ORIGINAL ARTICLE

Muscle damage induced by neuromuscular electrical stimulation (NMES) with frequencies of 30 Hz and 100 Hz

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ABSTRACT

Neuromuscular electrical stimulation (NMES) is the action of therapeutic electrical stimulation on muscle tissue in order to contract the muscle and consequently improve the muscle status. **Objective:** To evaluate the muscle damage stemming from isometric muscle contraction induced by NMES of low frequency (30 Hz) and high frequency (100 Hz). **Methods:** Experimental crossover study, randomized, unblinded. The study included 10 male college students, age 24.4 ± 6.0 years, weight 77.1 ± 11.8 kg, height 176.1 ± 5.6 cm, and BMI of 24.8 ± 3 4 kg/m^2. Two protocols (A) and (B) with an interval of 7 days between them. (A) - 20 minutes of NMES in the quadriceps at a frequency of 30 Hz (B) - 20 minutes of NMES at a frequency of 100 Hz. Measured lactate, creatine phosphokinase and lactate dehydrogenase before, immediately after, and 6 and 48 hours after the protocols. **Results:** Comparing 30 Hz *vs.* 100 Hz the following were observed: lactate (23.7 ± 6.7 *vs.* 13.4 ± 3.0 mg/dl, $p = 0.001$); CPK (195.4 ± 116.1 *vs.* 262.9 ± 153.6 IU, $p = 0.022$); LDH (374.3 ± 64 *vs.* 366.6 ± 84.1 IU, ns). The perception of contractile efficiency decreased significantly ($p = 0.016$) in the 100 Hz Protocol. **Conclusion:** Both the low-frequency NMES (30 Hz) and the high-frequency (100 Hz) elevate blood markers of muscle damage, most strikingly at the higher frequency. However, the achieved values reflect a normal response to a moderate-intensity exercise.

Keywords: Electric Stimulation, Muscle, Skeletal, Creatine Kinase, Lactate Dehydrogenases

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INTRODUCTION

Neuromuscular electrostimulation (NMES) is the action of therapeutic electrical stimuli applied to the muscle tissue seeking muscle contractions that produce forces between 25 and 90% of maximum voluntary contraction, in healthy as well as unhealthy individuals.[1-3] Its therapeutic use seeks primarily to accelerate the healing processes, gain muscle strength, improve general physical condition, and improve quality of life. It shows positive results regarding regaining strength, endurance, and muscle hypertrophy.[1]

Two important parameters - intensity and frequency - can be modulated for an efficient production of force during the application of NMES. The frequency is the rate of pulses per second that are applied to the muscle and it is expressed in pulses per second (pps) or in Hertz (Hz). Studies have demonstrated that low-frequency NMES (NMES-lf) promotes an improvement in localized muscle endurance,[4] whereas high frequency NMES (NMES-hf) improves muscle strength.[5] Some authors established that the structure of the muscle fibers changes after prolonged stimulation as a function of the frequency used; in other words, NMES-lf (< 50 Hz) induces a transformation in the muscle fibers from a fast (glycolytic) to slow (oxidative),[6-8] whereas stimulation at high frequency (over 50 Hz), although not well documented, induces a transformation from slow fibers to fast fibers.[8-11] In this way the frequency used in NMES is an extremely relevant factor in achieving the therapeutic or sports objectives (strength and/or muscle endurance).

However, the muscle contractions induced by NMES are, in principle, subject to the same injury mechanisms present in voluntary exercises,[12] in that they can contribute negatively to rehabilitation and training programs. Studies have compared muscle damage from NMNES with damage incurred by isometric exercises,[13] eccentric exercises,[14] and between different modalities of electrical current therapies.[15] However, there is no data giving the same attention to the use of different frequencies of NMES.

OBJECTIVE

This study sought to evaluate muscle damage stemming from isometric muscle contraction induced by NMES of low frequency (30 Hz) and high frequency (100 Hz).

METHOD

This was a crossover study, randomized and unblinded, and was approved by the Ethics In Research Committee from the University of Santo Amaro. All the participants were informed as to the procedures and the risks involved, and later signed the terms of free and informed consent.

The sample was made up of eight untrained, healthy, male college students not users of any type of medication and with no recent history of any osteo-muscular disease. The selection of the group sample was random and voluntary following verbal invitation and acceptance.

The sample population went through two experimental stages with a one-week interval in between. In Step I the individuals went through 20 minutes of neuromuscular electrostimulation at a frequency of 30 Hz, and in Step II they were submitted to neuromuscular electrostimulation at a frequency of 100 Hz. The extensor musculature of the knee was stimulated via four electrodes positioned on the medial third and the proximal portion of the belly of the vastus lateralis muscle, on the medial third of the vastus medialis muscle, and on the proximal portion of the rectus femoris. The Dualpex® 961 Sport pulse generator (Quark Produtos Médicos, Brazil) was used with a symmetrical biphasic square-wave pulse of 500 µsec, a frequency of either 30 Hz or 100 Hz, and with an on/off cycle of 10"/10". Self-adhesive electrodes were used of the brand PALS®, Platinum model, in a 5 x 9 cm rectangular format. At the beginning of the electro-stimulation (ES) phase, the highest current intensity tolerable by the volunteers was used and was readjusted every five minutes. The average initial and final intensity values are presented in Table 1.

In both phases (30 Hz and 100 Hz) an IV was positioned in the forearm vein of the dominant arm for the evaluation of Lactate, Lactate Dehydrogenase (LDH), and Creatine Kinase (CK). The individuals were kept seated with a 75º flexion of the hip joint and semiflexion of the knee joint for 15 minutes, followed by the collection of an 8 ml blood sample that was labeled Rest Phase. After the Rest Phase, the individuals underwent 20 minutes of NMES, and immediately thereafter another 8 ml blood sample was collected, named NMES Phase. At the end of the ES phase, the individuals remained at rest for another 20 minutes in what was called the Recovery Phase. More blood was collected 6 and 48 hours after the NMES for evaluation of CK and LDH.

For analysis of lactate, CK, and LDH, a spectrophotometer was used (Cobas Mira Plus; Roche®). To analyze lactate, a 3 ml sample of venous blood was used in a fluoride tube (kit from Katal Biotecnologia®). Reference reading of venous blood at Rest: 5.7 to 22.0 mg/dl.[16] For the CK, 1 ml of heparinized venous plasma was used (kit from Bioclin®). Reference reading of venous plasma at Rest: men = 24 to 195U/l.[17] For measuring LDH, 1.0ml of heparinized venous plasma was used (kit from BioTécnica®). Reference reading of venous plasma at Rest: 225 to 450 U/l.[17]

During the Rest, NMES, and Recovery phases the pulse, blood pressure, and discomfort were measured every five minutes using the 10-point Berg category-ratio (CR10). The circumference and skin folds were measured at the end of each phase, using a distance reference of 5 to 20 cm above the upper pole of the patella of both lower limbs. The circumference was measured using a tape measure and, for the skin folds, a caliper of the brand Accu-measure.

The normality of the data was verified by means of the Shapiro-Wilk normality test. The variables studied are represented by their measurements and standard deviation. The Anova was used to compare Phases I (30 Hz) and II (100 Hz) and between the moments, followed by the Scheffe post hoc and the *Student-t* tests for parametric data. Non-parametric data was compared using the Kruskal-Wallace test followed by the post hoc pairwise comparisons and the Wilcoxon test.

The SPSS 20.0 software (SPSS™, Chicago, IL) was used for statistical analysis adopting a level of significance of 5% ($p < 0.05$).

RESULTS

The evaluated individuals showed the following characteristics: age 24.4 ± 6.0 years, weight 77.1 ± 11.8 kg, height 176.1 ± 5.6 cm, and BMI 24.8 ± 3.4 kg/m^2. Table 1 shows the anthropometric alterations and the adjustments in NMES. Significant alterations were

Table 1. Anthropometric alterations and adjustments in the NMES parameters

	30 HZ		100 Hz		ANOVA
	Initial	Final	Initial	Final	*p* =
Proximal circumference (cm)	56.5 ± 3.9	58.1 ± 4.0	57.0 ± 3.5	58.8 ± 3.7	0.670
Distal circumference (cm)	44.9 ± 3.6	46.4 ± 3.4	45.0 ± 3.3	46.5 ± 3.1	0.662
Proximal SF (mm)	20.0 ± 4.7	20.0 ± 4.7	21.0 ± 5.2	21.0 ± 5.2	0.954
Distal SF (mm)	17.3 ± 3.8	17.3 ± 3.8	17.3 ± 4.1	17.3 ± 4.1	0.998
Intensity (mA)	36.8 ± 8.7	51.8 ± 8.9	30.8 ± 8.8	45.3 ± 9.5	0.001
IP $(mA.ms)^{-3}$	18.4 ± 4.3	25.9 ± 4.4	15.4 ± 4.4	22.6 ± 4.8	0.001

Data presented as average ± standard deviation. SF: Skin Fold; IP: product Intensity *vs.* Pulse. Variance analysis by ANOVA

only observed in the NMES adjustments and, at both frequencies, there was a significant increase in intensity between the initial and final NMES (30 Hz: 36.8 ± 8.7 *vs.* 51.8 ± 8.9, $p = 0.023$) and (100 Hz: 30.8 ± 8.8 *vs.* 45.3 ± 9.5, $p = 0.029$). The same behavior was observed in the product Intensity *vs.* Pulse (IP) (30 Hz: 18.4 ± 4.3 *vs.* 25.9 ± 4.4, $p = 0.023$) and (100 Hz: 15.4 ± 4.4 *vs.* 22.6 ± 4.8, $p = 0.029$).

The absolute values of CK alterations are shown in Figure 1A. In the 30 Hz protocol increases of 14.2 ± 8.5% and 37.4 ± 13.4% were seen, and in the 100 Hz protocol there were increases of 28.4 ± 53.6% and 85.0 ± 55.1%. Figure 1B shows the data after 6 and 48 hours. Differences between the 30 Hz and 100 Hz protocols were only observed after 48 hours, as much in absolute numbers as in delta percentage.

The changes in LDH in absolute numbers are seen in Figure 2A. Figure 2B shows the LDH rising 9.5 ± 2.7% and 26.3 ± 14.3% in the 30 Hz protocol, and 30.2 ± 16.7% and 41.2 ± 16.7% in the 100 Hz protocol after 6 and 48 hours, respectively. Differences between the 30 Hz and 100 Hz protocols were not observed when absolute numbers were analyzed. However, upon analyzing the delta percentages, greater increases were observed in the 100 Hz protocol, whether at 6 hours or 48 hours post-NMES (Figure 2B).

Significant elevations of lactate were seen in both protocols. Differences between the protocols were present in absolute numbers (Figure 3A) as well as in delta percentage (Figure 3B).

No significant differences were observed in perceived discomfort (CR10 - Borg) either when the moments or the protocols were compared (Figure 4A). However significant differences were observed in the perception of contractile efficiency (Figure 4B).

DISCUSSION

The main findings of the present study were: i - the significant increase of muscle damage markers (LDH and CPK) after the application of NMES; ii - the more accentuated increase of CPK in the NMES-hf; and iii - the significant increase of lactic acid with NMES-lf.

Increase in muscle damage markers

The increase in muscle damage markers is a common response to voluntary or electrically induced exercise. Various studies have observed important histological alterations such as disruption of the Z-line, infiltration of macrophages, and damage to the desmin filaments.[12,13,18] It is necessary to observe that the studies from Aldayel et al.[12] and Mackey et al.[18] use, respectively, 45 minutes of NMES and 180 muscle contractions-an exercise load that, regardless of the use of NMES, would be enough to cause muscular micro-lesions.[19]

However, Jubeau et al.[13] compared 40 muscle contractions induced by NMES with 40 voluntary contractions of the same torque, and found greater values of CPK with NMES. Similarly, one study that compared 50 muscle contractions either voluntary or induced by NMES observed, immediately after the contractions, a greater decrease in the force generated in the MVC.[20]

This reduction of force obtained in the maximal voluntary contraction or in any other mode of muscle contraction is considered to be the best marker of muscle damage.[21] In this way, the increase of blood markers of muscle damage appear to be a normal response after contractions induced by NMES.

Although the elevated CPK and LDH reflect some degree of cell membrane disruption or alteration of its permeability, it does not necessarily indicate muscle damage. There is a consensus that an elevation in CPK after physical exercise of up to 500 IU/L is considered normal.[22] The inability to classify the alterations of CPK and LDH in the present study as damage becomes more evident as we observe studies that evaluate the response to exhausting and high intensity physical exercise.

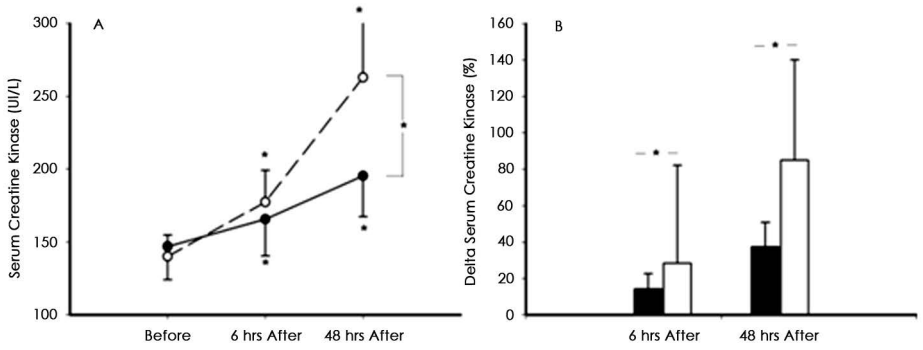
After prolonged exercises such as marathon or triathlon tryouts, the LDH levels double and the CPK levels can go up by a factor of 15, and may remain altered for weeks.[23] After high-intensity resistance exercises values of CPK have been seen of 1,349 IU for active individuals and 3,272 IU for sedentary.[22] In light of this, the data from the present study indicate normal responses in the elevations of CPK and LDH after NMES.

High *versus* low frequency in NMES

Comparing high and low frequency, the NMES-hf gave a more accentuated rise in CPK and LDH (Figure 1). The following mechanisms could explain this finding: i - the force-frequency relationship shows that a higher frequency results in higher force readings,[24] and a consequent increase in mechanical stress on the muscle fiber; ii - studies have demonstrated that, regardless of random recruitment and spatial dependence, NMES can present some degree of selective recruitment among the motor units.

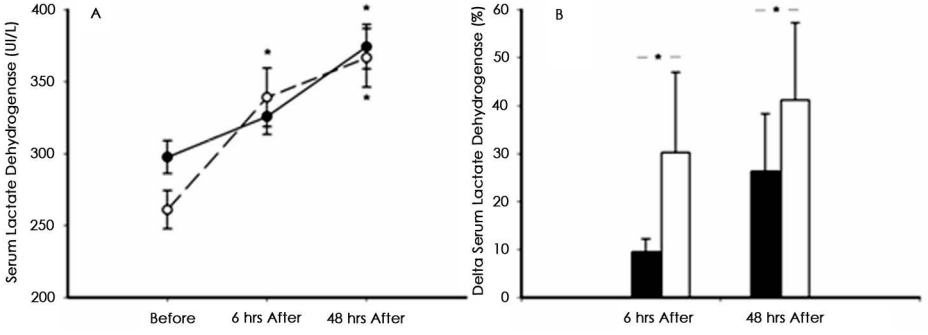
Cabric et al.[25] observed, in type-II fibers, an increase in the size of the fiber, the nuclear volume, and an increase in the mitochondrial fraction when submitting young college men to NMES-hf.[25] A preferential recruitment of fast-twitch motor units (type-II fibers) was also observed by Trimble et al.[26] The relevance of preferential recruitment of type-II fibers in NMES-hf to the results of the present study resides in the fact that type-II fibers, with low oxidative capacity, are more susceptible to damage after physical exercise.[27]

It has already been documented that a non-selective recruitment sequence, spatially dependent, and the synchronic pattern of the motor units, when used by NMES, contribute to a more rapid fatigue and important metabolic differences when compared to voluntary exercises for the same generated torque.[28] However, no significant differences have been observed in various systemic or local parameters such as VO_2, ventilation, pulse



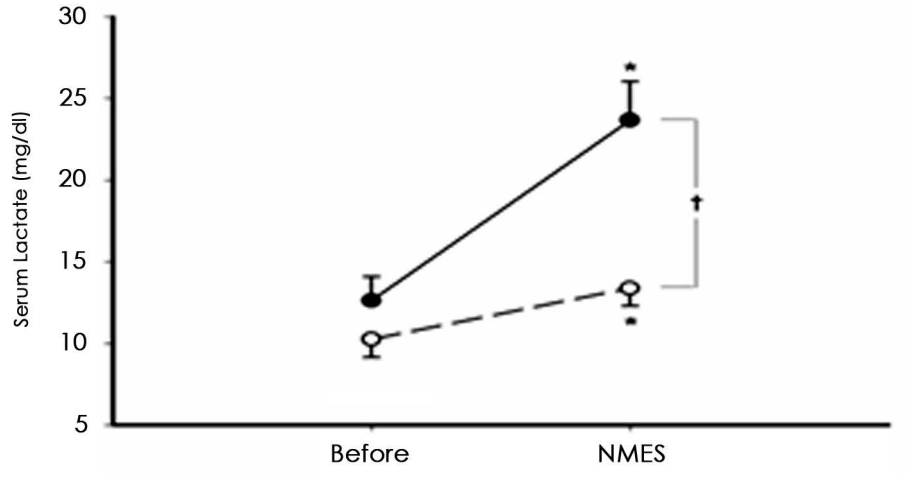
Black symbols for 30 Hz and white symbols for 100 Hz. Note the significant differences between the CPK deltas in the protocol with 100 Hz (B). * $p < 0.05$ in the intergroup analysis compared to the Rest values. † $p < 0.05$ in the analysis between groups

Figure 1. Behavior of serum Creatine Kinase as a function of time (A)



Black symbols for 30 Hz and white symbols for 100 Hz. Note the significant differences between the LDH in the protocol with 100 Hz (B). * $p < 0.05$ in the intergroup analysis compared to the Rest values. † $p < 0.05$ in the analysis between groups

Figure 2. Behavior of serum Lactate Dehydrogenase as a function of time (A)



Black symbols for 30 Hz and white symbols for 100 Hz. * $p < 0.05$ in the intergroup analysis compared to the Rest values. † $p < 0.05$ in the analysis between groups

Figure 3. Behavior of the serum Lactate before and immediately after NMES

rate, dyspnea, microcirculation, and tissue saturation of O_2 when comparing high and low frequency,[29,30] suggesting the same contractile and metabolic effectiveness at both high and low frequencies. Nonetheless, the present study recorded a higher formation of Lactate in the NMES-lf.

Converting the Lactate from the present study into mmol/L, we obtain 2.6 ± 0.7 mmol/L and 1.5 ± 0.3 mmol/L for NMES-lf and NMES-hf, respectively. These results are similar to what was observed by Aldayel et al.[15] of 2.1 ± 0.1 mmol/L and 2.6 ± 0.2 mmol/L when comparing alternating current and pulsed current, respectively.[15]

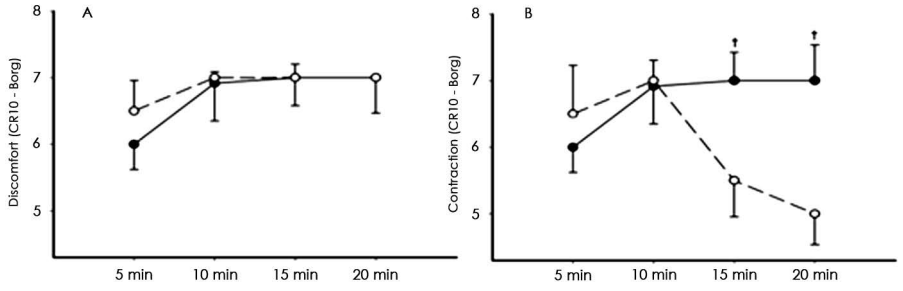
This behavior can be justified not by a greater contractile efficiency in NMES-lf but by the earlier onset of fatigue in NMES-hf. This hypothesis cannot be confirmed with the data from the present study. As cited previously, higher frequencies result in greater force, and therefore higher levels of fatigue.[24]

It follows that more vigorous muscle contractions followed by less contractile response due to precocious fatigue could justify less formation of Lactate at the end of the NMES-hf protocol. Two aspects can justify this hypothesis: i - the significant elevation of CPK in NMES-hf (Figure 1); and ii - the lower values of subjectively perceived intensity of muscle contraction in the final ten minutes of the NMES-hf, in spite of the similar levels of subjective perception of discomfort and intensity (mA) (Figure 4).

Kesar et al.[31] compared 30 Hz with 60 Hz and observed a significantly greater decline in force generated at 60 Hz.[31] Similarly, Gregory et al.[24] also observed a more accentuated drop in generated torque and greater fatigue at the higher frequency.[24]

CONCLUSION

Muscle contractions induced by NMES at low as well as high frequency induced a rise in blood markers of muscle damage. The greater LDH elevation in NMES-hf, despite less acidity and contractile response, must be considered in the light of the characteristics of the protocol adopted, as well as the tolerable intensity over a long period. The values observed allow the two intervention models to be used in rehabilitation programs.



Black symbols for 30 Hz and white symbols for 100 Hz. Note the significant reduction of contractile efficiency in the protocol with 100 Hz with no change in perceived discomfort (A). * $p < 0.05$ in the intergroup analysis compared to the Rest values. † $p < 0.05$ in the analysis between groups

Figure 4. Perception of discomfort (A) and of contractile efficiency (B) during the application of NMES

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