ABSTRACT
Background: The main limitation of neuromuscular electrical stimulation (NMES) is the muscle fatigue. According to previous studies, stimulation frequency has the greatest influence on rate of muscle fatigue. However, most of studies have been focused on high stimulation frequency but less than 100 Hz. We proposed that using a very high frequency may produce asynchronous motor unit recruitment and similar or less muscle fatigue.

Objectives: To examine the effects of very high (150 and 200 Hz) and low (50 Hz) stimulation frequencies on the declining stimulated muscle force.

Methods: Nine healthy participants underwent a fatigue test using 3 stimulation frequencies (50, 150, and 200 Hz) combined with wide pulse duration (0.9 ms) for 15 minutes fatigue test. Muscle fatigue was assessed using the normalized force values at the end of each stimulation frequency protocol.

Results: No difference in muscle fatigue was found when compared between very high frequency (150-200 Hz) and low frequency (50 Hz) combined with wide pulse duration conditions during a 15-minute fatigue test.

Conclusion: This study demonstrated that both very high and low frequencies combined with wide pulse duration showed no differences in muscle fatigue. Further studies with a larger sample size are needed for confirmation.

Keywords: stimulation frequency, muscle fatigue, neuromuscular electrical stimulation, quadriceps femoris, muscle force
Introduction

Neuromuscular electrical stimulation (NMES) is commonly used in rehabilitation programs to increase strength in healthy individuals and individuals with neurological impairment.\(^1\)\(^-\)\(^4\) The target force output during electrical stimulation depends on the current parameters, especially pulse duration, stimulation intensity and frequency. Although, NMES has been demonstrated to have benefit in strengthening\(^4\), muscle fatigue are the main limitation during NMES application.\(^5\) Therefore, using optimal current parameters that generates stronger force output and minimizes muscle fatigue are essential in clinical treatment.

Previous studies not only have shown that the higher stimulation frequency (60-100 Hz) generated stronger muscle contraction compared to the lower stimulation frequency (20-25 Hz), but also increased rate of muscle fatigue.\(^6\)\(^-\)\(^8\) On the contrary, a study of Matsunaga et al.\(^9\) showed that high frequency stimulation (100 Hz) produced less muscle fatigue than low frequency stimulation (20 Hz). Russ et al.\(^10\) demonstrated that the percentage of decline in peak force during the fatigue test was similar between 80 Hz and 100 Hz protocols, if the initial force was controlled. Thus, the relationship of frequency-fatigue remains inconclusive. Moreover, the term low or high stimulation frequency is relatively ambiguous. Most of previous studies\(^6\)\(^,\)\(^7\)\(^,\)\(^9\)\(^,\)\(^10\) defined lower stimulation frequencies in the range of 11-50 Hz and higher stimulation frequencies of 60 or 100 Hz. There is no previous research using the very high stimulation frequency especially higher than 100 Hz to test the fatigue effect.

In addition, most of the previous researches used a short PD (0.05-0.45 ms) to evaluate the effect of frequency on stimulated muscle force.\(^11\)\(^-\)\(^13\) Using a wide PD (0.5-1 ms), high frequency demonstrated a higher stimulated muscle force than expected by the direct activation of motor axons.\(^14\)\(^,\)\(^15\) This increasing stimulated force output that arises in addition to the direct response to motor axon stimulation has been referred to as from the central contribution which is similar to the natural recruitment pattern.\(^14\)\(^,\)\(^15\) However, the central contribution can be elicited only by a low stimulation intensity (10-20% of MVC).\(^16\) Martin et al.\(^16\) demonstrated that wide PD combined with high frequency (1 ms, 80 Hz) and low intensity protocol was a similar decrease in stimulating force compared with short PD with low frequency (0.05 ms, 20 Hz) after repetitive electrical stimulation protocol.

Thus, this study used a wide PD (0.9 ms), very high frequency (150 and 200 Hz) and low
current intensity (10-20% MVIC) to explore the effects of very high stimulation frequency on muscle fatigue. Based on physiological knowledge, the stimulation frequency which nerve and muscle can respond to stimuli depends on the duration of the refractory period.\(^1\)\(^7\) Because the stimulus which stimulates at the absolute refractory period will not produce any action potential, thus the muscle fiber will not contract.\(^1\)\(^7\),\(^1\)\(^8\) We proposed that electrical stimulation using very high stimulation frequency (more than 100 Hz) may asynchronously stimulate motor units if the motor axon is stimulated at the refractory period. Thus, the very high stimulation frequency (150 and 200 Hz) was chosen in this study, based on the reported refractory period of human nerve.\(^1\)\(^9\),\(^2\)\(^0\) For the frequency of 150 and 200 Hz, the pause duration between each pulse was varied from 5.77 and 4.10 ms, respectively. This duration may be short enough to stimulate a motor axon in the refractory period of human nerve.\(^1\)\(^9\),\(^2\)\(^0\)

Therefore, the purpose of this study was to examine the effects of very high (150 and 200 Hz) and low or conventional (50 Hz) stimulation frequencies with a wide PD (0.9 ms) on the decline in stimulated muscle force. We proposed that using very high stimulation frequency (150 and 200 Hz) would produce similar or less muscle fatigue than using low stimulation frequency (50 Hz).

Methods

Participants

Nine healthy participants (3 males and 6 females; age range 18-35 years) took part in this study. All participants had no history of lower extremity musculoskeletal, neurological or other diseases that might affect the contraction of muscle. They were informed of the research procedures and risks before signing a consent form. This study was approved by the ethics committee of the Faculty of Associated Medical Sciences, Chiang Mai University (AMSEC-58EX-034).

Procedures

All participants were asked to refrain from strenuous physical activities for at least 48 hours before taking part in the study. Each participant undertook in three sessions. The first session included the maximum voluntary knee extensor isometric contraction (MVIC) test, familiarization with the fatigue test protocol and the fatigue test. During the second and third session, one other stimulation frequency was tested, respectively. Each session was separated by at least 48 hours or until there was no perceived muscle soreness. The flowchart of the study procedures is shown in figure 1.
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3 trials of knee extensor isometric contractions, and the greatest peak force was recorded as the MVIC of knee extensor muscles, and used as the reference for calculating the percentage of maximum force produced during the fatigue test. After testing the MVIC, stimulation intensity was determined in all of the participants, which produced approximately 10-20% of MVIC, depending on the participants' tolerance for each frequency. This intensity was used in the fatigue test. This initial force (%MVIC) was controlled across the frequency of stimulation.

A 10.5x7.0 cm rubber carbon electrode was placed over each motor point of the rectus femoris and vastus medialis. A rectangular biphasic pulsed current, with a PD of 0.9 ms, frequency of 50, 150 or 200 Hz, and 2 s on time and 4 s off time was used (Phyaction Guidance E, Uniphy International, Holland). After determining the stimulation intensity, each participant became familiarized with the stimulation test, using 50, 150 and 200 Hz for a few minutes.

Following familiarization, a 15-minute rest period was taken to avoid muscle fatigue before the fatigue test. One of three stimulation frequencies (50, 150 or 200 Hz) was applied randomly to the quadriceps femoris muscle of the right leg.

The knee extensor isometric force outputs, produced by electrical stimulation, were recorded at the sampling rate of 1,000 Hz using the PowerLab® System (ADInstrument Ltd, Australia).
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Outcome measure
Muscle fatigue is defined as a comparison between the decline and initial stimulated force of an individual. Each stimulated force was determined by averaging the maximum stimulated force, which was sampled every second over a 2-s contraction time. Then, every 5 contractions of these stimulated forces were averaged and used for further data analysis. Thus, 30 data points of stimulated force from a 15-minute fatigue test were normalized to each participant’s initial force and compared with each stimulated frequency.

Statistical analysis
Normal distribution of data was analyzed using the Shapiro-Wilk test. A one way repeated measures analysis of variance (ANOVA) with a Bonferroni test was used to determine the significant differences of normalized force values at the end of a fatigue test, and current amplitude of each frequency. The Friedman’s test was used to determine the significant differences of muscle contraction intensity (%MVIC) at the beginning of each fatigue test. Statistical significance was set at a level of p < 0.05. Partial $\eta^2$ value was reported as measure of effect size (ES) for difference in the normalized force values at the end of each fatigue test. The SPSS version 17.0 for Windows was used for analysis of data.

Results
Nine participants (3 males, age = 24.11 ± 3.33 year with MVIC = 292.44 ± 82.80 N) were volunteered. Table 1 shows mean and standard deviation of the current amplitude and the % MVIC in each stimulation frequency. The current amplitude, which provided approximately 10-20% of MVIC, was greatest at 50 Hz when compared to 150 and 200 Hz. However, these differences were not statistically different among the three stimulation frequencies (p=0.051). The %MVIC of average peak forces at the beginning was slightly greater at the 150 and 200 Hz stimulation frequencies than that at the low stimulation frequency, but without statistical significance (p=0.459). All stimulation frequencies showed a similar decline in normalized stimulated force during the 15 minutes fatigue test (Figure 2). The normalized forces (Table 2) were not significantly different among the three stimulation frequencies (50, 150 and 200 Hz) at the end of the fatigue test (p=0.801; ES = 0.027).

Table 1 The mean and standard deviations of the current amplitude and % MVIC of average peak forces at the beginning (n=9)

<table>
<thead>
<tr>
<th>Variables</th>
<th>50 Hz</th>
<th>150 Hz</th>
<th>200 Hz</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current amplitude (mA)</td>
<td>44.22 ± 11.86</td>
<td>38.00 ± 11.19</td>
<td>38.78 ± 13.50</td>
<td>0.051</td>
</tr>
<tr>
<td>% MVIC of average peak forces at the beginning</td>
<td>12.85 ± 6.16</td>
<td>16.44 ± 6.27</td>
<td>15.37 ± 7.12</td>
<td>0.459</td>
</tr>
</tbody>
</table>

MVIC, Maximal voluntary isometric contraction
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Table 2. The normalized force (mean ± SD) at the end of the fatigue test in each stimulation frequency

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>Normalized force at the end of the fatigue test (% of participant's initial force)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>64.57 ± 42.76</td>
</tr>
<tr>
<td>150</td>
<td>59.95 ± 25.78</td>
</tr>
<tr>
<td>200</td>
<td>72.70 ± 43.84</td>
</tr>
</tbody>
</table>

Discussion

The present study was the first to compare the effects of very high (150 and 200 Hz) and low (50 Hz) stimulation frequencies on the decline in stimulated muscle force of quadriceps femoris muscle in healthy participants. We expected that using very high stimulation frequency (150 and 200 Hz) may asynchronously recruit motor units and would produce similar or less muscle fatigue than using low stimulation frequency (50 Hz). Our results found that the decline in stimulated force at the end of fatigue test was similar for all stimulation frequency conditions. The finding of the present study is supported by the study of Russ et al. which demonstrated that the percent declines in peak force during the fatigue test was similar for 80 Hz and 100 Hz protocols. Although, the study of Matsunaga et al. which found lesser declined in force during fatigue test at the high stimulation frequency (100 Hz) compared to low stimulation frequency (20 Hz) in healthy subjects. However, the off time between contractions in the present study was shorter than in Matsunaga et al.'s study (4 s versus 60 s, respectively).

Contrary to our findings, Dreibati et al. demonstrated that decreased muscle force at the...
end of the fatigue session was lesser for the low stimulation frequency (20 and 50 Hz) compared to high stimulation frequency (100 Hz). Compared with our study, the fatigue protocol tested by Dreibati et al.\textsuperscript{7} used much shorter PD (0.3 ms versus 0.9 ms in the present study). Thus, a possible explanation for the difference between findings is that the different stimulation parameters setting, that is, frequency and pulse duration, during repetitive electrical stimulation were used. It may be possible that wide PD would increase the central contribution which is similar to the natural recruitment pattern. Therefore, wide PD may minimize muscle fatigue when compare to short PD. However, previous studies\textsuperscript{14,22} reported that the central contribution from wide pulse stimulation was demonstrated only in some participants. The variability of the central contribution requires further study.

The present study using different combinations of frequency and wide PD (0.9 ms) during the fatigue test, so it is difficult to compare with the results of Kesar and Binder-Macleod.\textsuperscript{6} However, the similar decline of stimulated force during repetitive stimulation in our study showed a similar trend to the finding of Martin et al.\textsuperscript{16} which using 80 Hz with 1 ms PD protocol compared with 20 Hz with 0.05 ms PD. Kesar and Binder-Macleod\textsuperscript{6} used 60 Hz with 600 μs PD as the testing train and found the same decline in peak force between the low frequency (11 Hz 600 μs PD) and the high frequency (60 Hz 130 μs PD). The findings of Kesar and Binder-Macleod strongly suggested that the combinations of PD and frequency may effect on muscle fatigue during repetitive stimulation.

The advantage of using very high stimulation frequency is that the high frequency used current intensity smaller than the low frequency for generating muscle contraction and could generate the rapid muscle contraction.\textsuperscript{9} Therefore, therapists may apply very high stimulation frequency combined with wide PD as an optional parameter for NMES application. However, the present study did not compare discomfort level between high and low stimulation frequency protocols, thus the advantage in aspect of muscle force contraction and discomfort level should be confirmed in further study. Another limitation of this study is that the findings of this study were obtained from a small sample size of healthy individuals. In addition, further studies need to be performed on patients with quadriceps femoris muscle weakness.

Conclusion

This pilot study demonstrates that no difference in muscle fatigue was found when compared between very high frequency (>100 Hz) and low frequency (50 Hz) combined with wide pulse duration (0.9 ms) conditions during the repetitive electrical stimulation. Further studies with a larger sample size are needed for confirmation.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.
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