Intramuscular electrical stimulation was delivered using custom-made monopolar hook wire electrodes made of stainless steel (Fig. 3). Hook wire electrodes provide stable fixation within the muscle tissue, minimizing the risk of electrode migration during stimulation. Each electrode measured approximately 3 mm in length with a diameter of about 0.38 mm. They were constructed by coiling 24 turns of bare 38 AWG stainless steel 316 wire around a bare filament of the same material and gauge. The PFA insulation had an outer diameter of approximately 0.2 mm (32 AWG). For insertion, the electrodes were mounted inside a 20 G needle, as shown in Fig. 3. A conventional ECG electrode (3M Red Dot) placed on the lateral region of the hip served as the return electrode.

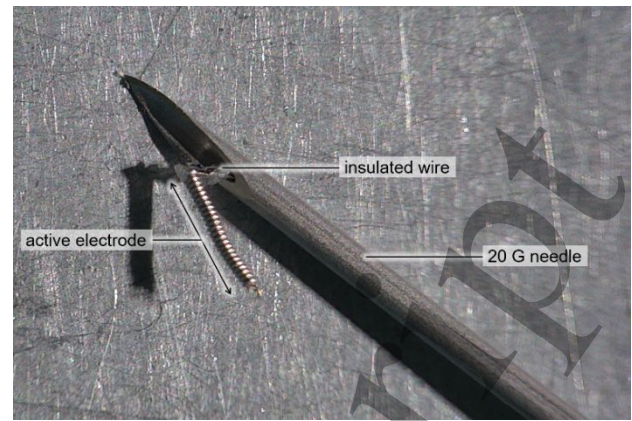


Fig. 3. Intramuscular hook wire electrode used in this study, shown mounted inside a 20 G insertion needle. Each electrode measured approximately 3 mm in length and 0.38 mm in diameter.

To determine the optimal location for the hook wire electrode placement, a rigid needle electrode (model 530607, Inomed Medizintechnik GmbH, Emmendingen, Germany) was initially used in conjunction with a hand-held electrical stimulator (Kegel8 Ultra 20). Through trial-and-error exploration, the motor point—the site eliciting the greatest desired force response—was identified. Once located, the needle electrode was replaced with the hook wire electrode, which was then connected to the previously described voltage-to-current converter.

All assays utilized current-controlled stimulation to ensure consistent activation, regardless of impedance changes. All pulses delivered were symmetrical biphasic pulses to mitigate electrochemical reactions at the electrode-tissue interface, thus reducing the risk of tissue damage. Each biphasic pulse had a duration of 250 μ s per phase, with no interphase delay.

The stimulation current was roughly optimized to maximize force generation without activating adjacent muscles. A current of 2 mA was used for the tibialis cranialis in the second session, and 3 mA for the extensor digitorum lateralis in the third session.

The optimal joint angle, and consequently the optimal muscle length, was determined by stimulating the muscle at the predefined current magnitude while positioning the limb at various levels of extension. The angle that produced the highest force response was identified as optimal. Once this position was established, the corresponding parts of the limb,

depending on the muscle being stimulated, were securely fixed to the baseplate and the load cell was positioned and aligned to maintain consistent positioning throughout the experiment.

The relationship between stimulation frequency and muscle fatigue was evaluated by delivering prolonged trains of electrical pulses at specific test frequencies. Each train had a maximum duration of 180 seconds (shorter if fatigue was detected), and a minimum rest period of 10 minutes was provided between trials to allow sufficient muscle recovery. The tested frequencies were 5, 7.5, 10, 12, 15, 20, 25, 30, 40, 50, 75, and 100 Hz. These values were selected to follow a logarithmic distribution, under the assumption that the most significant changes in fatigue dynamics would occur between 10 and 30 Hz. Occasionally, tests at a given frequency were repeated in order to 1) ensure that long-term fatigue or potentiation did not influence the results and 2) better characterize frequency regions where changes in fatigue dynamics were observed during the stimulation sessions.

For tibialis cranialis stimulation, the frequencies were applied in ascending order, beginning at 5 Hz and progressing through 7.5 Hz, 10 Hz, 15 Hz, and so on, up to 100 Hz. In contrast, a different stimulation order was used for the extensor digitorum lateralis to eliminate potential cumulative effects. Frequencies were alternated between low and high values, following this sequence: 5, 100, 7.5, 75, 10, 50, 12, 40, 15, 30, 20, and 25 Hz.

While previous studies have defined fatigue as a 50% reduction in muscle force from the maximum achieved during sustained stimulation [19,21,36] this threshold was not used in the present study. To avoid inducing complete fatigue, which would require extended recovery times, stimulation was halted once muscle force declined by approximately 25% from the peak value. This threshold was visually estimated in real time by monitoring the force–time curve, allowing timely interruption of the stimulation and prevent excessive muscle exhaustion. Using this approach allowed us to maximize the number of usable trials per animal while maintaining muscle responsiveness and ensuring animal welfare.

Due to time constraints, the two target muscles were stimulated in separate sessions, therefore, each muscle was studied in a different subject. Only muscles from the left posterior limb were tested, as switching the setup to the

contralateral limb was laborious and not feasible within the available session time. No alternation between extensors and flexors was performed, as they require different measurement setups; alternating between setups would have required considerably more time, which was not feasible and would also have increased variability in the measurements. Each preparation required considerable setup, and a minimum 10-minute rest was needed between trials to ensure recovery. Consequently, stimulation of multiple muscles or alternation between muscles within the same session was not possible.

2.4. Fatigue analysis

MATLAB2023b was used to process and analyze the force signals registered during the electrical stimulation of the muscles.

To analyze the dynamics of the force elicited during electrical stimulation and the onset of fatigue, low-pass filtering was applied first to remove noise and preserve the overall force trend. A fourth-order Butterworth low-pass filter with a cutoff frequency of 4 Hz was used, based on the fact that the lowest stimulation frequency was 5 Hz. Following filtering, the parameters shown in Fig. 4 were extracted using a set of custom-developed routines: i) peak force (with respect to baseline force), ii) time to peak force, iii) time from peak force to onset of fatigue, defined as a -25% force drop (from now on “time to fatigue”), and iv) slope of force decrease at fatigue onset. If a 25% decrease in force was not reached, the last three variables were not extracted.

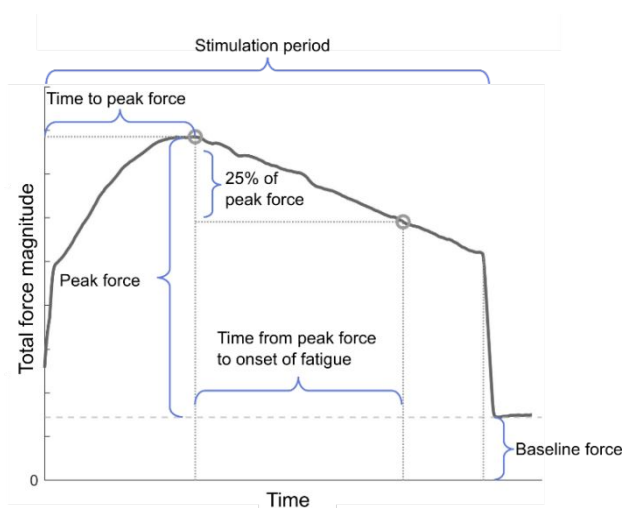


Fig. 4. An example of typical force recording obtained during stimulation and indication of the extracted parameters. Initially, potentiation occurs, leading

to an increase in force until the maximum force is achieved (peak force). Subsequently, the force begins to decrease as the muscle fatigues.

Complementary to the time-to-fatigue analysis, the total number of stimulation pulses applied until fatigue was reached was also computed to evaluate if the relationship between stimulation frequency and fatigue is driven by frequency itself or simply by the cumulative number of pulses delivered.

2.5. Fused force analysis

At low stimulation frequencies, muscle contractions appeared as separate twitches with distinct relaxation phases, producing visible fluctuations in force output (i.e., ripples). As stimulation frequency increased, these fluctuations progressively diminished, resulting in a smoother, fused contraction (Fig.5). To quantify this transition, the power of the force signal was computed [37], as it reflects the magnitude of force variability within the recorded trace. Higher signal power corresponds to greater force oscillations and thus a lower degree of fusion, whereas lower signal power indicates smoother, more continuous force production.

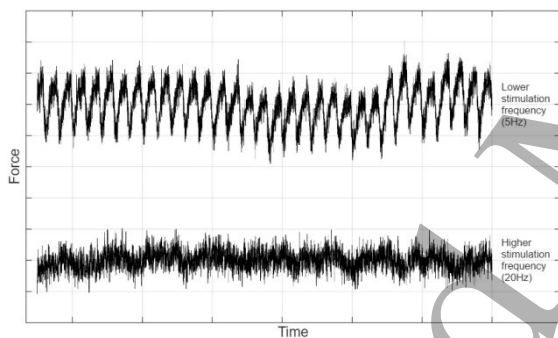


Fig. 5. Representative force traces recorded during electrical stimulation at lower 5 Hz (top) and higher 20Hz (bottom) stimulation frequencies at the extensor digitorum. At the lower frequency, individual twitches are clearly visible as large-amplitude oscillations in force, reflecting incomplete fusion of successive contractions. In contrast, at the higher stimulation frequency, these oscillations are substantially reduced, resulting in smoother and more continuous force output.

Force signals were processed and analyzed in MATLAB 2023b. A fourth-order Butterworth bandpass filter (3–140 Hz) was applied to remove slow drifts and high-frequency noise while preserving all components related to muscle contractions. Signal power (P) was then calculated for each

filtered trace using the equation below, where N is the number of samples and X is the acquired force signal.

$$P = \frac{1}{N} \sum_{n=0}^{N-1} (|X[n]|)^2$$

3. Results

In this section, all results are derived from only two out of the three animal subjects. Each muscle was studied in a single experimental session and in a different subject.

3.1. Fatigue analysis

For both muscles, peak force increased with stimulation frequency (Fig. 6), while the time to reach peak force decreased with frequency (Fig. 7). Notably, peak force in the tibialis cranialis showed a nearly logarithmic dependence on stimulation frequency. In contrast, the extensor digitorum lateralis exhibited a much abrupter relationship, with a sharp increase in peak force observed at frequencies above 30 Hz.

While only a single peak force value is shown for each frequency in the case of the extensor digitorum lateralis, multiple trials were conducted at the same test frequencies for the tibialis cranialis. These additional measurements were prompted by variations in peak force observed during the experimental session. That is, tibialis cranialis peak force measurements exhibited considerably greater variability than the extensor digitorum lateralis data. The cause of this increased dispersion remains unclear, but it does not appear to result from cumulative effects, as both increases and decreases in peak force were observed across repeated trials.

In the tibialis cranialis all stimulations that resulted in fatigue reached the maximal force value within less than 45 seconds. For stimulation frequencies from 30 Hz up to 100 Hz, the maximal force was reached nearly immediately after the stimulation started, indicating fast muscle potentiation. Specifically, when stimulating at 75 or 100 Hz, maximal force was reached within less than 2 seconds. Those stimulation frequencies that did not cause fatigue continued to show increasing force by the time the stimulation period elapsed.

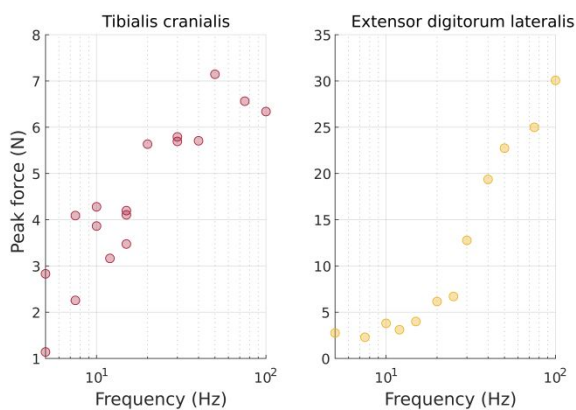


Fig. 6. Peak force relative to baseline during stimulation. Left subplot (red dots) shows tibialis cranialis measurements. Right subplot (yellow dots) extensor digitorum lateralis measurements.

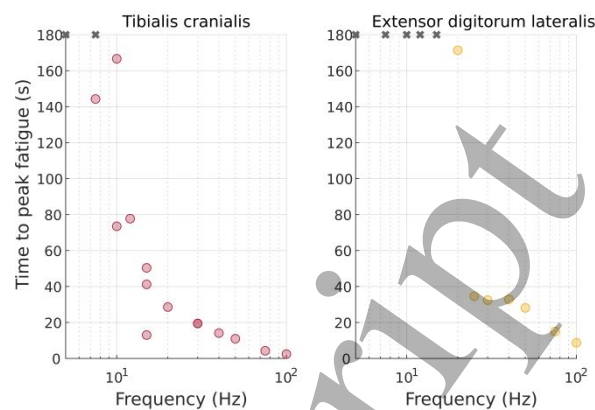


Fig. 8. Time to fatigue onset, defined as a -25% force drop. Left subplot (red dots) shows tibialis cranialis measurements. Right subplot (yellow dots) shows extensor digitorum lateralis measurements. Gray x marks indicate fatigue onset was not detected within the maximum 180 s stimulation period.

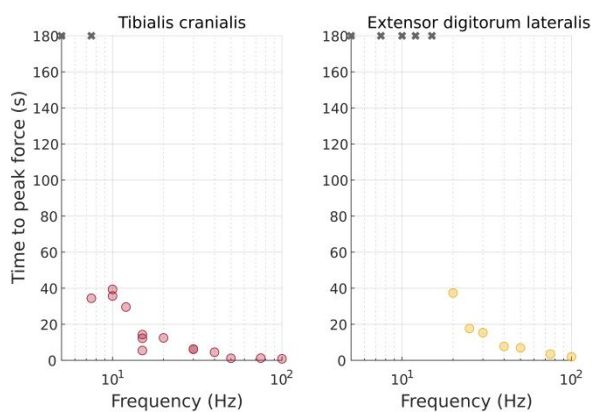


Fig. 7. Time to peak force after stimulation begins. Left subplot (red dots) shows tibialis cranialis measurements. Right subplot (yellow dots) shows extensor digitorum lateralis measurements. Gray X marks indicate cases in which no peak force was detected because force did not decrease significantly during the 180 s stimulation interval.

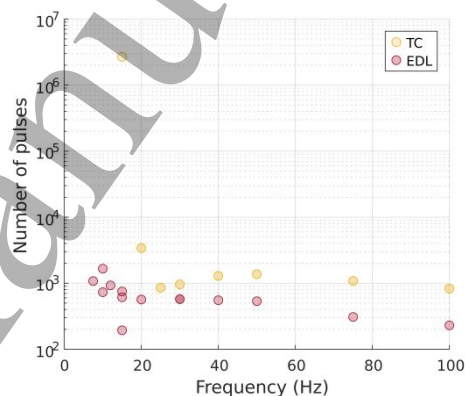


Fig. 9. Number of symmetrical biphasic pulses delivered until the -25% force drop. Data form trials where the -25% force drop was not reached are not included.

The tibialis cranialis did not exhibit fatigue after 180 seconds of stimulation at 5 Hz, and in one of the two trials at 7.5 Hz (Fig. 8). At higher frequencies, time to fatigue was strongly dependent on stimulation frequency, showing an approximately inverse proportionality. In the extensor digitorum lateralis, fatigue onset was not detectable at frequencies up to 20 Hz. At higher frequencies, time to fatigue also depended markedly on frequency, but the relationship is less straightforward than in the fibialis cranialis.

When evaluating the number of pulses applied before reaching 25% force decay, it can be observed (Fig. 9) that neither the tibialis cranialis nor the extensor digitorum lateralis reached the fatigue threshold with a similar number of applied pulses. Higher stimulation frequencies required fewer pulses to reach the same level of fatigue compared to low-frequency stimulation protocols.

The rate of force decline provides a more comprehensive view of the fatigue process (Figs. 10–13). In tibialis cranialis measurements (Figs. 10-13), the slope of force decline at fatigue onset reveals three distinct ranges: for frequencies below ~15 Hz, fatigue develops very slowly; between ~15 Hz and ~50 Hz, the fatigue rate increases gradually; and above 50 Hz, the fatigue rate rises sharply with frequency. The

increase in fatigue rate between ~15 Hz and ~50 Hz is much more gradual than the steep rise observed above 50 Hz. In fact, the slope of force decline shown on a linear frequency scale (Fig. 11) suggests that fatigue rate plateaus between 30 and 50 Hz. The extensor digitorum lateralis (Figs. 12–13) shows the same qualitative pattern but with frequency ranges shifted upward: the gradual increase occurs between ~20 Hz and ~75 Hz, and the steep rise appears only above 75 Hz. In this muscle, the plateau in normalized slope is even more evident, extending from 30 Hz to 75 Hz. It is also worth noting that, across the entire frequency range, fatigue develops more slowly in this muscle than in the tibialis cranialis (Fig. 13 vs Fig 11).

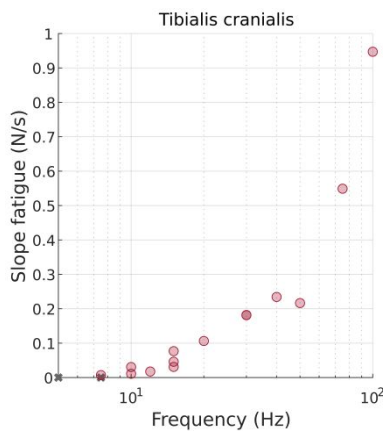


Fig. 10. Slope of force decline at fatigue onset from tibialis cranialis measurements. Gray x marks indicate no measurements because fatigue onset was not detected within the maximum 180 s stimulation period.

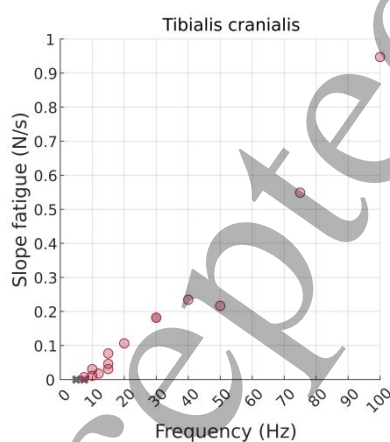


Fig. 11. Same as Fig. 10, but with frequency on a linear scale.

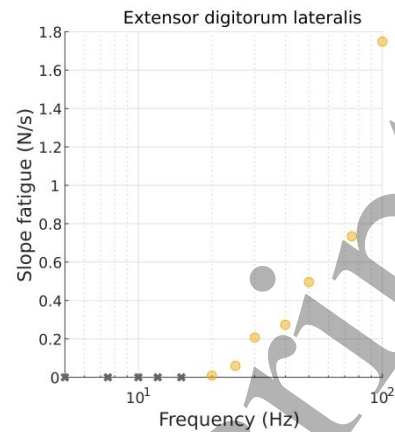


Fig. 12. Slope of force decline at fatigue onset from extensor digitorum lateralis measurements. Gray x marks indicate no measurements because fatigue onset was not detected within the maximum 180 s stimulation period.

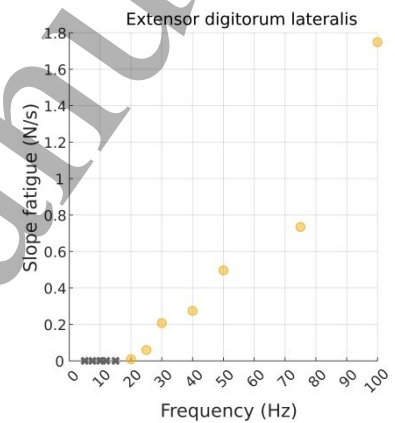


Fig. 13. Same as Fig. 12, but with frequency on a linear scale.

3.2. Fused force assessment

Both muscles exhibit a similar pattern of force ripple across stimulation frequencies (Fig. 14). At lower frequencies, the power of force fluctuations is higher because the muscle has sufficient time to relax between pulses, resulting in unfused contractions and greater amplitude oscillations. In both muscles, a stimulation frequency of approximately 12 Hz marks the threshold for fused contractions, defined here as force ripple power dropping below 5% of the maximum.

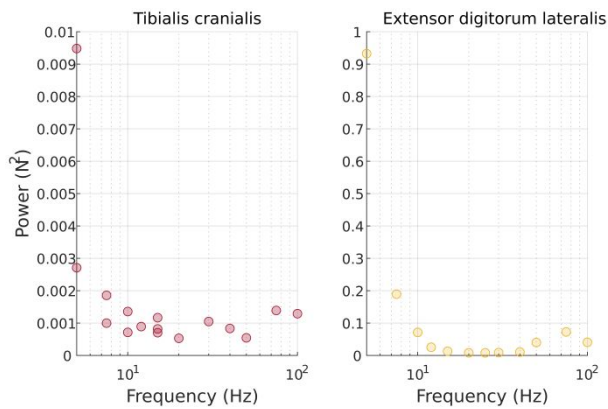


Fig. 14. Power of force fluctuations. Left subplot (red dots) shows tibialis cranialis measurements. Right subplot (yellow dots) shows extensor digitorum lateralis measurements. The muscles compared were obtained from different subjects.

It should be noted that the y-axis in Fig. 14 spans markedly different power ranges for the two muscles. The tibialis cranialis exhibits substantially lower power values than the extensor digitorum lateralis. Although this difference is reduced when power is normalized to the squared peak force, the extensor digitorum lateralis still produces significantly greater force ripple than the tibialis cranialis even after normalization.

4. Discussion

This study aimed to investigate the effect of stimulation frequency on muscle fatigue. Frequencies between 5 Hz and 100 Hz were applied to the tibialis cranialis and extensor digitorum lateralis muscles of sheep calves. The findings are consistent with previous research showing that higher stimulation frequencies increase contraction force, smoothness, potentiation, and fatigue. Although both muscles exhibited similar patterns across all measured parameters, certain differences warrant further discussion.

The frequently-dependent behavior of the extensor digitorum lateralis was shifted toward higher frequencies relative to the tibialis cranialis, impacting both the time to peak force (Fig. 7) and time to fatigue (Fig. 8). At any given stimulation frequency, the tibialis cranialis fatigued earlier than the extensor digitorum, but it also reached peak force sooner. These findings were initially unexpected given the muscle fiber composition described in [35], where it is indicated that the tibialis cranialis possesses a higher

percentage of type I muscle fibers (54.1%)—known for greater fatigue resistance—compared to the extensor digitorum lateralis (18.8%). However, it is important to note that during this study's stimulation protocols, muscle fatigue was deliberately limited by stopping stimulation upon a 25% decrease in measured force from peak force, as complete muscular fatigue was not desired. Since electrical stimulation activates both type I and type II muscle fibers simultaneously, the observed force loss in the tibialis cranialis and extensor digitorum lateralis likely reflects fatigue predominantly in type II fibers. Although these muscles differ in their proportion of type I fibers, the relatively small magnitude of force decline prevented any observable effect of fiber composition. Therefore, the influence of fiber type distribution remains inconclusive in this study. Hypothetically, a greater degree of force decay might reveal differences attributable to fiber composition. Future investigations could address this by examining the soleus muscle, which consists entirely of type I fibers [35].

Another significant difference between the two muscles is observed in contraction force ripple (Fig. 14). The tibialis cranialis produced markedly smoother contractions than the extensor digitorum lateralis, even after normalization for peak force. We suspect that this discrepancy does not arise from intrinsic muscle properties but rather from the combined influence of calf anatomy and the measurement setup. Specifically, force from the tibialis cranialis was transmitted through a mass with greater inertia (the entire foot versus the hoof), and the damping effect of the contact plate on the larger, softer, and hair-covered foot surface was likely greater than that on the stiff, less compliant hoof.

The most notable finding of this study is the distinct transition in fatigue behavior as a function of stimulation frequency. The slope of force decline at fatigue onset reveals three separate frequency ranges. Consistent with previous literature, higher frequencies induce faster fatigue, as clearly seen in the pronounced increase in fatigue rate at the upper frequency range for both muscles. What is particularly noteworthy, however, is the shift in slope behavior between the low and intermediate frequency ranges, occurring roughly between 10 and 20 Hz. This finding challenges the common binary classification of stimulation frequencies as simply “high” or “low” with respect to their effects on force

1
2
3 generation and fatigue, suggesting instead the existence of an
4 intermediate frequency range with distinct characteristics.

5
6 One could argue that the observed relationship between
7 fatigue and stimulation frequency is primarily driven by the
8 number of delivered pulses. Under this assumption, higher
9 stimulation frequencies would lead to faster fatigue simply
10 because pulses are delivered at a higher rate, resulting in a
11 more rapid accumulation of stimulation over time. In this
12 context, fatigue would depend mainly on the total number of
13 pulses rather than on frequency itself. However, the present
14 study shows that higher stimulation frequencies require fewer
15 pulses to reach the same level of fatigue compared to lower-
16 frequency protocols. This finding suggests that stimulation
17 frequency per se contributes to the development of fatigue,
18 and that fatigue cannot be explained solely by the cumulative
19 number of delivered pulses.

20
21 The results of this study suggest several practical
22 implications for the application of functional electrical
23 stimulation (FES).

24
25 It is well established that FES protocols should be tailored
26 to the functional demands of the task—whether it requires
27 high forces over a short duration, as in standing up, or lower
28 forces sustained over a longer period, as in maintaining an
29 upright posture. Consequently, fatigue prevention strategies
30 must account for the optimal stimulation frequency in relation
31 to the specific functional goal. Regardless of the task, FES
32 should aim to generate force as smoothly as possible,
33 particularly in situations where unfused contractions could
34 compromise stability, such as when maintaining an upright
35 posture.

36
37 In scenarios necessitating brief, high-force movements,
38 higher stimulation frequencies are appropriate. However, as
39 observed in our study, the highest frequency (100 Hz) did not
40 consistently yield superior outcomes. For instance, the tibialis
41 cranialis showed no significant force increase at 100 Hz
42 compared to 50 Hz, both reaching maximal force at similar
43 times. Notably, at 100 Hz, the muscle fatigued within 2
44 seconds with a steep force decay, suggesting potential total
45 fatigue imminent with slightly prolonged stimulation.
46 Conversely, the extensor digitorum exhibited a 7N force
47 reduction at 50 Hz compared to 100 Hz, but doubled the time
48 to reach a 25% decrease in maximal force. Evaluating whether
49 the force obtained at these frequencies suffices or exceeds

requirements is crucial. Adjustments may involve reducing
frequency for safety if excessive force is generated or
enhancing force via other parameters if insufficient.

According to the findings of this study, an optimal balance
of contraction force, smoothness, and fatigue resistance would
be achieved within the 20 Hz to 50 Hz frequency range, which
corresponds well with the typical frequencies used in FES.

Without increasing stimulation frequency, higher
contraction forces can be obtained by recruiting additional
axons innervating motor units. This can be achieved, to a
moderate extent, by increasing pulse duration, but most
commonly by applying higher stimulation currents [20].
However, in intramuscular stimulation scenarios, higher
currents carry the risk of stimulating adjacent muscles, thus
compromising specific muscle targeting. An alternative
approach, proposed in [14], is to stimulate simultaneously
with multiple electrodes distributed across the muscle, thereby
increasing recruitment and enhancing force output without
raising current intensity. Relatedly, when current was
adjusted to achieve a fixed target force, multi-electrode
stimulation was associated with longer endurance times [15].

The above concept of simultaneous (i.e., synchronous)
distributed stimulation is related to interleaved stimulation,
where phase-shifted or fully asynchronous low-frequency
stimuli are delivered across multiple electrodes to activate
motor units independently. Our findings—showing minimal
fatigue at stimulation frequencies below the fusion threshold
(12 Hz in this case)—suggest that high-frequency activation is
a primary driver of fatigue in FES. This underscores the
potential benefits of further exploring interleaved stimulation
to reduce fatigue while achieving smooth and sufficiently
strong contractions [28].

The main limitation of this study is that only one muscle
was evaluated per subject, which restricts the robustness of the
findings. This limitation stems largely from the time-
consuming nature of the procedure: both the preparation and
the stimulation phases required substantial time, making it
unfeasible to assess additional muscles within the same
session. Together with the associated economic constraints,
this limited the scope of the study to a single muscle per
session. Ideally, stimulating both muscles in each session
would have provided greater robustness without increasing the
number of animals used. Future studies should be performed

with an increased sample size, include muscles with different fiber-type compositions, and incorporate lateralization. If this approach is adopted and measurements from different subjects are obtained, the data should be standardized to account for inter-individual differences in muscle size. This was not included in the present study, as data for each muscle were obtained from a single individual.

5. Conclusion

Muscular fatigue is defined as a noticeable decline in the maximal force a muscle can generate. This study, using intramuscular neuromuscular electrical stimulation across eleven logarithmically spaced frequencies, examined how stimulation frequency affects fatigue development during isometric contractions. The slope of force decline at fatigue onset revealed three distinct ranges: below 15–20 Hz, fatigue develops very slowly; between ~20 Hz and ~50 Hz, the fatigue rate increases gradually and appears to plateau; and above 50–75 Hz, the fatigue rate rises sharply with frequency.

The finding of minimal fatigue at stimulation frequencies below the natural motor unit firing rates during voluntary moderate contractions [38] suggests that unnatural high-frequency activation is a primary driver of fatigue in functional electrical stimulation, highlighting the potential advantages of interleaved stimulation to mitigate fatigue.

Importantly, these findings are based on experiments conducted in two sheep. While the observed trends are consistent and suggest underlying physiological principles, their generalizability to broader populations, including other species or humans, remains likely but not definitively established and should be confirmed in future studies.

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Ethical statement:

The *in vivo* procedure (reference 22-018-AIC-11917) was approved by the local ethics committee, the Animal Experimentation Ethics Committee (CEEA) of the Comparative Medicine and Bioimage Centre of Catalonia (CEEA-CMCiB) of the Germans Trias i Pujol Research Institute - IGTP (Badalona, Spain), and by the Catalan Government through its Department of Territory and Sustainability.

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