FUNDACION H.A.BARCELO
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ELECTRICAL STIMULATION WITH DFIFERENT CURRENTS: THE EFFECTS ON FORCE, TOLERANCE AND FATIGUE IN HEALTHY SUBJECTS

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THEORETICAL FRAMEWORK

Electrical stimulation

Electrical stimulation is the use of electric currents in order to generate a muscle contraction. The objectives of this therapy are replacing insufficient voluntary contraction, prevention or treatment of atrophy, treatment of sarcopenia, muscle strengthening and performance. (1)

Neuromuscular electrical stimulation (NMES) is used when the peripheral nervous system is unharmed, acting on the nerve (1-3), aiming to generate muscle contraction with no voluntary activation or functional movement by the patient. (4).

The result of training, whether by electro-stimulation or by voluntary contraction, will be in relation to the force produced in the sessions. In order for muscle strength and trophism to be maintained, minimum contraction levels of 20% of the maximum voluntary isometric contraction (MVIC) must be repeatedly attained. Peaks of strength of 30 to 50 % of maximum voluntary isometric contraction (MVIC) are required to improve muscle performance (5). To achieve hypertrophy, values of 50 to 60% of MVIC must be reached (6). Other variables that affect strength gain with NMES are the type of contraction elicited and the angle at which the training is performed. (7)

The magnitude of the contractile response in neuromuscular stimulation is related to the parameters of the electrical current, such as pulse duration, intensity, frequency, and contraction and relaxation times (8). These variables should allow maximum force peaks to be generated with minimum nociceptive sensation (9).

Current variables used in electrical stimulation

Currents used in neuromuscular electrical stimulation have a pulse width of less than 1 ms (10) and an intensity between 1 and 120 mA. Pulsed currents can be classified, in terms of their waveform, as single-phase and two-phase. Two-phase currents, in turn, can be symmetric, compensated asymmetric and uncompensated asymmetric.

In low frequency pulsed currents it is possible to modify the phase duration or width, pulse duration or width and its frequency. (2, 3) New research also includes a variable called intra-pulse interval, but this is not yet available in conventional equipment. (8)

Medium frequency currents, on the other hand, have a carrier frequency (achieving pulses without in-between pauses) and a modulation frequency (the number of times the current is modulated or varies in one second). This modulation can be in on-off packages, determining a duty cycle (Duty Cycle = Emission Time / (Emission Time + Pause Time) or in intensity. Russian, Neo-Russian and Australian currents are modulated in on-off packages (also called burst modulated currents). (2, 3)

In addition, currents for electrical stimulation must have ramp-up, ramp-down, contraction time and relaxation time (pause), in order to resemble a voluntary contraction. These values are adjustable and their unit is seconds. (11)

The higher the intensity, pulse duration and/or frequency, the greater the contraction. Increasing these parameters will increase discomfort and fatigue. (12)

Medium frequency neuromuscular electrical stimulation

In the classical literature, medium frequency currents include Russian currents and interferential currents (11). Subsequently, Neo-Russian currents were added to this list, whose equipment emission differs in several technical aspects from conventional Russian currents (13) and, more recently, Aussie currents have been added as well (14).

Russian currents

Although Russian currents are very popular in Latin America, there is not much scientific evidence and the studies conducted by their inventor, Kots, are controversial. The author studied the strength gains, but the patient population comprised teenagers, which could explain why there was a large increase in this variable. Another controversial issue is that, in order to determine the optimal times of contraction and relaxation, instead of using Russian current, he used low-frequency monophasic currents of 1 ms pulse duration and then extrapolated this to Russian stimulation. This was called 10-50-10 (10-second contraction, 50-second pause, 10 repetitions) (15).

The classical Russian current has a sinusoidal waveform, with a pulse duration of 0.4 ms (400 us), a carrier frequency of 2500 Hz, modulation frequency of 50 Hz and a duty cycle of 50 %

(10 ms emission, 10 ms pause) (Fig. 4). It has achieved strength increases when applied in combination with isometric exercises (7, 11).

Neo-Russian currents

Neo-Russian currents have a symmetrical biphasic rectangular rather than a sinusoidal waveform, with the same pulse duration and carrier frequency as Russian currents. They differ from them in that their modulation frequency can also be changed (1 to 120 Hz) and different duty cycles can be chosen other than 50%. (13).

These features offer some advantages over Russian currents. Firstly, by varying their modulation frequency, they can stimulate different types of muscle fibers, such as I, IIa, IIb and IIc (1). On the other hand, by using duty cycles lesser than 50 % the motor response is higher (16) and fatigue is less (17).

Australian or Aussie currents

In 2010, Ibramed company released a new current called Aussie in the Latin American market, which claims to be more comfortable and to induce greater force than Russian and low frequency currents. It has a sinusoidal waveform and uses, in its electro-stimulation mode, a pulse width of 1 ms, 1000 Hz carrier frequency, modulated in burst of 4 ms on-time and 16 ms off-time when modulated at 50 Hz (14). Unlike the Russian current, this one does not consider the duty cycle, as it keeps the burst time steady and modifies the pause between one and the next to vary the modulation frequency (14, 18).

In order to determine the ideal carrier frequency, a first study was conducted using 1 and 35 KHz (18). Subsequently, the experience was repeated with frequencies from 0.5 to 20 KHz with different modulations (1 to 100 % duty cycle). The results showed that to achieve maximum torque, a carrier frequency of 1 KHz with a duty cycle of 20% should be used. When comfort is the objective, the use of a carrier frequency of 2500 Hz is recommended, as it provides a good balance between maximum voluntary isometric contraction (MVIC) and comfort (17).

Ward then compared low-frequency single-phase current with conventional Russian and Aussie currents (14). In this study, he used two phase durations for the single-phase ones, one of 200 us (0.2 ms), corresponding to the phase duration of the Russian current, and another of 500 us (0.5 ms), which coincides with the Aussie one (14). A weakness found in this work is that, being these unidirectional currents, the way of connecting the electrodes should have been standardized, since the negative electrode is more depolarizing than the positive one, thus affecting the force induced in the contraction. The negative pole should be connected to the

active electrode, on the motor point (4, 11). If this is the way it was done, it has not been properly described in the methodology. (14)

So far, then, no comparison has been made between Aussie and Neo-Russian currents (with a biphasic rectangular waveform) at different duty cycles, nor with RBS, which is the waveform most commonly used by good quality portable personal electro-stimulators. Unfortunately, Ward's studies also fail to include the fatigue variable, which is of utmost importance in daily practice (14, 18).

Low-frequency currents, rectangular biphasic symmetrical

Low frequency currents differ structurally from medium frequency currents in that they are made up of only one pulse every certain time rather than of a packet of pulses like medium frequency currents. Within the low frequency currents we can find the symmetrical biphasic (charge-balanced), the unbalanced asymmetrical biphasic, the monophasic and the balanced asymmetrical biphasic. All these are also called faradic. (3) It is now suggested that, instead of using these waveforms, low frequency rectangular biphasic symmetrical (RBS) waveforms should be used, which are available in higher-quality, and therefore more expensive, portable equipment. The waveform is important, even when these currents are used for electro-analgesia in its TENS (trans-cutaneous electrical nerve stimulation) mode, because it results in better analgesic effects. (19)

According to initial research, RBS are more tolerable and induce greater force than Russian currents (5, 9, 20, 21). Other authors, however, suggest that there is no difference in discomfort rates between medium and low frequency currents (22). Fukuda, on the other hand, claims he has found no difference in the force generated by each of these currents, but the intensity reached with Russian currents, as well as their tolerance, were higher (23).

Some authors have also studied the fatigue induced by currents, their findings indicating that low frequency currents cause less fatigue, which makes them more suitable, as they maintain the induced force at similar levels during more contractions along the session (20).

Force – Maximum force induced by electrical stimulation

Force is defined as "the ability of a muscle to actively generate tension, regardless of the specific condition under which such tension is being measured (slow-speed or fast-speed contraction, shortening or lengthening condition)" (24).

When electro-stimulation is applied, an increase in muscle tension is generated, even when the patient does not engage in any voluntary contraction. In research studies seeking to find out which one is the best current, the operator increases the current intensity to the maximum tolerable by the patient and measures the Electrically-Induced Contractions (MEIC) (12, 14, 16, 20, 21, 23, 25-30). Another variable having an influence on the MEIC is the frequency of the electrical current, being it directly proportional (31).

There is a directly proportional correlation between MEIC and the strength gain of a training session. The scalar magnitude of the force variable is the Newton (N) (5).

Force measurement or dynamometry

The force variable is measured with dynamometry. There are isometric and isokinetic dynamometers. The latter allows to measure force in a concentric, eccentric and isometric manner, thus providing a greater amount of data. The isokinetic dynamometer has been used in several of the articles where MEIC and MVIC are analyzed (5, 7, 23, 25, 32). Others, on the other hand, opt to use isometric dynamometers with load cells (9, 33). It has been noted that, despite their much lower cost, they are very reliable (6, 24, 34, 35).

The methodology used with isometric dynamometry to measure the MVIC consists in performing three maximum isometric contractions of 3 seconds duration (5), with a 120-second interval between one and the other (5, 9, 36). The best of the 3 repetitions should be considered (23). The subject should be seated in a therapeutic chair with the hip at 110° (23), with the trunk well stabilized with straps (37). The knee should be placed in 90° flexion (9, 26, 38-40). The patient is instructed to cross his arms over his chest and relax (5).

The MEIC is usually normalized with the MVIC, being expressed as a percentage of the latter (%MVIC). (20, 23)

Sensory, motor and pain thresholds in electrical stimulation and their correlation with tolerance

The sensory threshold was defined as the level of applied current, slowly increased, at which the subject first perceives cutaneous sensation. The motor threshold is the current intensity required to produce a threshold contraction. The pain threshold is the intensity producing an unpleasant sensation (18). Thresholds vary in women during the different phases of the menstrual cycle, regardless of whether they use contraceptives or not; therefore, in research involving this variable it is always convenient to recruit only men in order to homogenize the sample (41).

To measure the discomfort generated by the current, the visual analogue scale (VAS) is commonly used, thus establishing a quantitative variable (20, 21).

When intensity (mA) is used as a variable, it is important to verify that the intensity reported by the equipment is the one actually emitted. This measurement is made with an oscilloscope connected to the output of the equipment, with a resistive load of 1 KOhm (42).

Fatigue

Fatigue is a multidimensional concept combining physiological and psychological aspects. In physiology, fatigue is the exercise-induced decrease in the ability to generate strength due to central and peripheral changes. The movement itself is preceded and accompanied by brain activities related to the preparation and execution of the movement, which have been correlated with the perception of effort (43).

Muscle-specific (peripheral) fatigue can be measured using electro-stimulation through the MEIC (43).

Fatigue in electrical stimulation

During the application of NMES the force decreases. Some authors claim that the magnitude of fatigue is influenced by pulse width, frequency and intensity of electro-stimulation. As frequency is increased, the MEIC increases as well, which in turn increases metabolic consumption, thus generating fatigue (31).

Several authors have studied the effect of electro-stimulation with surface electrodes on fatigue, finding that medium frequency currents generate more fatigue than low frequency ones (5, 20).

There are various experimental models to study peripheral fatigue, but the one most used consists of applying electrical stimulation, reducing the central component (31).

There is no doubt that Laufer was one of the authors who researched on electro-stimulation-induced fatigue the most. To this end, she used a protocol which starts by measuring the MVIC to normalize the data. Then, she measures the MEIC during 21 repetitions, considering the first contraction as a hundred percent and the following contractions as a percentage of the first one (17). The next step is counting the number of contractions that were equal to or less than 50% of the initial strength, thus determining which current is more fatiguing (20).

Another option for calculating this variable is the determination of the Fatigue Index (FI), which consists of describing the percentage of reduction of the force induced between the first phase

(Pinit) and last phase (Pfinal), where a higher value indicates less fatigue and an FI = 1 implies that no fatigue has occurred. This formula is: FI=1-(Pinit-Pfinal)/Pinit (44).

OBJECTIVES

General

1. To compare induced isometric force, tolerance and fatigue in healthy subjects from 18 to 30 years of age, with Neo-Russian, Aussie and RBS currents, in the period from 11-30-2017 to 12-30-2017.

Specific

1.1. To evaluate the isometric force induced by Neo-Russian, Aussie and RBS currents by means of isometric dynamometry in healthy subjects from 18 to 30 years of age.

1.2. To compare the isometric force induced in healthy subjects aged 18 to 30 years by the different currents in order to determine which one induces greater force.

1.3. To evaluate the tolerance of Neo-Russian, Aussie and RBS currents using the Pain Visual Analogue Scale (VAS) in healthy subjects from 18 to 30 years of age.

1.4. To compare tolerance in healthy subjects aged 18-30 years to the three types of current in order to determine which one is less unpleasant.

1.5. To evaluate fatigue upon application of Neo-Russian, Aussie and RBS currents by means of a fatigue test with isometric dynamometry in healthy subjects from 18 to 30 years of age.

1.6. To compare the fatigue induced by the different currents in healthy subjects between 18 and 30 years of age in order to determine which of the three produces less fatigue.

MATERIALS AND METHODS

a. Design type

Experimental, crossover, randomized study (45).

b. Location

The data collection was carried out in the "*Ronzio Terapia Física Especializada*" Center, located at Mendoza 2096, 2 B, in the Autonomous City of Buenos Aires, Argentina, in the period from 11-30-2017 to 12-30-2017.

c. Universe

Students pursuing a degree in Kinesiology and Physiatry at the Instituto Universitario de Ciencias de la Salud Fundación H.A. Barceló (Autonomous City of Buenos Aires, Argentina), at Universidad Maimónides (Autonomous City of Buenos Aires, Argentina) and at the Universidad Nacional Arturo Jauretche (Florencio Varela, Buenos Aires, Argentina).

d. Population

Composed of 255 individuals, male (5, 41, 46), students pursuing a degree in Kinesiology and Physiatry at the Instituto Universitario de Ciencias de la Salud Fundación H.A. Barceló (Autonomous City of Buenos Aires, Argentina), at Universidad Maimónides (Autonomous City of Buenos Aires, Argentina) and at the Universidad Nacional Arturo Jauretche (Florencio Varela, Buenos Aires, Argentina).

e. Sample

Composed of 30 individuals according to the sample size estimate.

f. Sample size

To determine the required sample size, a pilot test was carried out prior to the study, in which the 3 types of electrical stimulation were randomly applied to 5 subjects. In order to estimate the variability of the sample, the global standard deviation of the 15 measurements was considered (SD=19.58%). A sample size for a Two-tailed T-Test was estimated, assuming an alpha error of 0.05, a power of 0.8 and a delta of 10%. For a crossover design only 1/2 of the calculated sample size is required, since the same subject contributes to all 3 groups. This results in a sample size of 30 subjects (47). The distribution coefficient was 1:1. (48)

g. Sampling:

Consecutive non-probability.

h. Criteria

a. **Inclusion:** Available, healthy, male volunteers between 18 and 30 years of age, with right-dominant lower limb, who are physically active (9, 41).

b. **Exclusion:** Having a right-dominant lower limb injury, skin lesions in the quadriceps area, having a pacemaker, cardiovascular or neurological diseases (18, 25). Subjects who have exercised 72 hours before the protocol (49, 50).
c. **Elimination:** Missing one or both appointments for evaluation, the patient's own decision to abandon the protocol while it is being carried out, intolerance to the procedure (51).

i. **Randomization:**

In order to minimize the potential bias of the order in which interventions are initiated (e.g., the effect of fatigue), the randomization sequence was generated prior to starting work (9, 23, 25, 52). The number 2 generator, available at http://www.randomization.com/, was used for situations where subjects must receive all interventions in random order, with balanced permutations, forming 5 x 6 blocks for the three currents of interest (53). The results of said distribution were as follows:

1. Aussie - RBS - Neo-Russian.
2. RBS - Neo-Russian – Aussie.
3. Aussie - Neo-Russian - RBS.
4. RBS – Aussie - Neo-Russian.
5. RBS - Neo-Russian – Aussie.
6. Aussie - RBS - Neo-Russian.
7. Aussie - Neo-Russian - RBS.
8. Aussie - Neo-Russian - RBS.
9. Neo-Russian – Aussie - RBS.
10. Neo-Russian – Aussie - RBS.
11. Aussie - RBS - Neo-Russian.
12. Neo-Russian – Aussie - RBS.
13. Neo-Russian - RBS – Aussie.
14. Aussie - Neo-Russian - RBS.
15. Neo-Russian - RBS – Aussie.
16. Neo-Russian - RBS – Aussie.
17. Aussie - RBS - Neo-Russian.
18. RBS - Aussie - Neo-Russian.
19. RBS - Aussie - Neo-Russian.
20. Aussie - RBS - Neo-Russian.

21. Neo-Russian – Aussie - RBS.
22. Neo-Russian - RBS – Aussie.
23. Neo-Russian - RBS – Aussie.
24. Neo-Russian – Aussie - RBS.
25. RBS - Aussie - Neo-Russian.
26. RBS - Neo-Russian – Aussie.
27. Aussie - Neo-Russian - RBS.
28. RBS - Aussie - Neo-Russian.
29. RBS - Neo-Russian – Aussie.
30. RBS - Neo-Russian – Aussie.

j. Blinding

The subject was blindfolded so that he would not watch the procedure. The study subject did not know the type of current applied and the operator was alien to the work and unaware of the hypothesis. The database was coded in order to conceal the interventions from the statistical analyst (25, 45).

k. Ethical aspects

This protocol was submitted to the Instituto Universitario De Ciencias De La Salud, Fundación H. A. Barceló as a research project and was approved by resolution number HSC 5745. In addition, it has the relevant clinical trial registration at Clinical Trials U.S.A. (www.clinicaltrials.gov), identifier: NCT03340337.

Participants were given a written document entitled "Information letter and Written Consent for Volunteer's Participation" and also an "Informed Consent" document explaining the objectives and purposes of the study, the experimental procedures, any known short or long-term risks, possible discomforts; benefits of the procedures applied; duration of the study; suspension of the study in the event of negative effects or when sufficient evidence of positive effects does not justify continuing with the study, and the subjects' freedom to withdraw from the study at any time they want. The document also indicated that the information provided by all participants in the study will be kept confidential in the event that the results are presented in scientific events and/or published. In case of acceptance, the subject proceeded to sign said documents.

l. Procedures

a. Checking the emission of the equipment and measuring the intensity

The emission of the equipment was corroborated by connecting a resistive load of 1 Kohm to a PC-based oscilloscope Owon brand, model VDS2062 of 60MHz and 100MHz bandwidth, then measuring the current intensity. (42) It was also verified that the waveforms emitted by the equipment correspond to the theoretical characteristics of the currents studied.

During the MEIC measurement, the intensity shown on the equipment's display was recorded and the real intensity was checked on the oscilloscope, considering the latter. Some equipment show on their display the peak to peak (PP) intensity, therefore, the value from the isoelectric line to the peak of the positive phase, called Vamp, was considered for all of them. (54)

Since the oscilloscope reports voltage, Ohm's law was used to calculate the intensity. (3, 11, 55)

b. Measuring MVIC

This data was used for normalization of the MEIC. A short 10-minute walk was taken to warm-up (23). An isometric dynamometer with load cell and computer interface, Fisiomove® brand, Isoforce® model, was used. The patient was positioned in a therapeutic chair with the hip at 110° (23, 31), stabilizing the trunk (at chest level) and the hip (at the level of the anterior superior iliac spines) with straps (37). The knee was placed in 90° flexion (9, 26, 31, 38-40). A goniometer was used to correctly position the joints at said angles (12). The patient was instructed to cross his arms over his chest and relax (5). The subject was blindfolded to prevent him from watching the procedure. The measuring system was attached to the distal end of the right lower limb by means of an ankle brace. The subject was instructed to perform 3 maximum 3-second contractions with an interval of 120 seconds between each of them (9, 23). The best of the 3 repetitions was considered (31, 52). Whenever the third one was the best, additional measurements were taken until a decrease in torque was obtained in order to determine the maximum (56).

Work and rest times were controlled by a chronometer and also by the dynamometer software. The best of the three contractions and, when necessary, the request for additional repetitions to obtain the best one, was automatically determined by the software, which also served to store the data that was later transferred to an Excel 2016 table.

c. Detecting motor points and positioning electrodes

The motor points were detected for the correct positioning of the electrodes, which allows to increase the generated force (9, 22). First, the skin was cleaned with a

cotton soaked in alcohol. To detect the points, a Globus brand 600 Pro model equipment was used, in which an ad hoc program was executed, with continuous emission at 5 Hz, 200 us. In order to locate the motor point of the internal vastus, a self-adhesive dispersive electrode of 2" x 4" (5.08 x 10.16 cm) was placed on the proximal third of the right thigh, to then search for it with the active electrode – metal strut-type, wrapped with cotton wool soaked in tap water and covered with conductive gel- until determining the point of greatest contraction at the same intensity. For the motor point of the anterior rectus, a self-adhesive dispersive electrode was placed on the motor point of the internal vastus of the right thigh, to then search for it with the active electrode, -metal strut-type, wrapped with cotton wool soaked in tap water and covered with a conductive gel- until determining the point of greatest contraction at the same intensity.

Once these points were marked, unused self-adhesive electrodes -Pro-Patch® brand, model ProM-030, 2" x 4" (5.08 x 10.16 cm) 2, 57)- were placed on them (52, 57). Two electrodes were used for each patient.

d. Measuring MEIC

After measuring the MVIC, the subject was allowed to rest for 120 seconds (while searching for motor points), to then perform the MEIC assessment with all three types of electrical currents, which were applied in random order so that the effects of one current would not influence the results of the next (23, 25). The randomization sequence is the one mentioned above, generated through www.randomization.com (53).

The same isometric dynamometer was used, positioning the subject as indicated above. (5, 9, 12, 23, 26, 31, 37-40).

The patient was asked not to make any voluntary contraction or collaborate in any way, but just let the electrical stimulation generate the contraction (20).

With each type of current, the intensity was increased until it reached the maximum tolerable by the subject, which situation was communicated to the operator (23, 25, 32, 52). Upon reaching said intensity, it was noted down accordingly, and then 3 contractions of 3 seconds duration were measured by isometric dynamometry, with a 120-second pause between them. Once the 3 contractions with the first current were measured, the second and then the third type of electro-stimulation followed, in the order as randomly determined (9, 23, 25, 52). An interval of 120 seconds was also taken between the determinations of the MEIC with each current, in order to minimize fatigue (9). The best of the 3 repetitions was considered (9, 23, 31, 52).

Whenever the third one was the best, additional measurements were taken until a decrease in torque was obtained in order to determine the maximum (56).

Work and rest times were controlled by a chronometer and also by the dynamometer software. The best of the three contractions and, when necessary, the request for additional repetitions to obtain the best one, was automatically determined by the software, which also served to store the data that was later transferred to an Excel 2016 table.

The following parameters were used to perform the MEIC tests (Table 1):

VARIABLE	NEO-RUSSIAN	AUSSIE	RBS
Emission mode	Synchronic	Synchronic	Synchronic
Waveform	Rectangular biphasic symmetrical	Sinusoidal	Rectangular biphasic symmetrical
Carrier frequency	2500 Hz	1000 Hz	-
Phase duration	200 us	500 us	200 us
Pulse width	400 us	1000 us	400 us
Modulation frequency	50 Hz	50 Hz	50 Hz
Duty cycle	50 %	20 %	-
Burst duration	10 ms.	4 ms.	-
Ramp-up	1 sec.	1 sec.	1 sec.
On time	3 sec.	3 sec.	3 sec.
Ramp-down	1 sec.	1 sec.	1 sec.
Rest time	120 sec.	120 sec.	120 sec.

Table 1: Parameters for electrical stimulation during MEIC measurement.

e. Normalizing MEIC data

In order to normalize the data, MEIC will be expressed as percentage of MVIC (% MVIC) (17, 20, 23, 52, 58).The calculation was done in an Excel 2016 table. The formula was the following:

$$\% \text{ MVIC} = (\text{MEIC} / \text{MVIC}) \times 100$$

f. Determining tolerance to each type of electrical stimulation

After recording the MEIC with each current, the subject was given a VAS scale and was asked to indicate how unpleasant he found the electro-stimulation, with 0 (on the left) being "no pain" and 10 (on the right) the equivalent of "crying in pain". Numerical data from the VAS was transferred to a Microsoft® Excel® 2016 table (20, 21, 57, 59).

Although an uncomfortable stimulus may not necessarily generate less force, what is sought is balance between these variables (14). That is why, considering that a current is more effective for electro-stimulation the more force it induces and the less discomfort it generates, the coefficient between the % MVIC and the VAS was calculated, thus establishing the "ratio" value through the following formula:

$$\text{Ratio} = \% \text{ MVIC} / \text{VAS}$$

g. Measuring fatigue

The fatigue induced by the three types of electrical stimulation was measured after one week of washout period (20).

The same isometric dynamometer was used, positioning the subject as indicated above. (5, 9, 12, 23, 26, 31, 37-40).

The motor points were detected and two unused self-adhesive electrodes were placed on them (9, 22, 52, 57).

The patient was instructed to cross his arms over his chest and to relax (5). The subject was blindfolded to prevent him from watching the procedure. The studied subject did not know the order of the procedures applied and the evaluator was not aware of the working hypothesis, thus defining a double-blind study (25, 45). The measuring system was attached to the distal end of the right lower limb by means of an ankle brace (9).

The patient was asked not to make any voluntary contraction or collaborate in any way, but just let the electrical stimulation generate the contraction (20). The intensity was increased until it reached the maximum tolerable by the subject, which situation was communicated to the operator (23, 25, 32, 52). Data was taken from the MEIC of 21 contractions (20), consisting of 1 second of ramp-up, 3 seconds of contraction, 1 second of ramp-down (60) and 5 seconds of rest time. The dynamometer was configured to collect data for 320 seconds and then the peak values of each of the 21 contractions were manually reviewed and transferred to Microsoft® Excel® 2016.

This variable was identified as maximum voluntary isometric contraction in fatigue test (MEIC-F).

The following parameters were then used to perform the fatigue test (Table 2):

VARIABLE	NEO-RUSSIAN	AUSSIE	RBS
Emission mode	Synchronic	Synchronic	Synchronic
Waveform	Rectangular biphasic symmetrical	Sinusoidal	Rectangular biphasic symmetrical
Carrier frequency	2500 Hz	1000 Hz	-
Phase duration	200 us	500 us	200 us
Pulse duration	400 us	1000 us	400 us
Modulation frequency	50 Hz	50 Hz	50 Hz
Duty cycle	50 %	20 %	-
Burst duration	10 ms.	4 ms.	-
Ramp-up	1 sec.	1 sec.	1 sec.
On time	3 sec.	3 sec.	3 sec.
Ramp-down	1 sec.	1 sec.	1 sec.
Rest time	5 sec.	5 sec.	5 sec.

Table 2: Parameters for electrical stimulation during fatigue test.

h. Normalizing data from the fatigue variable

Each repetition was expressed as a percentage of MVIC, using the acronym %MVIC-F, with the following formula:

$$\% \text{ MVIC-F}_x = (\text{MEIC-F}_x \text{ x } 100)/\text{MVIC}$$

Where "$_x$" is the repetition number.

i. Determining fatigue

After normalization and determination of %MVIC-F_x, repetition 1 (%MVIC-F_1) was considered to be 100 %. This variable was coded as R_1. Then, it was calculated what percentage of R_1 the subsequent repetitions corresponded to (where "$_x$" is the repetition number: $_x$ = 2, 3..., 21), by means of the following formula:

$$R_x = (\%MVIC\text{-}F_x \times 100) / MVIC\text{-}F_1$$

The number of repetitions equal to or lower than 50% was then counted, thus determining the fatigue induced by each type of electrical stimulation (20).

i. **Calculation of the fatigue index (FI):** The data collected during the fatigue test of the %MVIC-F were transferred to Microsoft® Excel® 2016 and the percentage decrease of the first as compared to the last contraction was calculated using the following formula (61):

$$FI = 1 - (\%MVIC\text{-}F_1 - \%MVIC\text{-}F_{21}) / \%MVIC\text{-}F_1$$

m. Flowchart

The experimental design is summarized in Figure 1:

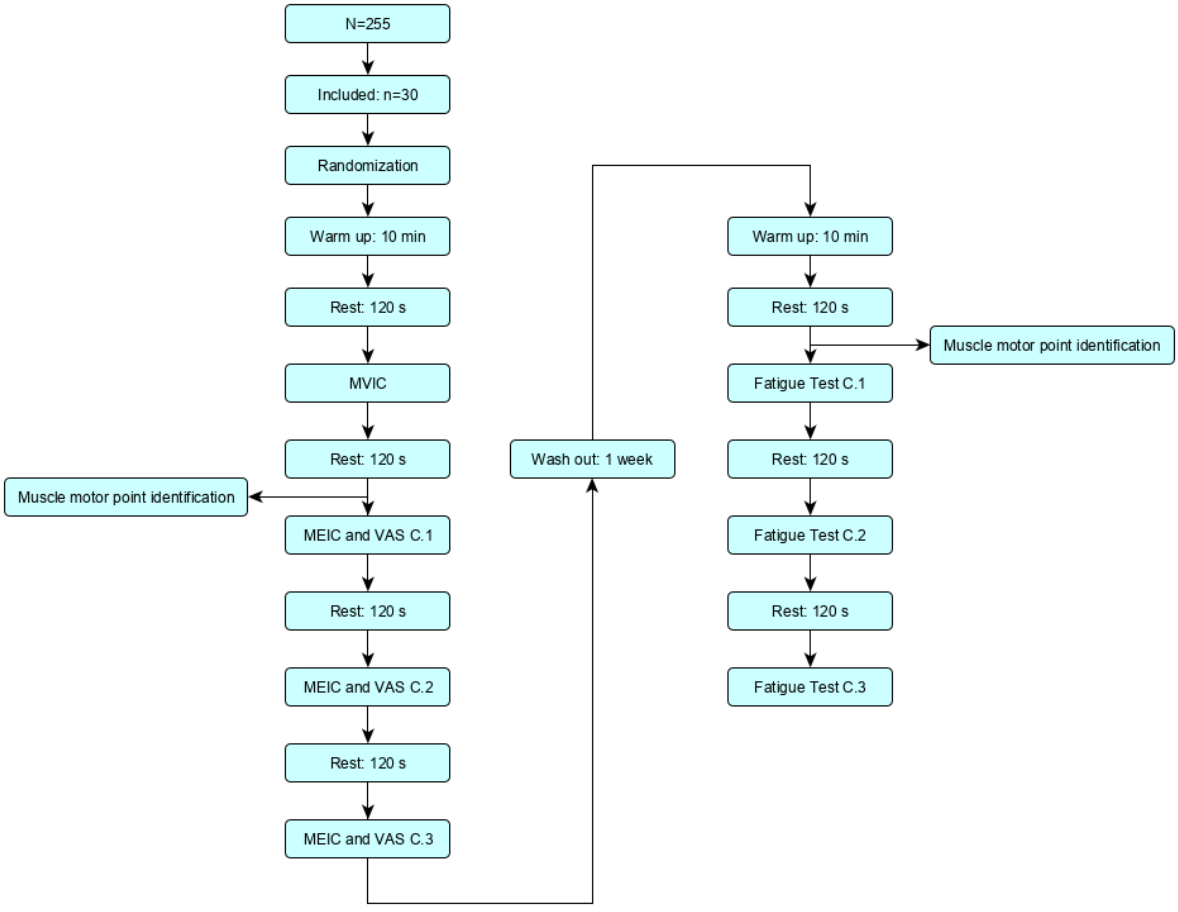

Fig. 1: Flowchart of the experimental design.

n. Statistical processing of data

The systematization was computerized and quantitative. The data was transferred to Microsoft® Excel® 2016, creating a database in which the data, tables and graphs were

normalized. IBM SPSS software, version 22.0 (IBM Corp., Armonk, NY, USA) was used for data analysis. A p-value < 0.05 was considered significant.

Continuous variables were expressed with their mean and standard deviation when assuming a normal distribution. Otherwise, the median and the interquartile range (IQR) were reported. Categorical variables were reported with their frequency and percentage.

To meet the specific objectives in 1.2, 1.4 and 1.6, an analysis of the variance of a repeated measurement factor was performed in order to determine the effect of three wave types (Aussie, NR and RBS) on the %MVIC, tolerance level and fatigue. Independent analyses were performed for each outcome variable. Bonferroni correction was applied for the *a posteriori* analyses, as appropriate. Results were expressed as mean difference and their respective 95% CI. The assumption of normality was evaluated using the Shapiro-Wilk test. The presence of outliers was determined by visual inspection of the box charts. The sphericity assumption was evaluated by means of the Mauchly test. When it was not possible to apply the parametric ANOVA test, the non-parametric Friedman test and the Wilcoxon test for Bonferroni-corrected samples were used for multiple comparisons.

To meet the specific objective in 1.2, it was analyzed which current shows the highest value of %MVIC (%MVIC-Neo-Russian vs. %MVIC-Aussie vs. %MVIC-RBS).

In order to comply with the specific objective in 1.4, it was analyzed to which of the currents the subjects referred a lower VAS value (VAS-Neo-Russian vs. VAS-Aussie vs. VAS-RBS) and which of the currents obtained a better ratio, calculated by using the formula mentioned above (Ratio-Neo-Russian vs. Ratio-Aussie vs. Ratio-RBS).

To meet the specific objective in 1.6, a comparison was made considering the number of repetitions ≤ 50% of the initial value between the 3 different waves. No assumption of normality was made at any of the levels as assessed by the Shapiro-Wilk test ($p<0.001$) for which the Friedman test was used for related samples. For post hoc comparisons the Wilcoxon Test for related samples was used with Bonferroni correction for multiple comparisons (R ≤ 50%-Neo-Russian vs. R ≤ 50%-Aussie vs. R ≤ 50%-RBS). In addition, a comparison was made between %MVIC-F_1 vs. %MVIC -F_{21} using the Wilcoxon Test for paired samples.

To compare fatigue rates between AU, RS and RBS groups, the Friedman non-parametric test was used with the Wilcoxon test for post hoc comparisons. Bonferroni correction was applied for post-hoc comparisons.

SOURCES AND INSTRUMENTS

- Notebook with Windows, Microsoft Office 2016 package, SPSS 22, Endnote X7.
- Owon PC-based oscilloscope, model VDS2062 60MHz and 100MHz bandwidth.
- Fisio-Move® brand computerized digital isometric dynamometer, model Isoforce® with its corresponding accessories and software.
- Bench made ad-hoc to fasten the dynamometer and position the patient.
- Velcro straps.
- Blindfolding eye mask.
- Constant current electrical stimulator, Neo-Russian current transmitter, Ibramed® brand, Neurodyn II® model.
- Constant current electrical stimulator, Aussie® current transmitter, Ibramed® brand, model Neurodyn Aussie Sport®.
- Constant current electrical stimulator, RBS wave transmitter, Globus® brand, model 600 Pro®.
- Self-adhesive electrodes, Pro-Patch® brand, model ProM-030, 2" x 4" (5.08 x 10.16 cm).

RESOURCES

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