The effect of neuromuscular electrical stimulation frequency on postprandial glycemia, current-related discomfort, and muscle soreness. A crossover study

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Applied Physiology, Nutrition, and Metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID</td>
<td>apnm-2018-0801.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>19-Dec-2018</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Guzmán-González, Benjamin; Universidad de Chile, Physical therapy Llanos, Pablo; Universidad de Chile, Physical therapy Calatayud, Joaquín; Universitat de Valencia, Department of Physiotherapy Maffiuletti, Nicola A.; Neuromuscular Research Laboratory, Schulthess Clinic Cruz-Montecinos, Carlos; Universidad de Chile, Physical therapy</td>
</tr>
<tr>
<td>Keyword:</td>
<td>electrical stimulation, blood glucose, muscle soreness, glycemic control, muscle damage, hyperglicemia</td>
</tr>
<tr>
<td>Is the invited manuscript for consideration in a Special Issue? :</td>
<td>Not applicable (regular submission)</td>
</tr>
</tbody>
</table>
Original Research Article

The effect of neuromuscular electrical stimulation frequency on postprandial glycemia, current-related discomfort, and muscle soreness. A crossover study.

Benjamín Guzmán-González¹, PT; Pablo Llanos², BSc; Joaquín Calatayud³, PhD; Nicola A. Maffiuletti⁴, PhD; Carlos Cruz-Montecinos⁵, PT, MSc

¹Laboratory of Clinical Biomechanics, Department of Physical Therapy, Faculty of Medicine, University of Chile, Santiago, Chile.
²Department of Physical Therapy, Faculty of Medicine, University of Chile, Santiago, Chile
³Exercise Intervention for Health Research Group (EXINH-RG), Department of Physiotherapy, University of Valencia, Spain; National Research Centre for the Working Environment, Copenhagen, Denmark
⁴Human Performance Lab, Schulthess Clinic, Zurich, Switzerland (NAM).
⁵Department of Physical Therapy, Faculty of Medicine, University of Chile, Santiago, Chile; Laboratory of Clinical Biomechanics, Department of Physical Therapy, Faculty of Medicine, University of Chile, Santiago, Chile; Laboratory of Biomechanics and Kinesiology, San José Hospital, Santiago, Chile

All correspondence and requests for reprints should be addressed to Carlos Cruz-Montecinos, PT, Laboratory of Clinical Biomechanics, Department of Physical Therapy, Faculty of Medicine, Universidad de Chile, Santiago, Chile. E-mail address: carloscruz@uchile.cl. Telephone number: (+56) 987687325
No conflicts of interest are reported by the authors or by any individuals in control of the content of this article.

ORCID:

BG: 0000-0002-5119-5106
CC: 0000-0002-3835-3368
JC: 0000-0002-8670-8346
NM: 0000-0001-5670-286X
Abstract

Consensus is lacking regarding optimal neuromuscular electrical stimulation (NMES) parameters for postprandial glycemic control. Therefore, the aim of this study was to determine the NMES frequency inducing the greatest hypoglycemic effect in healthy individuals. The secondary aim was to compare current-related discomfort and muscle soreness between different frequencies. We conducted an experimental clinical study with a randomized crossover design. Sixteen healthy and sedentary participants received NMES for 20 min at 5, 10, or 50 Hz (pulse duration: 400 µs, on-off ratio: 4:12 s) following a standardized meal. Glycemia, discomfort and muscle soreness during and after NMES were compared between conditions. 5-Hz NMES generated a significant hypoglycemic effect, contrary to 10 Hz and 50 Hz. 10-Hz and 50-Hz NMES resulted respectively in lower current-related discomfort and greater muscle soreness compared to the other frequencies. Women reported higher discomfort than men. These findings contribute towards the possibility of more efficient long-term NMES treatments in terms of glycemic response and patient tolerance.

Keywords: electrical stimulation, blood glucose, muscle soreness, glycemic control, muscle damage, hyperglycemia

Abbreviations:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>NMES</td>
<td>Neuromuscular Electrical Stimulation</td>
</tr>
<tr>
<td>MVC</td>
<td>Maximal Voluntary Contraction</td>
</tr>
</tbody>
</table>
**Introduction**

Neuromuscular electrical stimulation (NMES) consists of delivering electrical stimuli to muscles via skin electrodes to generate muscle contractions. It is mainly used for maintaining/restoring/improving neuromuscular function during/after disuse (Maffiuletti et al., 2018), with metabolic effects that are often overlooked (Nussbaum et al., 2017). Thus, NMES therapy is a potentially effective rehabilitation strategy for adults with advanced disease, such as chronic obstructive pulmonary disease, chronic heart failure, thoracic cancer, and orthopedic patients (Nussbaum et al., 2017). NMES parameters such as current amplitude, duration, frequency, and duty cycle can be modulated with marked effects over evoked force, discomfort, and fatigue (Glaviano & Saliba, 2016).

NMES metabolic effects include improvements in whole-body glucose uptake and lower glucose levels in healthy (Hamada et al., 2004) and diabetic individuals (Miyamoto et al., 2012; Van Buuren et al., 2015). In the same way, NMES has also been shown to improve insulin sensitivity in patients with type 2 diabetes (Joubert et al., 2014), and to decrease IL-6 in patients with fatty liver disease (Kawaguchi et al., 2011). In addition, increased oxygen demand, increased cardiorespiratory capacity, and decreased HbA1c levels have been observed after NMES training in diabetic patients (Van Buuren et al., 2015). Nevertheless, the current characteristics that generate the strongest hypoglycemic effect are not clear, and more particularly so for stimulation frequency (Hamada et al., 2004; Miyamoto et al., 2012; Van Buuren et al., 2015). Furthermore, how these parameters impact on patient-reported discomfort or soreness (i.e. the more common side effects of NMES) is unknown.

Even if greater muscle damage/soreness and fatigue are usually observed for frequencies \( \geq 50 \) Hz (Black & Mccully, 2008; Jubeau et al., 2008), there is little information on how current-related discomfort or muscle soreness are influenced by stimulation frequency/amplitude.
(Maffiuletti, 2010), particularly in relation to gender, since women have lower thresholds of sensory perception than men (Maffiuletti et al., 2008). Notwithstanding, too much discomfort could negatively affect glycemia levels (Moreau et al., 1995) and patient-reported tolerance/adherence, thus impacting the viability of administering NMES protocols with the required parameters over the long term.

Postprandial glycemic control is notably important for healthy subjects, particularly as related to preventing diabetes, obesity, and cardiovascular diseases. In addition, this could be particularly useful for the clinical community, since maintained hyperglycemia provokes insulin resistance, a pro-inflammatory environment, and high oxidative stress (Giri et al., 2018). Indeed, cardiovascular risks increase progressively with postprandial glycemia levels (characterized by a rapid, large and sustained increase in blood glucose levels), negatively impacting their evolution, especially for non-diabetic patients (Hsu, 2012). This is even more important for hospitalized patients whose physical activity is often limited or null, such as patients under prolonged bed rest. Due to these factors, their glycemic control is worsened (Dirks et al., 2016). Treatment is usually limited to some drugs and a strict diet, although this is often unable to terminate progression and damage from complications.

Therefore, the primary aim of this study was to compare the glycemic response to postprandial NMES exercise at three different frequencies (5, 10, and 50 Hz) in healthy individuals. A secondary aim was to compare current-related discomfort and muscle soreness induced by NMES. We used healthy subjects because their glucose metabolism is not affected by any pathology, nor is the discomfort/soreness they may perceive. These results may serve as a reference for both healthy subjects and patients with impaired glucose metabolism and consumption (Dirks et al., 2016) to propose new and effective methods to control glycemia with less discomfort and better adherence. Based on the already-documented proportionality between
stimulation frequency and muscle fatigue (Gregory et al., 2007), it was expected that 5-Hz NMES would result in the greatest hypoglycemic effect while 50-Hz NMES would provoke the lowest hypoglycemic effect and the highest levels of discomfort and soreness.

**Materials and methods**

**Participants**

The sample consisted of healthy subjects who voluntarily participated in this crossover clinical study. The inclusion criteria were as follows: aged 18 to 30, sedentary lifestyle (as verified with the long-form International Physical Activity Questionnaire; Craig et al., 2003) and able to understand and provide informed consent. Exclusion criteria were as follows: body mass index < 18.5 or > 35 kg/m², glycemia levels > 126 mg/dL in fasting or >200 mg/dL two hours after eating (considering these limits are related to a risk for diabetes; American Diabetes Association, 2014), metabolic diseases, use of any drugs that could affect glycemia levels, and any condition with a contraindication for NMES (such as skin infection/inflammation, pregnancy, or use of a cardiac pacemaker), compromised consciousness or an inability to understand instructions, deep vein thrombosis or active thrombophlebitis, or radiotherapy treatment over the stimulation area within the last six months (Rennie, 2010). All the procedures were performed in accordance with the Declaration of Helsinki after written informed consent was individually obtained. Approval for this study was granted by the Ethical Committee of the Northern Metropolitan Health Service (Santiago, Chile).

Regarding sample size, no prior research was found that provided the standard deviation for changes in glycemia induced by NMES in healthy subjects. Therefore, an NMES study conducted on diabetic carriers of Diabetes Mellitus 2 was selected (Sharma et al., 2010). The
sample size equation for comparing two means was used (Fernández, 1996), with an alpha error of 5%, a statistical power of 90% and the desired change of 21.5 ± 18.5 mg/dL. The resulting sample size was 16.

**Experimental protocol**

The effect of postprandial NMES exercise on glycemia, current-related discomfort and muscle soreness were compared between three different NMES frequencies. All subjects attended four separate sessions separated by one week, all of which began at 9:00 am in a fasting state. They were instructed to refrain from sports and from drinking caffeine or alcohol 24 h prior to and 72 h after each intervention. The first session (control condition) evaluated the glycemic response following digestion of the provided meal (Table 1), without the application of NMES. In the second, third, and fourth sessions (experimental conditions), postprandial glycemia was evaluated during and after each NMES protocol, which was randomly presented 30 min after the provided meal.

After the first session, the maximal voluntary contraction (MVC) force of the knee extensor muscles was measured separately for each leg according to the methodology described by Medeiros et al. (2015). Briefly, a load cell (resolution: 0.2 N, sampling rate: 500 Hz; FMON-1, ArtOficio, Chile) was placed behind the leg, approximately 3 cm from the lateral malleolus, using a rigid ankle brace. Measurements were taken with the subject in a sitting position, with the hip at 90° and both knees at 80° of flexion. Subjects first performed 20 submaximal contractions, followed by a 3-min rest period. Then, they were asked to complete 3 MVC of 5 s, with an interval of 3 min in between. Visual feedback of the exerted force was provided, and subjects also received verbal encouragement to exert the maximum force. Only the highest MVC force value was retained.
NMES exercise

Conventional NMES (pulse duration: 400 µs; on-off ratio: 4-12 s) was delivered for 20 min with the Quattro™ II (Compass Health Brands™, USA) device at three different frequencies: 5, 10, and 50 Hz. During NMES, subjects were in a supine position with both knees at 30° of flexion. The skin was first cleaned with alcohol, then two rubber electrodes (8.9 x 6.4 cm; Compass Health Brands™, USA) previously covered by wet sponges, were firmly secured with straps (so as to diminish burn risks; Nussbaum et al., 2017) and placed on each quadriceps according to the atlas of motor points established by Botter et al. (2011). Specifically, one electrode was placed on the proximal motor point of the rectus femoris, and the other electrode was placed on the distal motor point of the vastus medialis.

For each side, the current intensity was individually and rapidly adjusted to evoke at least 10 twitches of ~10% MVC, as assessed by the load cell. Due to the difficulty to assess knee extension force in the supine position, this procedure was completed before each NMES protocol in the same position used for the MVC assessment, and then it was reproduced with the legs resting without restraints over a soft wedge at 30° knee flexion. We used this supine position because it was comfortable for the subjects, it may refrain from an excessive amount of energy consumption and also to mimic a bed rest context. The aimed contraction level (~10% MVC force) was chosen because it was tolerated by all participants, contrary to a previously-tested higher intensity (30% MVC force), which was not tolerated by some of the participants, as also reported by Wiest, Bergquist, & Collins (2017).

Assessments
Glycemia was measured using a portable capillary glucometer (Freestyle Optium™, Abbott Diabetes Care™ Ltd., USA), which has been shown to be quick to administer, minimally-invasive, comfortable for users, and has a clinical accuracy comparable to laboratory methods using blood samples (Parwaiz et al., 2014). After cleaning the area with alcohol, a drop of blood was obtained by lancing the side of the fingertip. Glycemia measurements were taken by one of the researchers at the start of the NMES protocol (0 min), halfway through the NMES protocol (10 min), at the end of the NMES protocol (20 min), and 30, 60, and 90 min after the end of NMES. For the control condition, measures were taken at equivalent time points (i.e., 0, 10, 20, 50, 80, and 110 min). Three measurements were taken at each time point and averaged together.

Current-related discomfort was quantified using an 11-point verbal numerical rating scale graded from 0 to 10, where 0 represented “no discomfort” and 10 “intolerable discomfort”. Participants were asked to rate their sensation of discomfort when the 10% MVC force level was achieved (~0 min, the start of NMES), halfway through the NMES protocol (10 min), and when the NMES protocol was about to end (~20 min).

Muscle soreness was evaluated using a visual analog scale extending 50 mm, where the extreme left (0 mm) indicated “no pain” and the extreme right (50 mm) “extreme pain.” Participants were asked to rate their level of muscle soreness when performing self-palpation of each quadriceps, at the site of NMES application, with all five fingers exerting a constant pressure for 3 s, as described by Jubeau et al. (2008; i.e., sitting with hips at 90°, legs hanging, and 90° of knee flexion). This procedure was completed 90 min, 24, 48, and 72 h after each NMES session.

Statistical analyses
Data normality was verified with Shapiro-Wilk tests. Glycemia, discomfort and muscle soreness were analyzed using three-way repeated measures ANOVAs (condition × time × gender) followed by Bonferroni post hoc tests. Current intensity was analyzed using a one-way ANOVA. Partial eta squared ($\eta^2_p$) values were also calculated to determine the proportional effect of individual factors on glycemia, discomfort, and muscle soreness. Small, moderate and large effects were respectively defined as $\eta^2_p \geq 0.01$, 0.06 and 0.14 (Lakens, 2013). The level of significance for all tests was set at $p < 0.05$.

Results

Sixteen participants (8 women; age: 20.8 ± 2.4 years; BMI: 22.7 ± 2.8 kg/m²; height: 168.6 cm ± 11.4) were enrolled and successfully completed the study. There were no drop outs, and all subjects accomplished all testing sessions without any complications. No serious adverse events were recorded.

Glycemia

No significant condition x time x gender interaction was observed for glycemia. On the other hand, a significant main effect of condition ($p = 0.026; \eta^2_p = 0.40$) was observed. Regardless of time point and gender, glycemia was significantly lower for 5 Hz compared to control ($p = 0.016; \eta^2_p = 0.64$) and gender effect ($p = 0.002; \eta^2_p = 0.77$), and the condition by time interaction was also significant ($p = 0.005; \eta^2_p = 0.39$). In the control condition, glycemia was significantly reduced at the 50 and 110 min time points compared to baseline ($p = 0.011$ and $p = 0.001$, respectively). In the 5 Hz condition, glycemia was significantly reduced after 10 min (-13%; $p = 0.010$) and 20 min (-18%; $p = 0.041$) of NMES compared to baseline. Glycemia was basically unchanged
during the 10 Hz and 50 Hz protocols. After 10 min of NMES, glycemia was significantly lower at 5 and 50 Hz compared to control (p = 0.013 and p = 0.041, respectively). After 20 min of NMES, glycemia was significantly lower at 5 Hz compared to all the other conditions (p = 0.044-0.007; Figure 1).

**Discomfort**

No significant condition x time x gender interaction was observed for current-related discomfort. On the other hand, a significant main effect of condition (p = 0.013; η²p = 0.46) was observed. Regardless of time point and gender, discomfort was significantly lower for 10 Hz compared to 5 Hz (p = 0.044; Figure 2, small panel). We also observed a significant time (p < 0.001; η²p = 0.85) and gender (p = 0.010; η²p = 0.63) effect, with women showing significantly higher discomfort scores than men (p = 0.010). The interaction between condition and time was also significant (p = 0.006; η²p = 0.52). The discomfort induced by 50 Hz NMES was significantly higher compared to 10 Hz at the onset of exercise (p = 0.035), but thereafter decreased significantly (p < 0.001) and was comparable between all conditions (Figure 2).

**Muscle soreness**

No significant condition x time x gender interaction was observed for muscle soreness. On the other hand, a significant main effect of condition (p = 0.011; η²p = 0.59) was observed. Regardless of time point and gender, soreness was significantly higher at 50 Hz compared to the other frequencies (p = 0.031 for 5 Hz and p = 0.04 for 10 Hz; Figure 3, small panel). We also observed a significant time effect (p < 0.001; η²p = 0.62), and the condition by time interaction was also significant (p = 0.048; η²p = 0.34). Muscle soreness induced by 50 Hz NMES was
significantly higher compared to 5 Hz 24 h after the NMES session (p = 0.031), but thereafter decreased (p = 0.026) and was not significantly different from the other conditions (Figure 3).

**Current intensity**

No significant condition x gender interaction was observed for current intensity. On the other hand, a significant main effect of condition (p < 0.001; η²p = 0.9) was observed. Higher intensities were found for 5 Hz (mean 71.7 mA) compared to all other frequencies (10 Hz: mean 39.6 mA, p < 0.001; 50 Hz: mean 26.3 mA, p < 0.001). The intensity was also lower for 50 Hz compared to 10 Hz (p < 0.001).

**Discussion**

The primary objective of this study was to determine the frequency that would result in the greater hypoglycemic effect as a result of postprandial NMES exercise in healthy participants. The secondary aim was to also compare current-related discomfort and delayed onset muscle soreness (i.e., the main side effects of NMES) between different frequencies. Our main findings show that (1) NMES applied at 5 Hz generated a significant hypoglycemic effect, contrary to the 10 and 50 Hz conditions; (2) NMES applied at 10 Hz provoked less discomfort than 5 Hz (overall) and 50 Hz (onset), with women reporting higher discomfort scores than men; (3) NMES applied at 50 Hz induced greater levels of muscle soreness than 5 Hz and 10 Hz NMES; (4) NMES applied at 5 Hz required higher intensities to evoke the same force as 10 Hz and 50 Hz; 10 Hz required higher intensities than 50 Hz.

Our results regarding glycemia concur with the findings of Miyamoto et al. (Miyamoto et al., 2012), who reported a decrease in the postprandial glycemia of diabetic patients 30-90 min after stimulation and in the absence of muscle-damage indicators in the application of NMES at 4
Hz. As in the previously-cited study, our results regarding glycemia could be explained by a combination of high carbohydrate usage during muscle contraction during NMES at 5 Hz and the lower fatigue provoked by the unfused twitches of 5 Hz versus the partial (i.e., when twitch force does not return to zero before the next one) or complete fused twitches that occur with frequencies > 5 Hz, (Minogue et al., 2013) thus producing significant and sustainable levels of carbohydrate utilization during stimulation. On the other hand, and in contrast to the above-cited study, in the present investigation, we only observed a significant hypoglycemic effect during the application of 5 Hz NMES, but an increase in glycemia 30 min following exercise (see Fig. 1, 5 Hz condition). We believe this may be explained by the normal action of glucagon, i.e. glucagon levels increase during/after exercise as a way of providing energy and recovering consumed muscle glycogen (Ramnanan et al., 2011). Under this scenario, if muscle contractions are suddenly discontinued when NMES is stopped, the glucose liberated by the glucagon action would lead to a progressive increase in glycemia. It is worth mentioning that, in the present study, we evaluated sedentary, healthy subjects, in contrast to previous reports. For example, Miyamoto et al. (Miyamoto et al., 2012) applied an NMES protocol in diabetic patients receiving pharmacological treatments; this may have increased and prolonged the hypoglycemic effects of the tested NMES protocol, thereby preventing the post-exercise peak in glycemia we observed. In addition, the use of NMES with higher frequencies increases catecholamine levels (Moreau et al., 1995) and results in greater levels of muscle fatigue (Black & McCully, 2008; Jubeau et al., 2008), therefore we could expect less intense contractions by the end of the stimulation and a hyperglycemic response from catecholamines. These phenomena might explain, at least partly, why the hypoglycemic effect was exclusively observed at the lowest NMES frequency in the current study.
Postprandial glycemic control is notably important for healthy subjects, particularly as related to preventing diabetes, obesity, and cardiovascular diseases. As such, the obtained results could serve for optimizing glycemic control in both healthy and hospitalized individuals, thereby attenuating the risks associated to obesity, diabetes, cancer, cardiovascular disease, infection, wound healing, or other organ dysfunctions (Giri et al., 2018).

Regarding current-related discomfort, the 10 Hz protocol resulted in lower self-reported discomfort scores than 5 Hz (overall) and 50 Hz (NMES onset), with women reporting higher discomfort than men regardless of NMES frequency and time point. Since NMES discomfort depends on factors such as contraction intensity and afferent nerve stimulation (Delitto A et al., 1992), we believe that 5 Hz provoked more discomfort than 10 Hz because of its high current amplitude (p < 0.001), thereby contracting more and deeper fibers with more intensity, and highly stimulating afferent nociceptors. Somehow, in the same way, the high degree discomfort observed with 50 Hz during onset may be the result of the high-intensity tetanic contraction. On the other hand, the decrease in discomfort only observed during the 50 Hz stimulation compared to the other frequencies could be at least partly explained by the accelerated fatigue onset at 50 Hz, diminishing the contraction intensity and thus the perceived discomfort. The difference between genders matches findings where women showed a lower threshold of sensory perception than men (Maffiuletti et al., 2008), probably as a result of having greater epidermal nerve fiber density, as found by Gøransson (2004), thus affecting their tolerance to NMES.

Regarding delayed onset muscle soreness (24, 48 and 72 h after NMES), the 5 and 10 Hz protocols caused minimal soreness while 50 Hz NMES induced higher levels of soreness than 5 and 10 Hz, particularly 24 h post-stimulation. These results concur with the findings of previous studies on signs and symptoms of muscle damage up to 72 h after NMES at frequencies ≥ 50 Hz (Black & Mccully, 2008; Jubeau et al., 2008) but not, or less, at lower stimulation frequencies.
NMES is known to cause muscle damage and soreness, even more than voluntary exercise at the same exercise intensity (Jubeau et al., 2008), probably as a result from the superficial, synchronous, and spatially fixed recruitment provoked by NMES (Jubeau et al., 2008; Maffiuletti, 2010), imposing high metabolic demand and mechanical stress in muscle fibers. While not evaluated in our study, the differences between frequencies may reside on the tetanic contraction induced by the 50 Hz protocol; with lower intensities and higher frequency, we speculate that a more fatigable and superficial activation with fewer muscle fibers recruited may result, thus generating more contractile stress over the fibers and the extracellular matrix as seen by Mackey et al. after a single NMES bout (Mackey et al., 2011), thus provoking more muscle damage and soreness.

To our knowledge, this is the first study to compare the hypoglycemic effects and current related discomfort/soreness of three different frequencies in healthy patients. Moreover, the results will allow physiotherapists to apply NMES in a substantiated and safe manner, in addition to more precisely informing patients about the effects that NMES exercise may produce during and after postprandial stimulation for glycemic control. This will likely facilitate long-term applications with acceptable patient tolerance and significant improvements.

Like most experimental studies, this study is not without limitations. Since we only considered healthy volunteers, any extrapolation of the results to patient populations must be done with care. Furthermore, our findings could serve as a basis for conducting similar studies in diabetic individuals. In any case, more research is needed to clearly establish the link between the glycemic response and possible side effects of NMES, such as current-related discomfort and muscle soreness. For example, measurements of blood catecholamine concentrations could have indicated relationships between discomfort and variations in the glycemic curve. Also, the body position and knee angle used to measure the intensity needed to stimulate at 10% MVC were
different from the position used to apply the protocol, and evoked force was not measured during stimulation. The main reason for this was the complexity of measuring knee extension force from a supine position. While stimulation began at 10% MVC force for all the frequencies, possible fatigue-related reductions in evoked force over time were not recorded. Such occurrence may have affected glucose use throughout the 20-min NMES protocols, particularly at the highest frequency. MVC assessments during the stimulation protocol may help modulating the current intensity as the force output decreases due to adaptation to NMES and neuromuscular fatigue. While a continuous stimulation pattern might have been better, we used a 1:3 (25%) duty cycle since several subjects may not have tolerated the 10- or 50-Hz protocol. Future studies should analyze the efficiency of NMES with different current intensities, muscles and duty cycles, also considering these options may reduce tolerance and/or accelerate fatigue compared to our protocol (Glaviano & Saliba, 2016; Minogue et al., 2013; Packman-braun, 2018).

NMES side effects include soreness, discomfort, fatigue, electrical shocks, rashes, burns, or increased swelling (Nussbaum et al., 2017; Rennie, 2010), but also hypo/hyperglycemia, especially on impaired glucose metabolism contexts. Continuous glucose monitoring (CGM) could be a useful alternative to explore safety during the hours following NMES as it would show in-time monitoring of the glycemic curve (Sacks et al., 2011).

In summary, the application of low-intensity NMES at a frequency of 5 Hz resulted in a significant hypoglycemic effect in healthy subjects, contrary to 10 or 50 Hz, while 10 Hz and 50 Hz NMES resulted, respectively, in lower current-related discomfort and greater muscle soreness compared to the other stimulation frequencies. Low-frequency protocols may facilitate long-term applications of NMES in the clinical setting, with the goal of promoting an optimal glycemic response with acceptable patient tolerance.
Acknowledgments

We thank our colleague from the Kinesiology Department of the University of Chile Rigoberto Moya (PT) for providing the NMES device and the electrodes, as well as his insight and expertise in the NMES field.
REFERENCES


Giri, B., Dey, S., Das, T., Sarkar, M., Banerjee, J., & Dash, S. K. 2018. Chronic hyperglycemia mediated physiological alteration and metabolic distortion leads to organ dysfunction, infection, cancer


Minogue, C. M., Caulfield, B. M., & Lowery, M. M. 2013. Whole body oxygen uptake and evoked knee torque in response to low frequency electrical stimulation of the quadriceps muscles: VO2 frequency response to NMES. *Journal of Neuroengineering and Rehabilitation,* 95(9), 1750–1758.
https://doi.org/10.1016/j.diabres.2012.01.006


https://doi.org/10.1177/0004563213487893

https://doi.org/10.1111/j.1463-1326.2011.01454.x

https://doi.org/10.3138/ptc.62.5


TABLES

TABLE 1. Nutrition facts for the meal provided to participants.

<table>
<thead>
<tr>
<th></th>
<th>Carbohydrates (g)</th>
<th>kCal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green apple (200 g)*</td>
<td>29</td>
<td>112</td>
</tr>
<tr>
<td>Two slices of bread (76 g)</td>
<td>41.3</td>
<td>192</td>
</tr>
<tr>
<td>Orange juice (200 mL)</td>
<td>11</td>
<td>54</td>
</tr>
<tr>
<td>Jam (45 g)</td>
<td>12.3</td>
<td>51</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>409</strong></td>
</tr>
</tbody>
</table>

*Carbohydrate values and calories were determined from Schmidt Hebbel et al. (Schmidt-Hebbel, Pennacchiotti, Masson, & Mella, 1992).
FIGURE LEGENDS

FIGURE 1 Glycemia by condition and time point (mean data and SD). The small panel shows the main condition effect (*5 Hz > control at p < 0.05). For the same condition, significant reductions with respect to baseline are indicated by * (p < 0.05). For a given time point, significant differences between conditions are indicated by † (p < 0.05).

FIGURE 2 Current-related discomfort by condition and time point (mean data and SD). The small panel shows the main condition effect (*10 Hz > 5 Hz at p < 0.05). For the 50 Hz condition, significant reductions with respect to baseline are indicated by * (p < 0.05). Significant differences at baseline between 10 and 50 Hz are indicated by † (p < 0.05).

FIGURE 3 Muscle soreness by condition and time point (mean data and SD). The small panel shows the main condition effect (*50 Hz > 5 and 10 Hz at p < 0.05). For the 50 Hz condition, significant reductions with respect to the 24 h time point are indicated by * (p < 0.05). Significant differences at the 24 h time point between 5 and 50 Hz are indicated by † (p < 0.05).
TABLES

**TABLE 1.** Nutrition facts for the meal provided to participants.

<table>
<thead>
<tr>
<th>Carbohydrates (g)</th>
<th>kCal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green apple (200 g)*</td>
<td>29</td>
</tr>
<tr>
<td>Two slices of bread (76 g)</td>
<td>41.3</td>
</tr>
<tr>
<td>Orange juice (200 mL)</td>
<td>11</td>
</tr>
<tr>
<td>Jam (45 g)</td>
<td>12.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
</tr>
</tbody>
</table>

*Carbohydrate values and calories were determined from Schmidt Hebbel et al. (Schmidt-Hebbel, Pennacchiotti, Masson, & Mella, 1992).*
FIGURE 1 Glycemia by condition and time point (mean data and SD). The small panel shows the main condition effect (*5 Hz > control at p < 0.05). For the same condition, significant reductions with respect to baseline are indicated by * (p < 0.05). For a given time point, significant differences between conditions are indicated by † (p < 0.05).
FIGURE 2 Current-related discomfort by condition and time point (mean data and SD). The small panel shows the main condition effect (*10 Hz > 5 Hz at p < 0.05). For the 50 Hz condition, significant reductions with respect to baseline are indicated by * (p < 0.05). Significant differences at baseline between 10 and 50 Hz are indicated by † (p < 0.05).
FIGURE 3 Muscle soreness by condition and time point (mean data and SD). The small panel shows the main condition effect (*50 Hz > 5 and 10 Hz at p < 0.05). For the 50 Hz condition, significant reductions with respect to the 24 h time point are indicated by * (p < 0.05). Significant differences at the 24 h time point between 5 and 50 Hz are indicated by † (p < 0.05).