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Selective nociceptive modulation using a novel prototype of transcutaneous kilohertz high-frequency alternating current stimulation: a crossover double-blind randomized sham-controlled trial

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Abstract

Background Kilohertz high-frequency alternating current (KHFAC) stimulation has demonstrated to induce rapid and reversible nerve blocks without causing nerve damage. Previous studies have explored frequency-dependent effects using a transcutaneous approach in humans from 5 to 20 kHz. Nevertheless, its application in humans is limited by the lack of stimulators approved for frequencies above 20 kHz. Therefore, this study aimed to assess the effects and safety of transcutaneous KHFAC stimulation using a novel prototype stimulator, comparing interventions at 30, 40, and 50 kHz to sham stimulation on experimental pain, sensory, motor, and neurophysiological outcomes.

Methods A randomized, double-blind, sham-controlled crossover study involving 34 healthy participants was conducted. Four interventions (30, 40, 50 kHz, and sham) were administered, and stimulation was applied for 20 min to the median nerve of the non-dominant hand. A prototype stimulator capable of delivering frequencies between 1 and 50 kHz, with a maximum peak-to-peak output current intensity of 400 mA was designed. The intensity applied during the stimulation was below motor threshold, evoking a 'strong but comfortable' tingling sensation. Primary outcomes included heat pain threshold (HPT), pressure pain threshold (PPT), and adverse effects. The secondary outcomes included static two-point discrimination sensitivity, isometric pinch strength, and median sensory nerve action potential (SNAP).

Results Compared with the sham stimulation, all the active interventions exhibited a significantly greater increase in the PPT during and immediately after the stimulation, while only a significant increase was observed at 40 kHz (4.1 N/cm^2; 95%CI 0.3 to 7.9) at 15 min post-intervention. Compared to sham stimulation, the 40 kHz intervention had a significantly greater effect on the HPT at all time points, with the greatest difference (1.4 °C; 0.6 to 2.1) occurring immediately post-intervention. Adverse effects during active interventions included petechiae, erythema, and itching,

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which resolved at 24 h post-intervention. For secondary outcomes, only a significant reduction in the median SNAP velocity was observed in the sham stimulation group compared to the 50 kHz group.

Conclusions Active KHFAC stimulation, particularly at 40 kHz, delivered through a novel stimulator, effectively increased the PPT and HPT without affecting tactile or motor outcomes, inducing mild skin-related adverse effects. These findings have potential implications for people with pain-related pathologies.

Trial registration NCT05230836.

Keywords Kilohertz high-frequency alternating current, Nerve block, Peripheral nerve, Pain, Prototype

Background

Nociceptors are specialized peripheral sensory neurons responsible for detecting harmful mechanical, thermal, or chemical stimuli, which could trigger pain as a protective response to potential or actual tissue injury [1]. In healthy individuals, the nociceptive signals are transmitted to specific brain regions, eliciting a proportional pain response. Besides, nociceptive fibers are classified depending on their diameter, conduction velocity, and myelination, with Aδ and C fibers as the main types [2]. Aδ fibers, thinly myelinated, rapidly transmit sharp, localized pain (5–30 m/s) [2], while unmyelinated C fibers conduct slower, dull, diffuse pain (1.3–2.4 m/s) [3].

Selective blockade of nociceptive fibers through high-frequency electrical currents could provide an alternative and novel tool for pain treatment. Unlike conventional electrical stimulation modalities that use frequencies within the "physiological" range (< 500 Hz), kilohertz frequency alternating current (KHFAC) stimulation (> 1000 Hz) uses frequencies in a "supraphysiological" range [4]. Electrical stimulation above 1000 Hz exceeds the neuron's maximum firing rate, leading to a different neuronal response compared to conventional electrical stimulation [4]. Basic studies involving electrical currents above 1 kHz applied to peripheral nerves have demonstrated rapid reversible axonal blockage without causing structural or functional alterations [5].

Preclinical studies have shown that the frequency of the current could be a key parameter for selectively blocking different types of axons [5]. Joseph and Butera [6] reported in frogs that frequencies between 5 and 20 kHz were associated with a lower block threshold in large myelinated fibers (A fibers), whereas between 30 and 50 kHz, the block threshold was lower in unmyelinated fibers (C fibers) [6]. This could be of clinical interest in individuals with pain, as it would allow for the selective blocking of nociceptive fibers without affecting motor function, or tactile, or proprioceptive sensations.

Regarding KHFAC application, our research group has carried out several previous studies [7–10] in healthy volunteers via transcutaneous [7–9] and percutaneous [10] application of KHFAC to the median, radial and/or ulnar nerve, with frequencies ranging between 5 kHz and 20 kHz. The effects on the tactile threshold and/or muscle strength were quantified as indirect measures of large fiber function, without observing changes in the pressure pain threshold (PPT), as an indirect measure of A-delta fibers. Only two case series have applied KHFAC in subjects with pain, and both used a frequency of 10 kHz with implanted devices [11, 12]. One of them stimulated the dorsal root ganglion in six subjects with postamputation pain [12], and the other applied 10 kHz for low back pain in 10 participants [11]. Other studies have employed implanted devices for vagus nerve block in people with obesity, also with frequencies below 20 kHz, usually up to 5 kHz [13–16]. Nonetheless, studies in human volunteers have primarily examined the effects of KHFAC up to 20 kHz. This limitation lies in the unavailability of commercial stimulators capable of delivering KHFAC frequencies exceeding 10 kHz, as the application of such frequencies has not yet received approval for human use. In the field of physical medicine and rehabilitation, the effects of these high frequencies on nerve conduction remain largely unexplored.

Hence, the current study was designed considering the lack of human studies utilizing frequencies higher than 20 kHz, considering the previous evidence in animals indicating that frequencies between 30 and 50 kHz could induce a selective blockade of nociceptive fibers. To address this research gap, a novel KHFAC prototype stimulator was developed and used for targeting frequencies ranging from 30 kHz to 50 kHz. Our main objective was to investigate the effect of transcutaneous KHFAC stimulation at 30 kHz, 40 kHz and 50 kHz on the median nerve compared to sham stimulation on experimental thermal pain as an indirect measure of C fibers, and on mechanical pressure pain as an indirect measure of A-delta fibers in healthy volunteers. Additionally, the study aimed to assess the safety of the intervention using this new prototype device. The secondary objectives were to evaluate the effect of stimulation on the discriminative sensitivity, isometric pinch strength, and antidromic sensory nerve action potential (SNAP) of the median nerve. Finally, the success of the blinding of participants and evaluator was assessed.

Methods

Design of CHFS 500i prototype

Due to the absence of devices capable of producing KHFAC with frequencies higher than 20 kHz, a prototype was manufactured ad hoc (Neuromodest® CHFS 500i Cibertec S.A.; Madrid, Spain) and developed based on the patent (PCT/ES2017/070080) of our research group (Fig. 1A). A constant current square wave stimulator with outputs for needle and plate electrodes was designed. The stimulator generates a stimulation current in a frequency range of 10 kHz to 50 kHz which is determined by the user in the stimulation configuration process. The waveform of the delivered current is rectangular biphasic symmetrical. It incorporates two independent current output circuits depending on the type of electrode used for stimulation. One circuit is for invasive needle application (which is not used in this study) with a peak-to-peak maximum current of 50 mA. The other circuit is for application with plate-type electrodes and generates a peak-to-peak intensity up to 400 mA. The intensity should be adjustable during the application. The session time is adjustable in 1-minute intervals. The equipment always works as a constant current source, with the user configuring the stimulation current and the equipment itself selecting the voltage applied so that the desired current is supplied depending on the impedance of the electrode-patient combination. The electrical diagram of the device Neuromodest® CHFS 500i is shown in Fig. 1B.

Study design

A randomized, double-blind, sham-controlled crossover study involving healthy volunteers was designed. Each participant received four interventions in separate sessions, including three active interventions (KHFAC stimulation at 30 kHz, 40 kHz, and 50 kHz) and one simulated intervention (sham stimulation). The order of the interventions was randomized using R software (v4.3.1), resulting in 24 possible permutations in blocks of four (ABCD). The randomization sequence was concealed in opaque envelopes and known only by the investigator responsible for administering the intervention at the time of implementation.

Participants and setting

The sample for this study was recruited from students of Health Sciences at the "Universidad de Castilla-La Mancha" Campus in Toledo. The sample size was calculated using Epidat software (v4.1), considering the results from a previous study on PPTs [7]. Considering the expected difference in the change in PPT between the active and sham interventions of 4.7 N/cm^2, with a standard deviation (SD) of 8.8 N/cm^2, a type I error (α) of 0.05, and a statistical power of 80%, a sample size of 30 participants

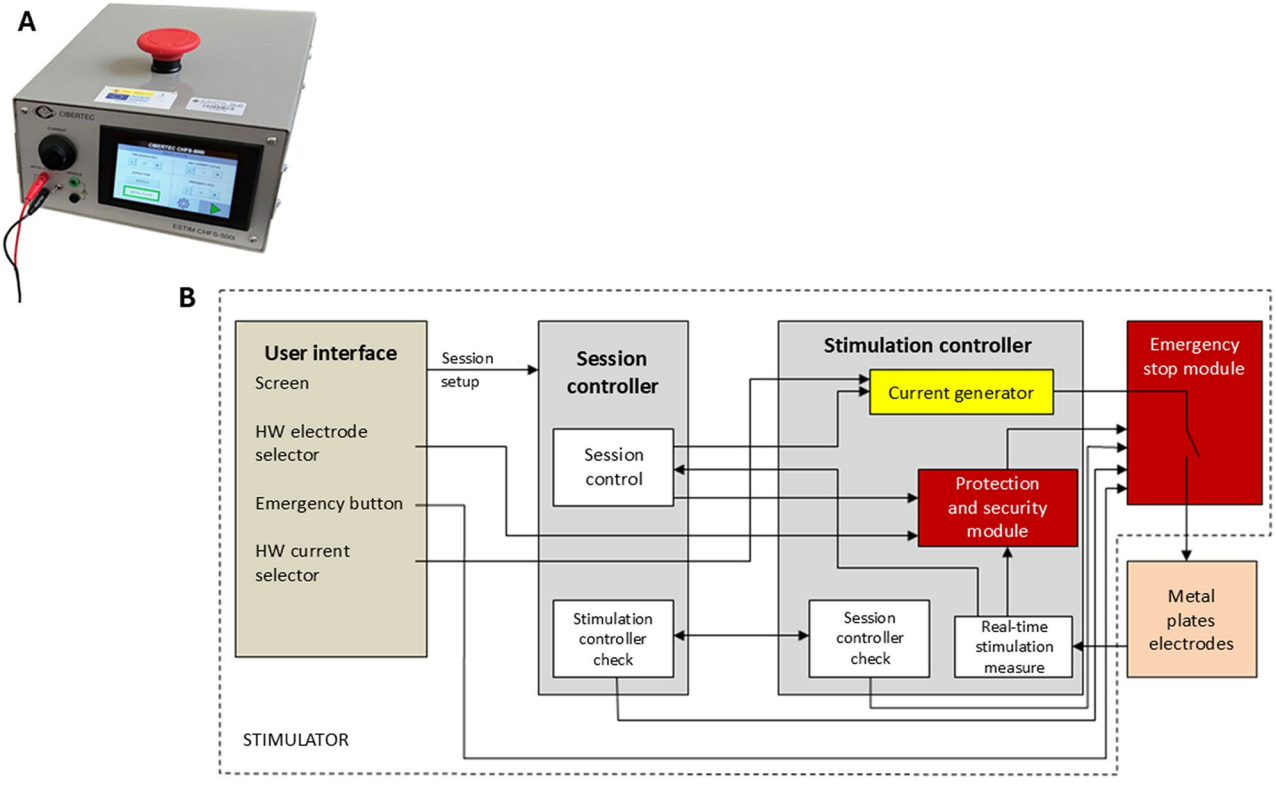

Fig. 1 **A**: Front view of Neuromodest CHFS 500i stimulator; **B**: Electrical diagram of Neuromodest® CHFS 500i. HW: hardware

($n = 30$) per group was estimated for paired groups. After accounting for potential loss to follow-up, a sample of 34 participants ($n = 34$) was established.

The inclusion criteria were healthy volunteers aged between 18 and 40 years who could understand the experimental procedures, absence of chronic pain conditions, and tolerance to transcutaneous electrical stimulation. The exclusion criteria were as follows: previous exposure to KHFAC stimulation, presence of pacemakers or other implanted electrical devices, epilepsy, pregnancy, tattoos or other external agents in the intervention area, medication intake or narcotic consumption during or 7 days prior to inclusion in the study, inability to attend experimental sessions, acute pain in any area, impaired sensitivity in the upper limb, history of cancer, history of trauma or surgical interventions in the upper limb, cardiovascular disease, diabetes, or history of neurological or neuromuscular disorders. Written informed consent was obtained from all participants before they participated in the study.

The study was conducted at the laboratory of the Faculty of Physiotherapy and Nursing, University of Castilla-La Mancha, with careful control of attenuated sound conditions and a constant temperature within the range of 22 °C to 26 °C.

Interventions

The participants included in the trial received a total of four interventions in a randomized order (KHFAC at 30 kHz, 40 kHz, 50 kHz, and sham stimulation). The duration of each intervention was 20 min, and a 24-hour washout period was established between interventions, considering that nerve blockade is rapidly reversible [5].

An active stimulation protocol with KHFAC at 30 kHz, 40 kHz, and 50 kHz was applied using two stainless steel electrode plates, each with a surface area of 9 cm^2 (3 cm × 3 cm), with a layer of conductive gel below them. The electrode plates were placed on the non-dominant hand near the transverse carpal ligament on the distal forearm, with a separation of 2 cm between them, targeting the median nerve. The intensity of the current was gradually increased until a 'strong but comfortable' tingling sensation was achieved, ensuring that it remained consistently below the motor threshold [8, 9] or below the tolerance threshold if the participant experienced discomfort. The current intensity was adjusted during the intervention due to habituation to the current.

Regarding the simulated intervention protocol, the device and the placement of the electrode plates were the same as those used in the active protocol. KHFAC at 10 kHz was selected; nonetheless, the intensity was increased at the beginning of the intervention until the motor threshold was reached and immediately decreased to 0 mA, maintaining this intensity throughout the intervention [10].

Outcomes

All the measurements were taken by a blinded assessor on the participants' non-dominant hand in a supine position, distal to the stimulation area. Measurements were taken at four time points: before intervention (T0), during intervention 5 min after the onset of stimulation (T1), immediately after intervention (T2), and 15 min after the end of stimulation (T3).

Main outcomes

The main outcomes were the PPT, an indirect measure of A-delta fibers [17]; the HPT, an indirect measure of C fibers [17]; and adverse events. The PPT was measured in N/cm^2 on the palmar region of the trapeziometacarpal joint. A digital algometer (Force Ten ™ FDX Wagner Instruments; Greenwich, USA) with a sensitivity of 0.1 N and a 1 cm^2 round rubber tip was utilized, applying a pressure ramp of 5 N/s. The PPT was the average of 3 measurements, with a 15-second rest period between them [18, 19]. Participants were instructed to tell the examiner when the sensation of pressure began to be painful [20]. The intraclass correlation coefficient (ICC) (0.92–0.96) and interrater reliability (ICC 0.94–0.96) of this measurement has shown excellent agreement [21].

The heat pain threshold (HPT) was measured in Celsius (°C) and was assessed using a 9 cm^2 Peltier thermode (TSA II MEDOC; Ramat Yishay, Israel) placed on the thenar eminence on the palmar surface of the hand. All trials began with the use of the thermode at 32 °C, which was increased at a rate of 1 °C/s with a safety limit of 50 °C. Participants were instructed to press the response unit button when the sensation of warmth began to be painful. The mean HPT was calculated using the average of 3 out of 4 measurements, excluding the first, with 30 s of rest between trials [22]. This outcome has shown good test-retest reliability (ICC 0.81), with a standard error of measurement of 1.26 °C on the hand of healthy participants [23].

The adverse effects and subjective perceptions of the participants were collected using a standardized ad hoc questionnaire that was completed at the end of each intervention [10]. This questionnaire included twelve items with two response options (YES/NO) related to skin alterations (i.e., erythema, petechiae, etc.), sensations (i.e., tingling, numbness, etc.), pain, sweating, stiffness, weakness, and an open question for other perceived effects or sensations of the participant. The unpleasantness of the intervention was also evaluated using a numerical scale from 0 to 10, where 0 corresponded to "not at all unpleasant" and 10 to "the most unpleasant". In addition, participants were informed that they should

report any abnormal sensations or effects in the four weeks following the intervention.

Secondary outcomes

The secondary outcomes were static two-point discrimination sensitivity (as an indirect measure of A-beta fibers), isometric pinch strength (as an indirect measure of efferent fibers), and SNAPs of the median nerve (as a direct measure of large A fibers). In addition, skin temperature was recorded for its potential influence on SNAPs, and the success of blinding was evaluated.

Static two-point discrimination was measured in mm using an aesthesiometer (BASELINE®; White Plains, USA) on the distal phalanx of the third finger. The protocol started at 10 mm, and the distance between tips was decreased by 1 mm intervals [24]. The intrarater reliability of this outcome on the hand of healthy individuals has shown good reliability (ICC 0.82) [25].

SNAPs of the median nerve were measured using the antidromic technique [26]. A stimulator (Digitimer DS7A; Letchworth Garden, UK), a digital data acquisition unit (Cambridge Electronic Design; Cambridge, UK), and an amplifier (ETH-256 iWorxs; Dover, USA) with a 3 Hz high-pass filter and a 2000 Hz low-pass filter were utilized for this measurement, applying stimuli with a pulse width of1ms and a frequency of 1 Hz. Two ring recording electrodes were placed in the metacarpophalangeal joints of the second finger, and the ground electrode was located in the radial styloid process [27]. A bipolar transcutaneous electrode with a fixed interelectrode distance was placed on the stimulation site over the median nerve, 40 cm from the recording electrode (distal and medial aspect of the arm) [27]. An average of 10 recordings was obtained, and the peak-to-peak amplitude was registered in microvolts (μV), while nerve velocity was measured in meters per second (m/s) using the peak latency and distance between the stimulation site and the cathode ring electrode [26, 27]. To record hand skin temperature, a laser-Doppler monitor (MOOR DRT4; Axminster, UK) was placed on the palmar surface of the third metacarpal head [28].

The success of the blinding of participants and evaluators was assessed after each intervention using a closed-ended question: "What type of treatment do you think you received?" with five response options: (1) "I strongly believe that I received an experimental treatment"; (2) "I somewhat believe that I received an experimental treatment"; (3) "I strongly believe that I received a placebo"; (4) "I somewhat believe that I received a placebo"; and (5) "Do not know, do not answer" [29, 30].

Statistical analysis

Descriptive statistics were performed for the baseline demographic variables. One-way repeated-measures ANOVA with a Bonferroni pairwise comparison post hoc test was performed for intragroup comparisons. To compare the change from baseline between interventions, a one-way repeated-measures ANOVA with a Bonferroni pairwise comparison post hoc test was also performed. In both ANOVAs, the sphericity of the outcomes was analyzed using the Mauchly test. When sphericity could not be assumed (Mauchly sphericity test $p < 0.05$), the Greenhouse–Geisser correction was applied. Adverse events were shown as the frequency and percentage, and nonparametric statistical tests were used (Pearson's chi-square test or Fisher–Freeman–Halton exact test) for intergroup comparisons. The data were analyzed using IBM SPSS Statistics software version 28.0.0 (IBM, NY, USA). The level of statistical significance (α error) was established as $p < 0.05$ for all outcomes.

To analyze the blinding outcome variable, James' blinding index (BI) [31] and Bang's BI [29] were obtained using Stata v15.0 (Stata Corp, TX, USA). James' BI is used to infer overall blinding success in randomized clinical trials. However, Bang's BI was used to characterize and evaluate the blinding situation in each trial arm independently. The James' BI ranges from 0 to 1 (0 representing total lack of blinding, 1 representing complete blinding, and 0.5 representing completely random blinding). To interpret the results, this study considered a lack of blinding if the upper bound of the confidence interval was less than 0.5. Bang's BI can be directly interpreted as the proportion of unblinding in each arm [29]. It ranges between −1 and 1, with 0 indicating the most desirable situation representing random complete blinding. Therefore, when the one-sided confidence interval did not cover the 0 value, the study was regarded as lacking blinding [29, 31].

Results

Thirty-four volunteers who met the inclusion criteria, received the interventions in randomized order and were included in the statistical analysis (Fig. 2, CONSORT). Table 1 presents the demographic data of all participants. The mean age of the participants was 20.8 years (SD 2.5); 67.6% ($n = 23$) were women, the dominant hand was the right hand in 88% ($n = 30$) of participants, and the mean body mass index was 23.5 kg/m^2 (SD 3.5). The mean intensity applied in the 30 kHz group was 192.0 mA (SD 54.7), 264.9 mA (SD 65.7) in the 40 kHz group, and 312.7 mA (SD 45.2) in the 50 kHz group, being these differences significant ($F = 82.6$; $p < 0.001$).

Table 2 shows the descriptive values of the primary and secondary outcomes. Intragroup comparisons showed a significant increase in the PPT for the active groups during the intervention, immediately after the intervention, and at 15 min postintervention with respect to baseline. However, no changes were observed in the sham stimulation intervention. HPT increased at all three time points

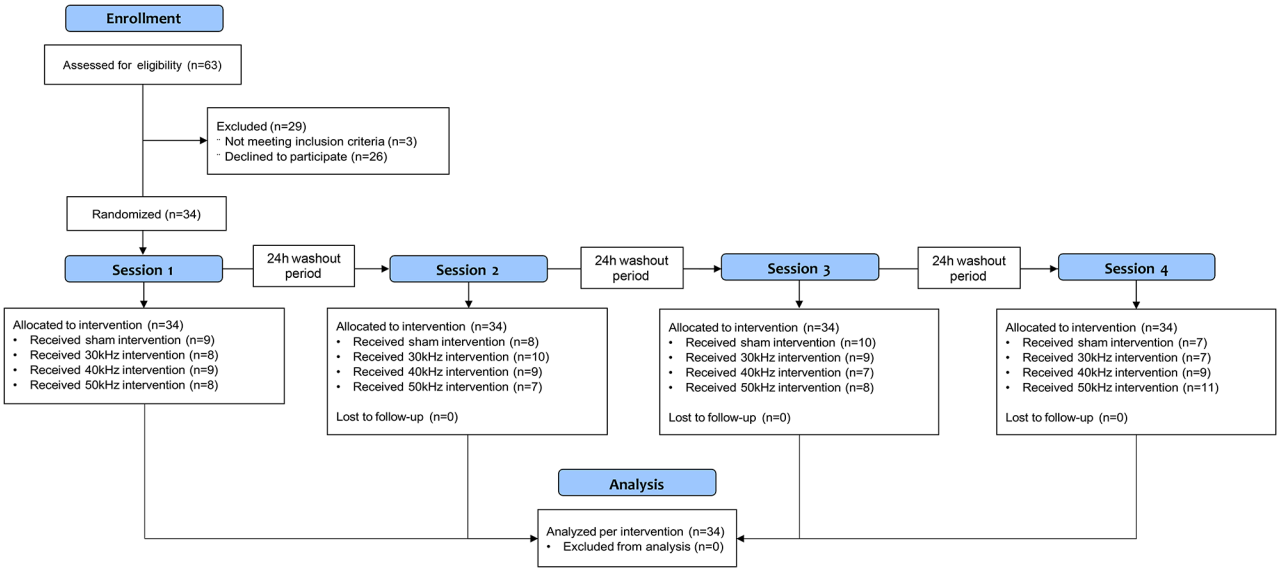

Fig. 2 Study CONSORT Flow Diagram, an adapted version for crossover trials

Table 1 Demographic characteristics of participants

Demographic Characteristics (*n*=34)	
Outcomes (units)	**Mean (SD)**
Age (years)	20.8 (2.5)
Gender (F/M, n)	23/11
Height (m)	1.7 (0.1)
Weight (Kg)	68.6 (13.8)
Body Mass Index (Kg/m^2)	23.5 (3.5)
Handedness (L/R, n)	4/30

Data are expressed in mean and standard deviation (SD). *F/M* female/male, *L/R* left/right, *n* number of participants

from baseline in the 30 kHz and 40 kHz interventions, and in the sham stimulation after the intervention and at 15 min, and in the 50 kHz group only after the intervention. Conduction velocity showed a slight decreasing tendency in all interventions with respect to baseline, but it reached statistical significance only in the sham and 40 kHz interventions. Similarly, skin temperature tended to slightly decrease from baseline, reaching statistical significance in the sham stimulation, 40 kHz stimulation and 50 kHz stimulation, at 15 min after the intervention. No intragroup changes with respect to baseline were observed for the other outcomes.

Figure 3 shows the intergroup comparisons of changes in the main outcomes from baseline. A greater effect was observed on the PPT during the intervention for 30 kHz stimulation (6.7 N/cm2 (95% CI 4.7 to 8.7)), 40 kHz stimulation (9.8 N/cm2 (95% CI 6.2 to 13.3)) and 50 kHz stimulation (6.8 N/cm2 (95% CI 3.5 to 10.1)) when compared to the sham stimulation. Immediately after the intervention, significant differences in the effect were observed for the three active interventions compared to the sham stimulation group (30 kHz stimulation: 6.6 N/cm2 (95% CI 3.5 to 9.7); 40 kHz stimulation: 10.8 N/cm2 (95% CI 7.1 to 14.5); and 50 kHz stimulation: 5.7 N/cm2 (95% CI 1.9 to 9.5)). However, only 40 kHz stimulation had a greater effect than sham stimulation at 15 min postintervention (4.1 N/cm^2 (95% CI 0.3 to 7.9)). Moreover, the PPT showed a greater change in 40 kHz stimulation than in the 30 kHz stimulation (3.1 N/cm2 (95% CI 0.02 to 6.1)) during the intervention and was close to reaching statistical significance after the intervention (4.2 N/cm2 (95% CI -0.1 to 8.6); $p=0.06$). The effect in the 40 kHz group was also greater than that in the 50 kHz group after the intervention (5.1 N/cm2 (95% CI 1.9 to 8.4)) (Fig. 3A).

The effect on the HPT was greater with the 40 kHz stimulation than with the sham intervention during stimulation (1.2 °C; 95% CI 0.4 to 2.0), postintervention (1.4 °C; 95% CI 0.6 to 2.1), and 15 min (1.3 °C; 95% CI 0.5 to 2.2). In the 30 kHz stimulation, the change was greater than that in the sham stimulation only at postintervention (1.2 °C; 95% CI 0.1 to 2.3), and no difference was observed between the 50 kHz and sham stimulation (Fig. 3B).

Table 3 shows the intergroup comparisons of the mean change from baseline for the secondary outcomes. No difference was observed between the active interventions and the sham stimulation for the secondary outcomes, except for the velocity of sensory nerve action potentials, which slightly decreased more in the sham group than in the 50 kHz group after the intervention and at 15 min postintervention.

Table 4 shows the frequencies of adverse effects and comparisons between active interventions and sham stimulation. Erythema, petechiae and itching were the most common, and their proportions were greater in

Table 2 Descriptive outcome values mean and standard deviation (SD) of the four intervention groups

Outcomes (Units)	Sham group				30 kHz group				40kHz group				50kHz group			
Mean (SD)	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3
Pressure Pain Threshold (N/cm^2)	39.8 (11.1)	40.4 (12.4)	41.9 (14.3)	42.0 (13.5)	41.3 (10.7)	**48.6 (13.6)**	**50.0 (13.6)**	**45.1 (12.8)**	38.0 (9.4)	**48.3 (14.2)**	**50.9 (14.9)**	**44.3 (13.7)**	40.4 (10.5)	**47.8 (12.6)**	**48.2 (13.7)**	**44.5 (12.5)**
Heat Pain Threshold (ºC)	44.2 (3.3)	44.4 (3.4)	**44.8 (3.4)**	**44.9 (3.3)**	43.6 (3.4)	**44.7 (2.9)**	**45.5 (2.6)**	**45.6 (2.5)**	43.5 (2.8)	**44.9 (2.8)**	**45.5 (2.8)**	**45.6 (2.8)**	44.5 (3.1)	44.9 (3.4)	**45.6 (2.9)**	45.5 (3.2)
Two-point discrimination (mm)	2.5 (0.9)	2.4 (0.8)	2.4 (0.9)	2.6 (0.9)	2.4 (0.7)	2.4 (0.8)	2.3 (0.8)	2.3 (0.8)	2.4 (0.7)	2.4 (0.7)	2.5 (0.7)	2.4 (0.8)	2.4 (0.8)	2.5 (0.9)	2.4 (0.9)	2.4 (0.9)
Pinch Strength (Kg/f)	5.4 (1.6)	5.4 (1.9)	5.4 (1.7)	5.4 (1.5)	5.5 (1.4)	5.3 (1.5)	5.4 (1.5)	5.5 (1.5)	5.5 (1.4)	5.4 (1.4)	5.4 (1.4)	5.4 (1.4)	5.3 (1.8)	5.4 (1.7)	5.1 (1.5)	5.4 (1.5)
SNAP amplitude (μV)	14.6 (9.3)	16.3 (10.5)	16.8 (10.8)	16.9 (10.8)	17.2 (11.4)	20.0 (13.9)	19.8 (12.7)	23.2 (17.7)	18.8 (12.4)	22.4 (21.5)	22.5 (28.1)	22.9 (28.0)	18.6 (15.5)	21.4 (14.2)	21.3 (21.2)	21.7 (18.5)
SNAP velocity (m/s)	45.9 (4.2)	**45.1 (4.2)**	**45.0 (4.4)**	**44.6 (4.5)**	44.7 (5.7)	44.2 (5.2)	44.2 (5.4)	44.0 (5.7)	45.3 (4.1)	**44.4 (4.0)**	44.6 (4.0)	**44.0 (4.4)**	45.4 (4.8)	45.0 (4.8)	45.3 (4.7)	45.0 (4.9)
Skin temperature (ºC)	31.6 (4.0)	31.3 (4.4)	31.1 (4.4)	**30.6 (4.4)**	30.4 (3.8)	30.5 (4.1)	30.3 (4.1)	29.9 (4.1)	31.0 (3.9)	30.5 (4.0)	30.3 (4.2)	**29.7 (4.1)**	31.9 (3.0)	31.7 (3.1)	31.6 (3.3)	**31.1 (3.3)**

Data are expressed as mean and standard deviation. Bold values denote statistically significant differences at the $p <$ level with respect to baseline. T0: at baseline, T1: during intervention at 5 min., T2: immediately after the intervention, T3: 15 min after de intervention. SNAP: sensory nerve action potentials. Bold *values denote statistically significant difference* at the $p < 0.05$ level respect to baseline

the active electrical stimulation interventions than in the sham stimulation. These adverse effects were observed only below the electrodes, and all of them were resolved 24 h after the interventions, including the tingling sensation, which was reported during and immediately after the stimulation.

Table 5 shows the results of participant blinding, and Table 6 shows the results of assessor blinding. The overall analysis via James' index [31] determined the lack of blinding of the participants and the correct blinding of the assessor when 30 kHz and 50 kHz were applied. The blinding analysis by the intervention group using Bang's index [29, 30] revealed a lack of blinding in the active groups of the participants, whereas correct blinding was obtained for the assessor. In the sham group, the Bang's index revealed complete blinding of participants and a lack of blinding of the assessor.

Discussion

To the best of our knowledge, this study is the first to investigate the effects of transcutaneous KHFAC applied at frequencies of 30 kHz, 40 kHz, and 50 kHz in healthy participants on somatosensory and motor outcomes. The main findings demonstrated a greater effect on both the pressure and heat pain thresholds in the 40 kHz stimulation than in the sham stimulation during, immediately after, and 15 min postintervention. Furthermore, 40 kHz stimulation also had a greater effect on the PPT than 30 kHz intervention during stimulation and when compared to 50 kHz on the PPT immediately poststimulation. Regarding stimulation safety, all the interventions were well tolerated, and only mild and expected adverse events were found in the active groups compared to the sham group for the appearance of erythema, petechiae and an itching sensation.

Although the effects on the PPT with 30 kHz and 50 kHz also achieved statistically significant differences, only the stimulation at 40 kHz reached the minimal clinically important difference (> 10 N/cm^2) [32, 33]. Regarding the HPT, although the 40 kHz stimulation was more effective than sham stimulation at all measured time points, the difference did not reach the minimal clinically significant difference, which has been established in the range of 3.5 to 5.3 °C for noninjured subjects [23, 34]. The observation of more pronounced changes in the PPT than in the HPT could be attributed to the optimality of 40 kHz as a frequency for A-delta fibers, while it may be suboptimal for C fibers. Future studies should assess differences between A-delta and C-fiber blockade.

The observed effects on mechanical and thermal pain thresholds without any changes in motor function or tactile sensitivity in the present study are consistent with previous preclinical evidence from KHFAC. Some studies have shown that the frequency of the current can be

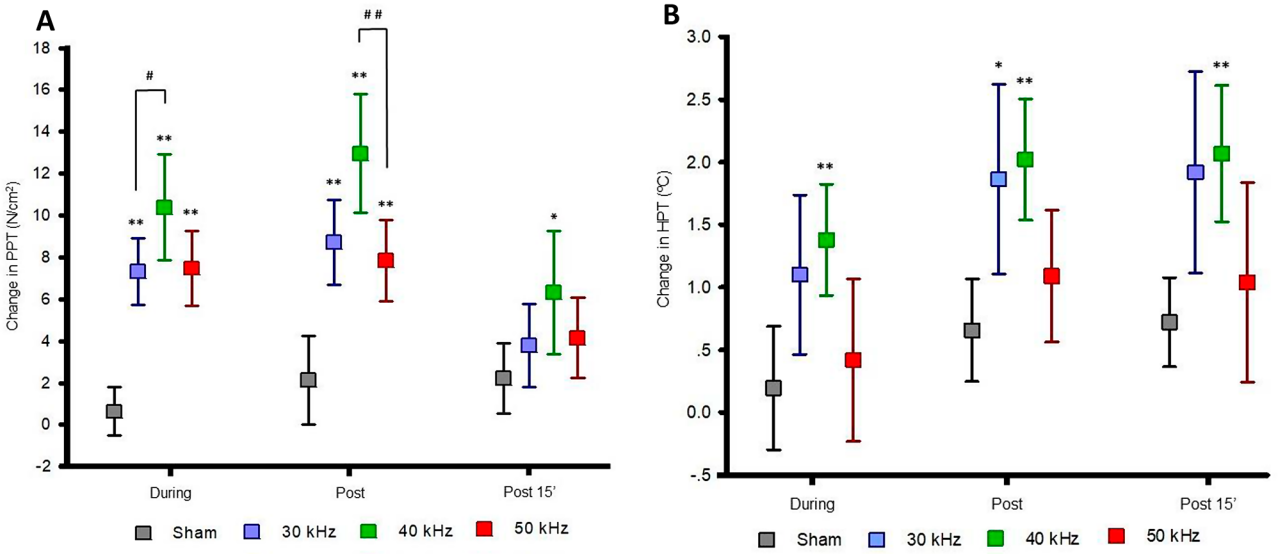

Fig. 3 Pressure and Heat Pain Threshold changes from baseline. Legend. **A**: (Left): Intergroup comparison of changes with respect to baseline in pressure pain threshold, expressed as mean and 95% confidence interval. Differences between sham and active groups are indicated with (*) $p < 0.05$ and (**) $p < 0.01$. Differences between active groups are indicated with (#) $p < 0.05$ and (# #) $p < 0.01$. **B** (Right): Intergroup comparison of changes with respect to baseline in heat pain threshold, expressed as mean and 95% confidence interval. Differences between sham and active groups are indicated with (*) $p < 0.05$ and (**) $p < 0.01$. *PPT* pressure pain threshold, *HPT* heat pain threshold

Table 3 Intergroup secondary outcomes

Outcomes (units) Mean (CI 95%)	Change 30 kHz minus Change Sham			Change 40 kHz minus Change Sham			Change 50 kHz minus Change Sham		
	During intervention (T1)	Post intervention (T2)	15 min after intervention (T3)	During intervención (T1)	Post intervention (T2)	15 min after intervention (T3)	During intervención (T1)	Post intervention (T2)	15 min after intervention (T3)
Two-point discrimination (mm)	0.0 (-0.3 to 0.3)	-0.1 (-0.4 to 0.2)	-0.2 (-0.7 to 0.2)	0.0 (-0.3 to 0.4)	0.1 (-0.3 to 0.5)	-0.1 (-0.5 to 0.2)	0.1 (-0.2 to 0.4)	0.1 (-0.2 to 0.3)	-0.1 (-0.5 to 0.3)
Pinch Strength (Kg/f)	-0.2 (-0.7 to 0.4)	-0.1 (-0.7 to 0.6)	0.0 (-0.7 to 0.7)	-0.1 (-0.7 to 0.4)	-0.1 (-0.8 to 0.6)	-0.1 (-0.8 to 0.6)	0.1 (-0.5 to 0.6)	-0.2 (-0.8 to 0.5)	0.0 (-0.6 to 0.7)
SNAP amplitude (μV)	1.1 (-4.6 to 6.8)	0.4 (-4.2 to 4.9)	3.7 (-4.7 to 12.2)	1.9 (-4.0 to 7.8)	1.6 (-6.0 to 9.1)	1.8 (-6.7 to 10.3)	1.1 (-3.9 to 6.1)	0.5 (-3.8 to 4.8)	0.9 (-3.1 to 4.9)
SNAP velocity (m/s)	0.4 (-0.4 to 1.1)	0.4 (-0.4 to 1.2)	0.6 (-0.1 to 1.4)	0.0 (-0.8 to 0.7)	0.3 (-0.4 to 1.1)	0.1 (-0.8 to 1.1)	0.4 (-0.4 to 1.1)	**0.8* (0.2 to 1.3)**	**0.9* (0.2 to 1.6)**
Skin temperature (ºC)	0.4 (-0.4 to 1.3)	0.3 (-0.7 to 1.3)	0.6 (-0.5 to 1.6)	-0.1 (-0.8 to 0.6)	-0.2 (-1.0 to 0.7)	-0.2 (-1.1 to 0.7)	0.1 (-0.5 to 0.8)	0.1 (-0.6 to 0.8)	0.2 (-0.6 to 1.0)

Data are expressed as mean and 95& Confidence Interval. Bold *values denote statistically significant difference* * $p < 0.05$. SNAP: sensory nerve action potentials

a key factor in the block threshold of different types of fibers [5]. The blocking threshold linearly increases with the applied frequency for larger diameter fibers, i.e., at a higher frequency, more intensity is needed to reach the blocking threshold. Smaller-diameter fibers have a higher block threshold than large fibers at lower frequencies. However, smaller diameter fibers exhibit an inverted U shape, and with frequencies ≥ 30 kHz, the blocking threshold decreases. Therefore, frequencies ≥ 30 kHz can block small-diameter fibers without blocking large-diameter fibers [6, 35, 36]. Studies in healthy volunteers have shown changes in motor response and tactile thresholds (larger-diameter fibers) without changes in pain thresholds (smaller-diameter fibers) when KHFAC is applied at frequencies lower than 30 kHz (10 and 20 kHz) [8–10]. However, in a recent study in which KHFAC was applied percutaneously at 30 kHz in healthy volunteers, changes in the PPT were observed, without changes in tactile thresholds or motor function [37].

Peña et al. [38] provided a potential explanation for the mechanism underlying the inverted U-shaped block threshold observed in small-diameter fibers. KHFAC devices used at higher electrical charge can distort waveforms and introduce amplitude and frequency-dependent direct current offsets. Franke et al. [39] also observed that electrical current generators at high frequencies could produce undesirable contamination of the KHFAC waveforms by direct current. This selective-specific block threshold at high frequencies could be due to the direct current offset. However, Peña et al. [38] only stimulated

Table 4 Frequency of adverse events and comparison between the active groups and sham group

Adverse Events	Sham Group (*n* = 34)	30 kHz Group (*n* = 34)	40 kHz Group (*n* = 34)	50 kHz Group (*n* = 34)	Differences with Sham Group (*p* value)
Erythema n (%)	0 (0.0)	33 (97.1)	33 (97.1)	33 (97.1)	**(p < 0.001)[a]**
Petechiae n (%)	0 (0.0)	25(73.5)	24 (70.6)	29 (85.3)	**(p < 0.001)[a]**
Itching n (%)	0 (0.0)	16 (47.1)	15 (44.1)	25 (73.5)	**(p < 0.001)[a]**
Tingling n (%)	2 (5.9)	8 (23.5)	5 (14.7)	3 (8.8)	($p = 0.17$) [a]
Numbness n (%)	6 (17.6)	9 (26.5)	12 (35.3)	6 (17.6)	($p = 0.27$) [a]
Warmth sensation n (%)	0 (0.0)	1 (2.9)	0 (0.0)	1 (2.9)	($p = 1.00$) [a]
Cold sensation n (%)	0 (0.0)	1 (2.9)	2 (5.9)	1 (2.9)	($p = 0.90$) [a]
Pain n (%)	0 (0.0)	1 (2.9)	2 (5.9)	0 (0.0)	($p = 0.62$) [a]
Heaviness n (%)	1 (2.9)	2 (5.9)	3 (8.8)	4 (11.8)	($p = 0.70$) [a]
Weakness n (%)	3 (8.8)	5 (14.7)	3 (8.8)	3 (8.8)	($p = 0.89$) [a]
Muscular stiffness n (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)	($p = 1.00$) [a]
Hand sweating n (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)	($p = 1.00$) [a]
Unpleasantness. VAS cm Mean (SD)	1.4 (1.6)	1.8 (1.6)	1.8 (1.4)	2,1 (1.7)	($p > 0.09$) [b]
Pain. VAS cm Mean (SD)	0.8 (1.3)	1.0 (1.2)	1.3 (1.2)	1.4 (1.7)	($p > 0.07$) [b]

Data are expressed as percentages or mean and standard deviation. Bold fonts indicate p-values below 0.05. Superscript letters indicate statistical tests used for each variable: (a) Chi-Cuadrado De Pearson o *Fisher's exact test* for expected values less than 5. (b) *one factor repeated measures ANOVA with* Bonferroni post-hoc test

Table 5 Statistical analysis of blinding assessment of participants

Methods	Index	*p*-value	95% Confidence interval	Conclusion
Participants blinding 30 kHz				
James' BI	0.35	$p < 0.001$	0.26 to 0.44	Unblinded
Bang's BI-Active/ 2×5	0.5	$p < 0.001$	0.29 to 0.70	Unblinded
Bang's BI-Placebo/ 2×5	0.02	0.41	-0.18 to 0.24	Blinded
Participants blinding 40 kHz				
James's BI	0.30	$p < 0.001$	0.22 to 0.39	Unblinded
Bang's BI-Active/ 2×5	0.66	$p < 0.001$	0.52 to 0.80	Unblinded
Bang's BI-Placebo/ 2×5	0.02	0.41	-0.18 to 0.24	Blinded
Participants blinding 50 kHz				
James's BI	0.28	$p < 0.001$	0.19 to 0.36	Unblinded
Bang's BI-Active/ 2×5	0.66	0	0.53 to 0.78	Unblinded
Bang's BI-Placebo/ 2×5	0.02	0.41	-0.18 to 0.24	Blinded

Data are expressed as 95& confidence interval. BI: blinding index

Table 6 Statistical analysis of blinding assessment of assessors

Methods	Index	*p*-value	95% Confidence interval	Conclusion
Assessor blinding 30 kHz				
James' BI	0.52	0.65	0.42 to 0.62	Blinded
Bang's BI-Active/ 2×5	-0.10	0.80	-0.30 to 0.09	Blinded
Bang's BI-Placebo/ 2×5	0.42	$p < 0.001$	0.23 to 0.62	Unblinded
Assessor blinding 40 kHz				
James's BI	0.37	0.01	0.27 to 0.46	Unblinded
Bang's BI-Active/ 2×5	0.14	0.10	-0.04 to 0.34	Blinded
Bang's BI-Placebo/ 2×5	0.42	$p < 0.001$	0.23 to 0.62	Unblinded
Assessor blinding 50 kHz				
James's BI	0.48	0.43	0.38 to 0.58	Blinded
Bang's BI-Active/ 2×5	-0.10	0.79	-0.31 to 0.10	Blinded
Bang's BI-Placebo/ 2×5	0.42	$p < 0.001$	0.23 to 0.62	Unblinded

Data are expressed as 95& confidence interval. BI: blinding index

myelinated fibers, and specific threshold-frequency relationships in response to KHFAC with direct current offsets may differ in unmyelinated axons.

The effects on the PPT and HPT in the experimental interventions were found during and immediately after KHFAC stimulation, with effects lasting up to 15 min in the 40 kHz group. This effect is consistent with the rapid reversible block after stimulation that has been observed in preclinical studies.

It is important to highlight the differences in the mechanisms of action between low-frequency analgesic currents and the currents applied in this study. While conventional or high-frequency TENS, typically applied in the 75–150 Hz range, aims to stimulate A-beta fibers to achieve presynaptic inhibition based on gate control theory, and low-frequency TENS promotes endogenous opioid release, KFHAC application produces a partially and rapidly reversible nerve block [40]. It is also essential to note that, as observed in preclinical studies, the higher the frequency, the greater the blocking threshold; therefore, higher frequencies require increased intensity. However, previous studies have not shown axonal damage in the nerve, so KFHAC can be considered a safe technique.

Although, there is no consensus on the neurophysiological mechanisms that produce nerve blocks via KHFAC [40], it has been reported that, depending on the axon model, the blockage could be due to the inactivation

of sodium channels or the activation of potassium channels [40]. With respect to the observed effect at 15 min, Yang et al. (2017), in a study of the sciatic nerve in frogs, proposed that the time to restore intra-axonal ion concentrations via the sodium-potassium pump may underlie the poststimulation effect [41]. Rapeaux et al. (2022) proposed the accumulation of periaxonal K^+ due to the activation of potassium channels for poststimulation effects [42]. Future studies should focus on assessing the sustained impact of KHFAC stimulation beyond the initial 15-minute timeframe in people with pain-related pathologies. It seems reasonable that short, repetitive applications could have the same effect as continuous applications. However, further studies are needed to investigate this aspect. This applies to both isolated sessions and programs involving repeated sessions.

The effect observed on nociception in our study with transcutaneous KHFAC stimulation at 40 kHz could have potential clinical applicability for pain relief. Nevertheless, there are no studies on people with pain-related pathologies using frequencies ≥ 20 kHz. There are only two case studies in people with pain with promising results where KHFAC stimulation was used, but these studies were performed with implanted electrodes and at frequencies ≤ 10 kHz [12, 43]. Duncan et al. applied KHFAC at 4 kHz in a patient with neuropathic pain, and Soin et al. applied KHFAC at 10 kHz in 10 patients with postamputation pain.

Standard transcutaneous electrical stimulation with low-frequency currents, known as transcutaneous electrical nerve stimulation (TENS), which has been used for decades to relieve pain, causes changes in the tactile threshold [7] that can influence functionality. However, a KHFAC current > 30 kHz could be advantageous since it selectively affects nociception without affecting tactile thresholds or motor function. Furthermore, the adverse effects observed are mild and similar to those that occur with TENS current [44].

Limitations

This randomized crossover trial has certain limitations. The first point is the use of indirect measures to assess the blockade of nerve A-delta and C fibers. Another point is the maximum intensity output at 350 mA of the stimulator used, which was reached by some participants during stimulation at 50 kHz without reaching the desired "strong but comfortable" tingling sensation. An underdose of current intensity could underestimate the effect of 50 kHz stimulation. Regarding the blinding assessment of the participants, it should be noted that although Bang´s Index specifically determined the correct blinding for the sham stimulation, active stimulation sessions at 30 kHz, 40 kHz, and 50 kHz and the general blinding determined by James´ Index were associated with incorrect blinding. Conversely, regarding the blinding assessment of the assessor, only the specific analysis of Bang´s Index for the active group determined successful blinding for 40 kHz stimulation, while 30 kHz and 50 kHz stimulation were generally blinded to the results determined by the James´ Index. Future studies should optimize the sham stimulation protocol to correctly blind participants and assessors.

Conclusion

The application of transcutaneous KHFAC to the median nerve through this novel stimulator prototype, particularly at 40 kHz, elicited an increase in both pressure and heat pain thresholds in comparison to sham stimulation without affecting the tactile threshold or motor function. Furthermore, the adverse effects on the skin caused by KHFAC are mild and similar to those caused by standard low-frequency transcutaneous electrical stimulation used in the clinical context for pain management. Future studies should address the clinical impact of transcutaneous KHFAC at 40 kHz in people with pain. The effects of multiple repeated sessions and comparisons with standard electrical stimulation protocols are also needed.

Abbreviations

KHFAC	Kilohertz High-Frequency Alternating Current
SNAP	Sensory Nerve Action Potential
SD	Standard Deviation
TENS	Transcutaneous Electrical Nerve Stimulation
HPT	Heat Pain Threshold
PPT	Pressure Pain Threshold
BI	Blinding Index

Supplementary Information

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Supplementary Material 1

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Author contributions

The study conception and design were formulated by JAC, JGS, JJFP, and DSM. DMCA and JJFP implemented the study and collected the data. JAC and DSM completed the data processing and statistical analysis. JAC, JGS, DSM, and JJFP wrote the main manuscript text and prepared the figures and tables.

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Data availability

The datasets generated and analyzed during the current study are available in the Zenodo repository (Link: https://doi.org/10.5281/zenodo.10477710).

Declarations

Ethics approval and consent to participate

Before the initiation of the study, the local research ethics committee (reference number: 805 – 24/11/2021) and the Spanish Agency of Medicines and Medical Devices (reference number: 965/22/EC-R) approved this study. All the participants gave and signed an informed consent to participate in this study.

Consent for publication

Not applicable.

Competing interests

JAC and JGS are the patent holders of the prototype stimulator used in this study.

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