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Soleus H-reflex modulation following transcutaneous high- and low-frequency spinal stimulation in healthy volunteers

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ABSTRACT

The main aim of this work was to investigate the difference in the excitability of the soleus H-reflex in healthy volunteers following spinal transcutaneous electrical nerve stimulation (TENS) and high-frequency alternating current (HFAC) at a frequency of 10 kHz applied at the lower thoracic spinal level (T10–T12). A double-blind, randomized, crossover, controlled clinical trial was designed. Participants received three randomized interventions (TENS, 10 kHz, and sham stimulation) during 40 min. The amplitude and latency of the soleus H-reflex were registered prior to, during, and 10 min following stimulation. Twenty-four participants completed the study. A significant inhibition of H-reflex amplitude was observed following transcutaneous spinal TENS (12.7%; 95% CI 1.5–22.2%) when compared with sham stimulation (5.5%; 95% CI 3.6–14.5%; $p = 0.03$). An increase in H-reflex latency was also observed following transcutaneous spinal stimulation at 10 kHz (2%; 95% CI 1.4–2.5%) as compared with sham stimulation (0.7%; 95% CI 0.07–1.3%; $p < 0.01$). No differences were found between TENS and 10 kHz for H-reflex modulation. Transcutaneous spinal TENS and HFAC at a frequency of 10 kHz had a modulatory effect on the soleus H-reflex when compared to sham stimulation; however, no differences were found between these two interventions.

1. Introduction

The Soleus Hoffmann reflex (H-reflex) is a monosynaptic reflex elicited by the stimulation of the tibial nerve, although a recent study suggests that polysynaptic components are also present (Burke, 2016). The H-reflex is evoked by the activation of group Ia afferent fibers from muscle spindles (Cramp et al., 2000), and its amplitude is determined by the excitability of spinal motoneurons, and by the activity of spinal interneuronal systems, such as presynaptic inhibitory mechanisms and spinal activity evoked by Ib afferent inputs (Schieppati, 1987). The H-reflex amplitude has been found to have a high intrasubject reliability (Hardy et al., 2002), and provides an objective measure that has traditionally been used to determinate the influence of short- and long-term interventions on spinal cord excitability (Cramp et al., 2000; Gómez-Soriano et al., 2012; Goulet et al., 1994; Hardy et al., 2002; Joodaki et al., 2001; Kim and Yoon, 2003; Oza et al., 2017; Walsh et al., 2000). The amplitude of the H-reflex mirrors the level of synchronized α-motoneuron activation, which is in turn modulated by several afferent fiber systems and which also depends on the number of motor units recruited by the external peripheral electrical stimulation. In certain neurological dysfunctions, such as spasticity, it has been shown that H-reflex amplitude is abnormally increased (Hardy et al., 2002; Little and Halar, 1985).

Typically, transcutaneous electrical nerve stimulation (TENS) has been used for the treatment of pain; however, certain studies have investigated its effectiveness on the excitability of the nervous system, due to the clinical usefulness of the treatment in spasticity (Fernandez-Tenorio et al., 2016). TENS can be applied both peripherally (Aydin et al., 2005; Bajd et al., 1985; Cho et al., 2013; Goulet et al., 1996; Goulet et al., 1994; Hardy et al., 2002; Joodaki et al., 2001; Kim and Yoon, 2003; Levin and Hui-Chan, 1992; Martins et al., 2012; Ng and Hui-Chan, 2007; Potisk et al., 1995; Walsh et al., 2000) and spinally (Hofstoetter et al., 2014; Serrano-Munoz et al., 2017; Simorgh et al., 2008), with peripheral stimulation being the most studied. Although there are many modalities of TENS, it is usually applied at a frequency of 50–150 Hz and an intensity below the motor threshold (Fernandez-Tenorio et al., 2016). Accordingly, some studies have addressed the effect of TENS on H-reflex modulation in both healthy volunteers

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(Cramp et al., 2000; Goulet et al., 1994; Hardy et al., 2002; Joodaki et al., 2001; Kim and Yoon, 2003; Serrano-Munoz et al., 2017; Simorgh et al., 2008; Walsh et al., 2000) and subjects with spasticity (Aydin et al., 2005; Goulet et al., 1996; Levin and Hui-Chan, 1992; Martins et al., 2012). When TENS is applied over the peripheral nerve to modulate the H-reflex, the main effect after the stimulation is a decrease in the H-reflex amplitude (Aydin et al., 2005; Hardy et al., 2002; Joodaki et al., 2001; Levin and Hui-Chan, 1992). There is little evidence regarding spinal TENS application, and to date, only two studies (Serrano-Munoz et al., 2017; Simorgh et al., 2008) have assessed the soleus H-reflex following spinal TENS, in which a decrease in H-reflex amplitude was observed that led to lower excitability. Hofstoetter et al. (2014) applied spinal TENS in incomplete spinal cord injury patients with spasticity and observed a decrease in spasticity values and an increase in gait speed. It has been suggested that transcutaneous spinal stimulation can modify the excitability of neural circuits and cause synaptic interactions of afferent fibers in the dorsal column, resulting in the inhibition of type I motoneurons (alpha motoneurons) (Hofstoetter et al., 2014; Simorgh et al., 2008).

In recent years, studies have applied high-frequency alternating current (HFAC) without modulation to produce a fast, reversible nerve conduction block. Several animal studies have demonstrated that nerve excitability is decreased or abolished at the level of the stimulated area (Avendaño-Coy et al., 2018). Other studies have demonstrated that the optimal frequency for conduction block is approximately 4–5 kHz (Bhadra and Kilgore, 2005; Boger et al., 2008; Gaunt and Prochazka, 2009; Joseph and Butera, 2011; Tai et al., 2005; Waataja et al., 2011). There have been very few studies focusing on peripheral transcutaneous HFAC application in humans. It has been shown that the stimulation frequency needed to achieve a nerve block depends on the nerve diameter (Avendaño-Coy et al., 2018). While Avendaño et al., found a similar effect between 5 kHz-HFAC and TENS with respect to increasing somatosensory thresholds (Avendaño-Coy et al., 2017), a recent study published by our group has shown a higher decrease in handgrip strength following 10-kHz intervention as compared with 5 kHz and sham stimulation (Serrano-Munoz et al., 2018). There exist no human studies that have applied spinal HFAC transcutaneously; however, certain studies have applied HFAC at a frequency of 10 kHz using epidural electrodes to reduce lower back and leg pain (Kinfe et al., 2016; Reddy et al., 2016), but no neurophysiological measures were assessed. It has been hypothesized that the reversible nerve conduction block produced by the application of HFAC could be helpful to reduce the undesirable increase in neural activity in clinical conditions such as spasticity or chronic pain (Tai et al., 2011).

In the present study, we hypothesize that the transcutaneous spinal application of HFAC at 10 kHz would have a larger modulatory effect on soleus H-reflex than TENS and sham stimulation. The frequency of 10 kHz was chosen based on the effect of this stimulus on peripheral nerve activity (Ackermann et al., 2009; Gerges et al., 2010; Tai et al., 2004, 2005) and also on the effect of epidural stimulation of the human spinal cord (Kinfe et al., 2016; Reddy et al., 2016). No study has compared the effect of TENS and HFAC using the transcutaneous spinal stimulation technique; therefore, the purpose of the present study was to compare the neurophysiological effects of TENS and HFAC at a frequency of 10 kHz by transcutaneous spinal stimulation on soleus H-reflex excitability in healthy volunteers as compared with sham stimulation. The idea of comparing both stimulation techniques was based on the need to contrast the efficacy of a classic intervention (TENS) with the efficacy of the new HFAC paradigm of intervention.

2. Methods

2.1. Design

A double-blind, randomized, crossover, controlled clinical trial was designed. Participants received three randomized (www.randomizer.org) interventions (TENS, 10 kHz, and sham stimulation) at the thoracic spine level. The duration of the intervention was 40 min, and three measurements of the H-reflex were recorded throughout the experimental session: (i) prior to the intervention, (ii) during the intervention at min 33, and (iii) 10 min following the intervention (Serrano-Munoz et al., 2017). A stimulation duration of 40 min was selected because this reflected a minimum of 30 min which was the time expected to produce an effect (Fernandez-Tenorio et al., 2016), and an additional 10 min to perform measures "during the intervention"..

A washout period of 48 h was selected between interventions, since the stimulation was higher than that used in similar previous studies (Aarskog et al., 2007; Buonocore and Camuzzini, 2007; Chen and Johnson, 2009; Dean et al., 2006). The present study was approved by the local Toledo Ethical Committee (Ref. No. 23; 18/02/2011) and the clinical trial was registered in the ClinicalTrials.gov Protocol Registration System (NCT02718989).

2.2. Subjects

The sample size was calculated based on H-reflex latency as the main variable. When a mean difference of 0.4 ms with a standard deviation of 0.4 ms was taken into account, with a type I error (α) of 0.05 and a power of 80%, a sample size of 20 subjects was calculated. However, to account for possible subject and data loss, the sample was increased by 20% (n = 24) (Joodaki et al., 2001)). Twenty-four healthy volunteers ≥ 18 years old, without any problems of the central or peripheral nervous systems, were recruited via non-probabilistic convenience sampling. Exclusion criteria were musculoskeletal pathology in the lower limbs, inability to tolerate electrical current, and allergy to the electrode material. Volunteers were informed of the protocol and signed the informed consent approved by the local ethics committee.

2.3. Procedures

The procedures were similar to a previous study performed by our group (Serrano-Munoz et al., 2017). Participants were placed in the prone position with the right lower limb at 120° knee flexion. Two self-adhesive surface electrodes (9 × 5 cm) (ValuTrode, Axelgaard Manufacturing Co, LTD, Fallbrook, USA) were fixed to the back, over the soleus metamere (S1 − S2), which corresponded to bone level T10 − T12. The stimulator used for the three interventions was an Enraf Nonius stimulator, Myomed 932, the Netherlands, which was calibrated using a digital oscilloscope before the stimulation session.

TENS stimulation: The therapist applied a symmetrical biphasic current of 200 µs pulse-width at a frequency of 100 Hz. The intensity was set to a sensation of "strong but comfortable, just below the motor threshold", and as such, the intensity was gradually increased until a minimally visible contraction was observed and subsequently decreased until it disappeared. This sensation remained throughout the session. To avoid habituation to the stimulus, participants were asked every 2 min (Claydon et al., 2013) to corroborate the perceived sensation, and the intensity was increased if requested. Current density was calculated in units of mA/cm^2 as presented in our previous study (Serrano-Munoz et al., 2017).

10-kHz stimulation: The therapist applied a high-frequency sinusoidal current, without modulation, at a frequency of 10 kHz. The current intensity was adjusted as with TENS intervention and was also increased if required to avoid the habituation phenomenon.

Sham stimulation: Sham stimulation consisted of the same parameters used for TENS stimulation except for the intensity, which was increased until the sensory threshold and subsequently decreased to zero, where it remained until the end of the session. The therapist informed the participant that the intensity may be below the sensory threshold, with the possibility that the participant may or may not feel the current. This method has been previously validated as an appropriate sham (Deyo et al., 1990; Petrie and Hazleman, 1985).

2.4. Soleus H-reflex recording

The soleus H-reflex was elicited by 1-ms rectangular electrical pulses applied to the tibial nerve using a bipolar electrode placed in the popliteal fossa. Electromyographic activity (EMG) was recorded using bipolar silver chloride electrodes (×1000 amplification) and filtered by a built-in 20–450 Hz bandpass filter (Signal Conditioning Electrodes v2.3, Delsys Inc., USA). Electrodes were placed over the soleus muscle at 2/3 of the way between the medial condyles of the femur and the medial malleolus, following SENIAM recommendations. In order to control for movement of the stimulation and recording electrodes, the initial electrode position was marked on the skin with a non-permanent marker pen, with the location maintained throughout the entire protocol. EMG activity was sampled at 10 kHz (MicroPlus 1401, Cambridge Electronic Design). Maximal peak-to-peak amplitude of the H-reflex (Hmax amplitude) was recorded following 1-mA increments in intensity. The latency of the Hmax was also recorded as an outcome of the study. The H-reflex onset latency was determined at the time after the stimulus when evoked reflex EMG activity exceeded background activity by 15%. The maximal peak-to-peak amplitude of the M response (Mmax response) was also monitored using the same procedure as with Hmax.

2.5. Statistical analysis

Statistical analysis was performed using the commercial software package Sigmaplot 12.0 for Windows (Systat software, Inc., Germany). The Shapiro-Wilk test was performed to determine the Gaussian distribution of each group of data. The effect of each intervention was calculated by the difference between the respective data "during-test" and "post-test" minus the data obtained "pre-test". These outcomes were named the "during-test effect" and "post-test effect", respectively. A two-way repeated-measures ANOVA, with the "time" factor (during-test effect and post-test effect) and the "intervention" factor (TENS, 10 kHz, and sham stimulation) being performed to compare differences in Hmax amplitude, Hmax latency, and Mmax response. Bonferroni's post-hoc multiple comparison test was used to reveal specific differences. A p value of ≤ 0.05 was considered statistically significant.

3. Results

Twenty-four healthy volunteers completed the study. Fourteen were males (58%) and 10 were females (42%), with a mean age of 24.7 years old (SD 5.6), a mean weight of 69.4 kg (SD 12.5), a mean height of 1.71 m (SD 0.09), and a mean body mass index of 23.7 kg/m^2 (SD 3.4). Regarding current density for the same current perception, that received for the TENS intervention was significantly lower (1.31 mA/cm^2; SD 0.29) than that received for the 10-kHz intervention (2.12 mA/cm^2; SD 0.21) ($p < 0.001$). There was not any drop out in participants throughout the trial. The Mmax response remained stable in all trials performed. No significant differences were found in any comparison considering either the "time" factor (F (1,23) = 0.0; p = 0.97), the "intervention" factor (F (2,22) = 1.8; p = 0.18), or the "intervention-time" interaction (F (2,22) = 1.2; p = 0.32). Specifically, Mmax response was 1.55 mV (SD 0.97) before the TENS stimulation, 1.51 mV (SD 0.94) during TENS stimulation and 1.49 mV (SD 0.91) after TENS stimulation. For the 10 kHz stimulation, the Mmax response was 1.56 mV (SD 1.08), 1.62 mV (SD 1.13) and 1.63 mV (SD 1.14) respectively. Finally, for the sham stimulation intervention the Mmax response was 1.44 mV (SD 0.88), 1.45 mV (SD 0.87) and 1.47 mV (SD 0.87) for before, during and after stimulation respectively.

3.1. Effect on peak-to-peak soleus H_{max} amplitude

Fig. 1 illustrates an H-reflex recording of a typical subject and the effect following interventions. Table 1 shows the absolute Hmax amplitude and the effect of the interventions on the baseline recording. When absolute Hmax amplitude values at the baseline were compared among the interventions, no significant differences were found (p = 0.19). When the effects on Hmax amplitude were compared, no significant differences were observed for either the "intervention" factor (F (2,22) = 1.9; p = 0.17) or the "time" factor (F (1,23) = 0.02; p = 0.89). However, significant differences were observed for the "intervention-time" interaction (F (2,22) = 4.1; p = 0.03). When the post-test effect of TENS (-0.08 mV; 95% CI −0.14 to −0.01) was compared with the post-test effect of sham stimulation (0.03 mV; 95% CI −0.02 to 0.08), a greater inhibition of the Hmax amplitude was observed (p = 0.03), while no significant differences were observed in the comparison between 10-kHz and sham stimulation (p = 0.21) or between 10-kHz stimulation and TENS (p = 0.50).

3.2. Effect on H_{max} latency

Table 2 shows absolute Hmax latency values and the effect of the interventions on the baseline value. No significant differences were found in the baseline absolute Hmax latency values among interventions (p = 0.72). When the effects on Hmax latency were compared, no significant differences were observed for the "time" factor (F (1,23) = 3.8; p = 0.06). However, significant differences were observed for the "intervention" factor (F (2,22) = 5.98; $p < 0.01$) and the "intervention-time" interaction (F (2,22) = 3.69; p = 0.04). When the effects on Hmax latency were compared over time (post-test effect minus during-test effect), a significant increase of 0.23 ms (95% CI 0.08 to 0.39; $p < 0.01$) was observed in the 10-kHz stimulation, while in the TENS and the sham stimulation there were no differences; 0.0 ms (95% CI −0.14 to 0.13; p = 0.9) and 0.02 ms (95% CI −0.10 to 0.15; p = 0.68), respectively. When the during-test effects on Hmax latency were compared among interventions, no significant difference was observed; however, when the post-test effects were compared, a greater Hmax latency was observed in the 10-kHz stimulation (0.58 ms; 95% CI 0.41–0.76) than in the sham stimulation (0.20 ms; 95% CI 0.02–0.39; $p < 0.01$), whereas in the other comparisons between interventions, no significant differences were observed.

3.3. Adverse effects

No adverse effects were reported in any of the participants; thus, the stimulations were determined as safe procedures. However, 6 participants (25%) following the application of 10-kHz stimulation, 5 participants (21%) following TENS, and 2 participants (8%) following sham stimulation reported that the sensation of micturition had increased considerably in comparison with that prior to intervention.

4. Discussion

The present study shows that spinal TENS applied transcutaneously over the soleus metamere decreased the amplitude of the H-reflex, while spinal HFAC stimulation applied using the same procedures but at a frequency of 10 kHz significantly increased the latency of the H-reflex. Both findings were observed 10 min following intervention but not during the stimulation, as compared with sham stimulation. No differences were found between TENS and HFAC with respect to the latency or amplitude of Hmax. This is the first study to address the effects of spinal application of HFAC transcutaneously, and the results are in accordance with previous studies that implanted electrodes or stimulated over peripheral nerves (Avendaño-Coy et al., 2017; Fisher et al., 2015; Kinfe et al., 2016; Reddy et al., 2016).

Although the spinal approach could be useful due to its impact on certain central neural mechanisms, few studies have applied spinal TENS (Hofstoetter et al., 2014; Serrano-Munoz et al., 2017; Simorgh et al., 2008). The results of the present study are consistent with previous findings (Serrano-Munoz et al., 2017; Simorgh et al., 2008)

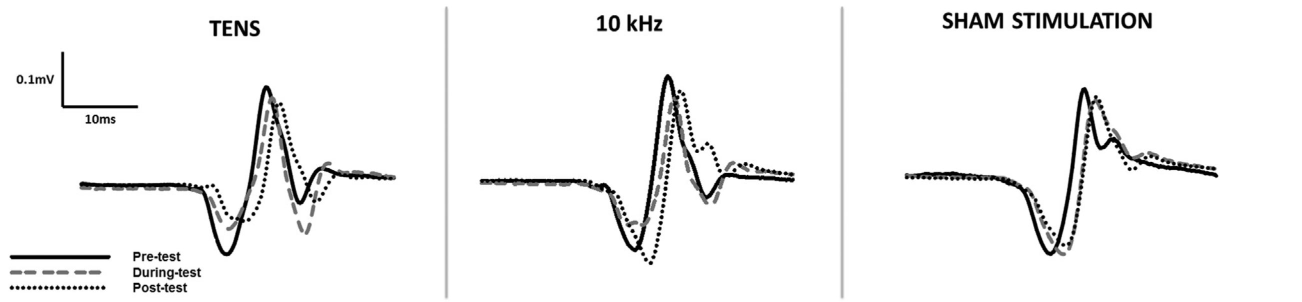

Fig. 1. H-reflex recording of a typical subject and the effect following interventions.

showing a decrease in peak-to-peak soleus H-reflex amplitude following application of spinal TENS. Furthermore, Hofstoetter et al. (2014) demonstrated a decrease in the spasticity index and an improvement in gait speed following a 30-min spinal TENS (50 Hz and 200 µs pulse width) in subjects with incomplete spinal cord injury; however, neurophysiological measures were not recorded. Nevertheless, the present study found no differences in latency following the application of spinal TENS, which is consistent with a previous report by Martins et al., (Martins et al., 2012) following TENS applied over the dermatome S1 − S2 based on the intensity for sensory perception of the stimulus in participants with spasticity after stroke, using an adjustable frequency and pulse duration between 5 and 100 Hz, and 2 to 200 µs respectively. Peripheral application has demonstrated an increase in H-reflex latency (Cramp et al., 2000; Joodaki et al., 2001; Walsh et al., 2000); however, amplitude parameters of the H-reflex have been shown to be variable following TENS application (Goulet et al., 1994; Walsh et al., 2000). Interestingly, Goulet et al., observed no effect on the H-reflex amplitude following TENS stimulation (99 Hz and 250 µs pulse) (Goulet et al., 1994); however, Joodaki et al., found a decrease in the H/M ratio and an increase in latency after application of TENS (99 Hz and 250 µs pulse) (Joodaki et al., 2001). This variability could be due to several factors such as the TENS parameters chosen, or the intensity received. Hardy et al. (2002) showed that an increase in the H-reflex amplitude was inversely correlated with stimulation intensity, with lower intensities of TENS causing greater H-reflex amplitudes. This observation has been supported by several studies (Avendaño-Coy et al., 2017; Moran et al., 2011; Serrano-Munoz et al., 2017). Our previous study (Serrano-Munoz et al., 2017) found that TENS was only effective in inhibiting the H-reflex amplitude when greater current densities were applied. No difference was found in the soleus H-reflex amplitude when a current density of 0.71 mA/cm^2 was applied; however, a significant inhibition was observed when 1.30 mA/cm^2 was applied, the current density of which is very similar to that applied in the present study (1.31 mA/cm^2). The current density received in the 10-kHz intervention was 2.12 mA/cm^2, which is in accordance with our previous hypothesis that higher frequencies require higher intensities to achieve the same perceived current sensation, since the higher frequencies have less resistance to the skin and subcutaneous tissues (Avendaño-Coy et al., 2017; Beatti et al., 2011).

HFAC stimulation is known to produce a fast and reversible conduction block. The vast majority of studies have been developed using computer simulations or peripheral nerve animal models (Avendaño-Coy et al., 2018). In human studies, only Avendaño-Coy et al. (2017) recorded neurophysiological measures, assessing a compound action potential (CAP) prior to and 20 min following intervention. An increase in the CAP latency was observed only 20 min after stimulation with HFAC at 5 kHz over the radial nerve, when compared with sham stimulation; while TENS showed no significant difference. These results are in line with the increase in Hmax latency observed in the present study at 10 min after the HFAC applied at 10 kHz. The modification of nerve conduction can be supported by a change in the channel dynamics (Avendaño-Coy et al., 2018) as the main mechanism, as evidenced in previous preclinical studies (Tai et al., 2011; Wang et al., 2008; Zhang et al., 2006). However, the observed effect on CAP or Hmax latency after but not during the application of the stimulation, suggests the implication of unknown mechanisms that have not been identified as yet in preclinical studies, which have focused instead on temporary reversible blocking effects during the stimulation period. On the other part, the present study showed no effect on Hmax amplitude following the application of HFAC stimulation at 10 kHz, which was also reported by Avendaño-Coy et al. (2017) following peripheral application over the radial nerve.

The mechanisms involved during spinal stimulation with HFAC or the optimal frequency to achieve a nerve block are unknown. A study developed in primates (adult baboons) (Fisher et al., 2015) showed a transient near-complete block of pathways in the central nervous system following the application of a sinusoidal HFAC at 2 kHz through a single sharp metal microelectrode over the corticospinal tract at the cervical spinal cord. Similar results were found to previous studies performed on the peripheral nervous system (Ackermann et al., 2011; Tai et al., 2004). Although a complete block can be achieved under optimal conditions, it is important to be aware that variation in

Table 1
Maximal peak-to-peak soleus H-reflex amplitude (Hmax) following TENS, 10 kHz, or sham stimulation, and comparison among times and interventions.

	Intervention (mV) Mean (SD)			Difference within intervention (mV) Mean (95% CI)		Comparison between times (mV) Mean (95% CI)	Comparison between interventions (mV) Mean (95% CI)		
	Pre-test	During-test	Post-test	During-test effect	Post-test effect	During-test effect vs post-test effect		During-test effect	Post-test effect
TENS	0.63 (0.40)	0.59 (0.32)	0.55 (0.30)	−0.04 (−0.12 to 0.05)	−0.08 (−0.14 to −0.01)	−0.04 (−0.08 to 0.01)	TENS vs SHAM	−0.04 (−0.15 to 0.08)	**−0.11* (-0.19 to −0.02)**
10 kHz	0.61 (0.31)	0.59 (0.29)	0.59 (0.32)	−0.02 (−0.01 to 0.04)	−0.02 (−0.08 to 0.04)	0.00 (−0.04 to 0.05)	10 kHz vs SHAM	−0.02 (−0.1 to 0.06)	−0.05 (−0.11 to 0.02)
SHAM	0.55 (0.33)	0.55 (0.32)	0.58 (0.34)	0.00 (−0.05 to 0.05)	0.03 (−0.02 to 0.08)	0.03 (−0.01 to 0.07)	10 kHz vs TENS	0.02 (−0.11 to 0.15)	0.06 (−0.05 to 0.17)

During-test effect = during-test minus pre-test; post-test effect = post-test minus during-test; significant differences are represented in bold. *$p < 0.05$.

Table 2
Latency of apparition of the soleus H-reflex following TENS, 10 kHz, or sham stimulation, and comparison between times and interventions.

	Intervention (ms) Mean (SD)	Difference within intervention (ms) Mean (95% CI)		Comparison between times (ms) Mean (95% CI)			Comparison between interventions (ms) Mean (95% CI)		
	Pre-test	During-test	Post-test	During-test effect	Post-test effect	During-test effect vs post-test effect		During-test effect	Post-test effect
TENS	29.91 (2.2)	30.30 (2.2)	30.30 (2.1)	0.39 (0.24–0.56)	0.39 (0.23–0.55)	0.0 (−0.14 to 0.13)	TENS vs SHAM	0.21 (−0.02 to 0.45)	0.19 (−0.03 to 0.39)
10 kHz	29.95 (2.3)	30.30 (2.3)	30.53 (2.3)	0.35 (0.13–0.57)	0.58 (0.41–0.76)	**0.23*** **(0.08–0.39)**	10 kHz vs SHAM	0.17 (−0.12 to 0.45)	**0.38*** **(0.11–0.65)**
SHAM	29.98 (2.3)	30.16 (2.2)	30.18 (2.3)	0.18 (0.05–0.31)	0.20 (0.02–0.39)	0.02 (−0.10 to 0.15)	10 kHz vs TENS	0.04 (−0.4 to 0.3)	0.19 (−0.09 to 0.49)

During-test effect = during-test minus pre-test; post-test effect = post-test minus during-test; significant differences are represented in bold. $^*p < 0.05$.

electrode placement or interindividual differences may lead to incomplete block in certain cases. These factors could explain the absence of a change in the results regarding Hmax amplitude in the present study, since it was carried out transcutaneously and the distance between the electrode and the target tissue was higher and the skin and bone were present as a barrier.

It is known that a peripheral HFAC nerve block depends on the frequency applied (Ackermann et al., 2011). In peripheral applications with implanted electrodes in small mammals, frequencies lower than 10 kHz can be sufficient to achieve a peripheral nerve block (Bhadra and Kilgore, 2005; Gaunt and Prochazka, 2009; Tai et al., 2005, 2004). However, Ackermann et al. (2011), determined that the minimum frequency required to reach a peripheral nerve block using implanted electrodes in the median nerve of primates, the diameter of which is very similar to humans, was 20 kHz. For spinal stimulation, a frequency of 10 kHz was useful in two clinical trials in reducing lower back and leg pain; however, the application was implanted electrodes on the cord (Kinfe et al., 2016; Reddy et al., 2016). Further studies using transcutaneous spinal HFAC stimulation are required to assess different frequencies in order to support the nerve block observed in animal and human studies with the use of implanted electrodes and peripheral applications. A recent systematic review regarding spinal cord stimulation for the management of spasticity (Nagel et al., 2017) concluded that this line of research should be resurrected due to the improvements observed in the modulation of the motor component of the spinal cord, in addition to the many technological advances in recent years. This approach could afford the possibility of a potential use in humans for numerous pathologies that are characterized by an undesirable increase in nerve activity, producing pathological muscle activity or sensations, such as chronic or neuropathic pain and spasticity. The nerve block produced by HFAC stimulation is fast and reversible as compared with other treatments for nerve hyperactivity such as blocks by radiofrequency or injections of botulinum toxin. Unlike implanted electrodes, surgery is not necessary with transcutaneous electrodes, and there are less associated risks due to the non-invasive nature of the application.

The observations related to the micturition sensation in certain participants (although not significant) can be explained by the fact that the soleus metamere is the same as the bladder metamere, and the electrical current is deep enough to stimulate both systems. In addition, it is known that HFAC stimulation has the capacity to reach deeper tissues due to the low impedance of the skin and subcutaneous tissues (Beatti et al., 2011). This can support the feasibility of the transcutaneous approach to spinal cord stimulation due to the fact that the electrical current must have reached the target tissue. It is possible that higher frequencies reach deeper into the tissues than lower frequencies; however, there were no differences in the increase in micturition sensation between TENS and HFAC. Further studies addressing the depth of these electrical currents should be designed to clarify their mechanism of action and effectiveness.

Among the main limitations of this work, we must highlight the fact that although neurophysiological measures are the most relevant for addressing objective changes in nerve conduction, it is important to also assess other indirect clinical measures such as sensory thresholds and muscle strength, since it has been evidenced in pilot studies. Although sample size was calculated to attain a statistical power of 80%, an increased variability could mask additional statistical differences. However, the sample size is higher than similar studies in this field, where they used ten participants (Goulet et al., 1994; Joodaki et al., 2001; Simorgh et al., 2008). It is necessary to develop clinical trials in participants with chronic pain or spasticity to evaluate the therapeutic effect of HFAC currents. One important limitation of the present study is related to certain concerns regarding HFAC stimulation. Furthermore, the intensity limit of the device was 100 mA. For TENS intervention no participant reached this limit; however, in the 10-kHz intervention, 17 of 24 participants reached the intensity limit, thus the absence of higher effects could be related to this issue. Further studies should be performed using devices that allow a greater increase in current intensity.

5. Conclusions

Transcutaneous application of spinal TENS and HFAC stimulation at a frequency of 10 kHz have a modulatory effect on the soleus H-reflex following stimulation. Although no differences were found between TENS and HFAC, TENS reduced the amplitude of Hmax and HFAC showed a higher latency of soleus H-reflex as compared with sham stimulation. Future studies are required with a greater sample size to allow the detection of possible differences between HFAC and TENS intervention and to address the therapeutic feasibility of transcutaneous spinal stimulation by HFAC.

6. Author's contribution

DSM, EBE and IGA contributed in participant recruitment, data collection and drafting manuscript. JAC, JGS, GAM and JT conceiving study concept, results interpretation and supervised the whole project. All authors read and approved the final manuscript.

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Conflict of interest

Gómez-Soriano, Taylor, and Avendaño-Coy have a patent related to a device that evokes electrical current between 10 kHz and 50 kHz (Patent number: P201630168); although, this device has not used in the present study. The remaining authors have no conflicts of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jelekin.2019.03.004.

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