NEUROMUSCULAR ELECTRIC STIMULATION AND MANUAL PASSIVE STRETCHING WHEN RECOVERING MECHANICAL PROPERTIES OF IMMOBILIZED GASTROCNEMIUS MUSCLES

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SUMMARY

We evaluated the influence of immobilization, free remobilization, remobilization with manual passive stretching, remobilization with neuromuscular electric stimulation (NMES) and remobilization with electric stimulation and associated passive stretching on some mechanical properties of the gastrocnemius muscle of female rats. Sixty female rats were assessed, being distributed into 6 experimental groups. One of these groups served as control. The animals of the five remaining groups had their right posterior limb immobilized for 14 consecutive days. From the five groups, one was sacrificed right after the immobilization period, a second group was released from immobilization, a third was submitted to the manual passive stretching technique for 10 consecutive days, a fourth was submitted to NMES for 10 consecutive days and the last one was submitted to NMES and manual passive stretching for 10 consecutive days. We found that the immobilization caused a significant reduction of the mechanical properties values evaluated on the muscle. The free remobilization could not reestablish any of the properties. The remobilization by manual passive stretching restored the mechanical properties of stretching at the proportionality limit, stiffness and resilience. The remobilization stimulated by NMES reestablished all of studied properties. The remobilization by electric stimulation and passive stretching reestablished the mechanical properties of stretching at the maximum limit, proportionality limit, and stiffness.

Keywords: *Electric stimulation; Immobilization; Skeletal muscle.*

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INTRODUCTION

The immobilization of a body segment is considered as the local, continuous and stiff rest, used in cases of injuries such as fractures, dislocations, muscular trauma, surgical manipulations, including other illnesses[1]. Changes induced by the immobilization of a segment are not restrained only to the muscle, because disorders of innervation, circulation, bone atrophy, ligament changes, connective tissue augmentation, edema, joint stiffness, and muscle fibers morphometric and histological changes are usually seen[1-6].

In literature, few studies assess how muscle strength is recovered after immobilization and, especially, after immobilization followed by remobilization[7-9].

Passive stretching is often used in the rehabilitation of segments submitted to immobilization and also in sports practice. This technique can prevent connective tissue proliferation, muscular atrophy and the serial loss of sarcomeres in immobilized muscles, influencing its mechanical response such as increased resilience[5,7,10,11].

Neuromuscular electric stimulation (NMES) is a versatile electrotherapeutic modality, usually considered as an integral part of post-immobilization sequels treatment plans. This technique can induce the skeletal striated muscle to histological, histochemical and biomechanical changes[9,12]. Literature reports involving NMES are concerned to assess changes as muscular function improvement, enhancement of the ability to generate torque[1,5], as well as this stimulation´s action over contractile properties and over muscle metabolism[13].

Few authors targeted the assessment of the mechanical properties of electrically-stimulated muscles submitted to passive stretching[2,9,14].

Our objective was to assess the effects of neuromuscular electric stimulation (NMES) associated to manual passive stretching on some mechanical properties of the gastrocnemius muscle of female rats submitted to immobilization by cast conducting longitudinal traction mechanical assays.

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MATERIALS AND METHODS

Groups

This study was approved by the Committee of Ethics for Animal Use (CEUA) – University of São Paulo – Ribeirão Preto campus – SP, Brazil.

Sixty female Wistar rats were divided into 6 groups. The groups were characterized as Control (CG, n=10) where the animals were kept in cages and spared from any intervention; Immobilized (IG, n= 10) where the animals had their right posterior paw immobilized for 14 days; Immobilized/ stretched (ISG, n=10) where the animals were immobilized and then submitted to manual passive stretching technique for 10 consecutive days; Immobilized/Electro stimulated (IEG, n= 10) where the animals were immobilized and then submitted to low-frequency NMES followed by passive stretching for 10 consecutive days, and; the immobilized/ released group (IRG, n= 10), which was submitted to immobilization followed by release of the animals from their cages for 10 days.

Immobilization technique

The immobilization of animals´ right posterior paws was provided with cast including the pelvis, the hip and the knee at full extension as well as ankle joint at plantar flexion. The animals were previously anesthetized with *Tiopental®* (*Sodium Tiopental,* 1g of salt diluted into 40ml of 0.9% NaCl saline solution). Then, they had their right posterior limb and pelvic band wrapped by a tubular mesh and cotton bandages in order to avoid ulceration. Subsequently the same region was wrapped with fast-dry 2.5cm-wide cast bandage, applied by the usual method. Cast was replaced whenever necessary. The animals remained immobilized for 14 consecutive days and kept in plastic cages containing 2 animals each with water and ration ad libitum. Cast immobilization did not prevent them from eating or walking in the cages.

Manual passive stretching technique

The manual passive stretching technique was performed with the knee joint at full extension, because at this position the gastrocnemius muscle stretching is more effective, as its origin is at femur. A non-quantified strength was applied on the plantar portion of the right posterior paw performing a dorsiflexion movement of the ankle joint, promoting passive stretching of the gastrocnemius muscle of the animals for a 15-second period (counted). The stretching technique was performed as a single series of 10 passive exercises with 30-second rest intervals, for 10 consecutive days. The activity was conducted always at the same time of the day, at morning.

Neuromuscular electric stimulation technique

For the therapeutic intervention with NMES, an IBRAMED *NEURODYN II Compact®* current generator was employed. This equipment has the following features: Variable pulse frequency rate (R): 10-166Hz; cycle-on 2-20 seconds (s); cycle-off 3.5 – 38 s; pulse train up time: 2.5s; pulse train down time: 0.5s; amplitude range: 0-80mA; pulse duration ranging automatically from 25 to 280μs and the wave format is asymmetric two-phase squared.

The parameters employed for animals' stimulation were: frequency: 50 Hz, cycle-on: 13s, cycle-off: 22s; and power of approximately 1mA (100 Ω resistance) able to foster a sustained and visible contraction of the muscle. The 50Hz frequency is recommended by some authors as able to induce muscular strength gain[12,15].

Prior to the applications, all animals had their dorsal-inferior and ventral portions of the right gastrocnemius muscle trichotomized aiming to enable the electric stimulus transmittance. Whenever the animals received the NMES, anesthesia was provided with intraperitoneal *Tiopental®*. The intervention period using the NMES technique encompassed 10 consecutive days, with 10 minutes/day of stimulation, always performed at mornings.

Current was transmitted to animals through cords and electrodes (carbon-silicone). Firstly, a thin layer of hydrophilic, neutral pH gel approximately 1mm thick was applied to a rectangular 5x3 cm electrode fixated on the dorsal inferior region of the animal. We developed another electrode used for local stimulation of the gastrocnemius muscle. This electrode was a smaller-scale 5.4mm-wide handpiece, also applying a gel layer for producing an appropriate chain driver to the muscle.

Stretching plus neuromuscular electric stimulation technique

The group submitted to these techniques followed the same standards adopted when each technique was employed separately. Regarding the sequence in which the techniques were employed, NMES was provided first, followed by manual passive stretching technique.

Traction assay

For the mechanical assay on the gastrocnemius muscle, a universal assay machine EMIC®- DL10000 model belonging to the Bioengineering Laboratory of USP – Ribeirão Preto Medical School, featuring a 50kgf load cell was employed. The universal assay machine is directly connected to a microcomputer equipped with a software program able to accurately obtain the values for load and deformation.

A special accessory was used for fixating the muscles and conducting traction assays so that the muscle could be submitted to the assay by preserving its origin at femoral region and muscle insertion at the calcaneus.

After muscle fixation to the accessories of the universal assay machine, this was submitted to axial traction. The parameters adopted for the assay were a 0.30kgf pre-load, the assay speed was 10mm/min, load and stretch limits were 8.00kgf and 25mm, respectively.

For each load increment applied to the muscle, a resultant value for stretching was achieved, and this enabled us to build graphs representing the load *versus* stretching using a *Microsoft Excel 2000®* application.

Statistical analysis

Data obtained from traction assays were submitted to statistical analysis. For a simultaneous analysis of the groups, the ANOVA test was employed, and for intergroup's comparison, the Tukey-Kramer test was used, adopting for both a significance level of 5%.

RESULTS

Load at proportionality limit

The mean value and the standard deviation for load at proportionality limit were the following: control group valor (26.08 ± 2.28)N; immobilized group (14.64 ± 2.47)N; immobilized/ stretched (21.85 ± 3.54)N; immobilized/ electro-stimulated (25.95 ± 5.23)N; immobilized/ electro-stimulated/ stretched (20.56 ± 3.24)N and immobilized/ released (16.46 ± 1.93)N.
The control group showed a statistically significant difference compared to all other groups, except for the immobilized/ electro-stimulated group ($p<0.05$). The immobilized group showed a statistically significant difference compared to immobilized/ stretched, immobilized/ electro-stimulated, and immobilized/ stretched/ electro-stimulated groups ($p<0.05$).

Stretching at proportionality limit

The mean value and the standard deviation for stretching at proportionality limit obtained by the mechanical assay were the following: control group $(7.41 \pm 0.89)x10^{-3}$m; immobilized group $(5.78 \pm 1.10)x10^{-3}$m; immobilized/ stretched group $(7.19 \pm 0.99)x10^{-3}$m; immobilized/ electro-stimulated group $(7.79 \pm 1.69)x10^{-3}$m; immobilized/ electro-stimulated/ stretched group $(6.75 \pm 0.59)x10^{-3}$m, and immobilized/ released group $(5.23 \pm 0.94)\ x10^{-3}$m.
For that property, the control group showed a significantly superior value compared to the immobilized and immobilized/ released groups ($p<0.05$). The immobilized group showed significant difference only when compared to the electro-stimulated group ($p<0.05$).

Load at maximum limit

The mean value and the standard deviation for load at maximum limit achieved on mechanical assays on animals´ gastrocnemius muscles were the following: control group (33.45 ± 1.34)N; immobilized group (19.04 ± 1.96)N; immobilized/ stretched group (25.23 ± 3.63)N; immobilized/ electro-stimulated group (31.55 ± 5.01)N; immobilized/ electro-stimulated/ stretched group (26.75 ± 3.15)N and immobilized/ released group (21.84 ± 3.51)N.
For that property, the control group showed superior results compared to the immobilized, immobilized/ stretched, immobilized/ electro-stimulated/ stretched, and immobilized/ released groups ($p<0.05$). The immobilized group showed a significant difference when compared to immobilized/ stretched, immobilized/electro-stimulated/ stretched groups ($p<0.05$).

Stretching at maximum limit

The mean value and the standard deviation for stretching at maximum limit were the following: control group $(12.73 \pm 1.19)x10^{-3}$m; immobilized group $(8.77 \pm 1.51)x10^{-3}$m; immobilized/ stretched group $(9.24 \pm 1.04)\ x10^{-3}$m; immobilized/ electro-stimulated group $(11.43 \pm 2.32)\ x10^{-3}$m; immobilized/ electro-stimulated/ stretched group $(11.36 \pm 1.99)x10^{-3}$m and immobilized/ released group $(9.47 \pm 2.05)x10^{-3}$m.
The control group showed a significant difference ($p<0.05$) when compared to the immobilized, immobilized/ stretched, and immobilized released groups. The immobilized group showed a statistically significant difference compared to immobilized/ electro-stimulated and immobilized/electro-stimulated/ stretched groups ($p<0.05$).

Stiffness

The mean value and the standard deviation for stiffness of the assessed muscles were the following: control group $(3,66 \pm 0,33)x10^{3}$N/m; immobilized group $(2.31 \pm 0.64)x10^{3}$N/m; immobilized/ stretched group $(3.34 \pm 0.57)x10^{3}$N/m; immobilized/ electro-stimulated group $(3.40 \pm 0.74)x10^{3}$N/m; immobilized/ electro-stimulated/ stretched group $(3.20 \pm 0.59)x10^{3}$N/m and immobilized/ released group $(2.83 \pm 0.42)x10^{3}$N/m.
The control group showed a statistically significant difference compared to immobilized and immobilized/ released groups ($p<0.05$), while the immobilized group showed a statistically significant difference compared to immobilized/ stretched, immobilized/ electro-stimulated and immobilized/ stretched/ electro-stimulated groups ($p<0.05$).

Resilience

The mean value and the standard deviation for resilience were the following: control group $(97.15 \pm 22.30)x10^{-3}$ J; immobilized group $(52.78 \pm 17.09)x10^{-3}$ J; immobilized/ stretched group $(81.95 \pm 18.31)x10^{-3}$J; immobilized/ electro-stimulated group $(93.71 \pm 26.62)x10^{-3}$J; immobilized/ electro-stimulated/ stretched group $(70.06 \pm 13.70)x10^{-3}$J and immobilized/ released group $(44.68 \pm 12.71)x10^{-3}$J.
For this property, the control group showed a statistically significant result compared to immobilized, immobilized/electro-stimulated/ stretched and immobilized/ released groups ($p<0.05$), while the immobilized group showed a significant difference when compared to immobilized/ stretched and immobilized/ electro-stimulated groups ($p<0.05$).

DISCUSSION

The responses achieved in this study show that the adopted immobilization model was effective, with changes occurring on the assessed mechanical properties, thus confirming the results evidenced by other studies[7-16].
In general, techniques such as the manual passive stretching and neuromuscular electric stimulation (NMES) are frequently employed on physical rehabilitation programs in an attempt to prevent muscular atrophy, a reduced muscle and soft parts stretching and the reduced range of motion[2,13,17].
The evaluation of the load at maximum limit shows similarity between control group and immobilized/ electro-stimulated groups; however, immobilized/ stretched and immobilized/ electro-stimulated/ stretched groups do not show such similarity, but do present a trend towards recovering this property. Such fact may be evidenced by the difference between those groups when compared to the immobilized group.
For stretching at maximum limit, the results suggest that a muscle that is remobilized only with passive stretching shows an inferior ability to stretch, although presenting the same ability to bear similar loads to the ones remobilized by using other techniques.

For the evaluation of load at proportionality limit, our results show that this property was not recovered on the muscle by employing remobilization techniques and by releasing it for one of two weeks. As evidenced by Carvalho et al.[16] free remobilization for a 3-week period can recover this property to levels similar to those of the control group. Stretching and electric stimulation plus stretching techniques were also unable to re-establish this property to similar levels to control group within a 2-week period.

The results found for proportionality limit suggest that remobilized muscles by passive stretching and electric stimulation plus passive stretching techniques are able to bear lighter loads, although they stretch similarly to control muscle. Matheus et al.[9] reported, in their study, that the electric stimulation performed during the immobilization period prevents the reduction of the values for the mechanical properties of gastrocnemius muscle.

By assessing the mechanical properties of a striated skeletal muscle, we can see that, regardless of the position in which it is immobilized – whether in a stretched or shrunk position – stiffness reduction is present. In our study, stiffness was significantly reduced on immobilized muscles, i.e., the muscle stretched more with a lighter load and, when submitted to the three remobilization techniques, this property was re-established. According to Järvinen et al.[7], this property is very important for the muscle, because its reduction indicated that the muscle is stretching more in the presence of lighter loads, which makes it more susceptible to injuries. The results found by those authors corroborate the ones found in our study, which demonstrates the need of making some considerations during the rehabilitation process in order to avoid potential injuries caused by overload to this structure.

The results suggest that the remobilization provided by the three techniques contributes to the re-establishment of the stiffness property.

The energy absorbed during the elastic phase – or resilience – was higher on control, immobilized/ stretched and immobilized/ electro-stimulated groups. When a segment is immobilized with the muscle shrunk, resilience is about 64% lower compared to muscles immobilized in a stretched position. However, a healthy muscle submitted to NMES at 16 and 64Hz frequencies increases the resilience compared to muscles not submitted to electric stimulation[19]. In our study, a 50 Hz frequency was employed, evidencing the re-establishment of this property to control patterns.

Immobilizing a segment at a shrunk position leads to an increased amount of connective tissue on the muscle, which could leave it less elastic[7]. When performing the NMES and stretching techniques, the muscle did not stand still, suggesting that these techniques performed alone may have contributed to the re-establishment of this property. Garret et al.[19] reported in their study that NMES with variable frequency ranges and on healthy muscles is able to increase the values for this mechanical property. However, in our study, the evidences suggested that when used in association to passive stretching, the NMES does not contribute to the re-establishment of this property.

A muscle's ability to absorb energy without rupturing is important for avoiding injuries. When energy is excessively absorbed, the material may fail, causing a rupture in a given site[10]. In such case, a muscle that shows a better ability to absorb energy during the elastic phase would be less likely to be injured upon extreme efforts.

CONCLUSION

We conclude that immobilization significantly reduces the values of the mechanical properties assessed on the gastrocnemius muscle. When remobilization was established with manual passive stretching, properties such as stretching at proportionality limit, stiffness and resilience were re-established to the levels of the control group; however, this technique cannot re-establish the properties of load at proportionality limit, load and stretching at maximum limit. Remobilization by NMES promoted the re-establishment of all mechanical properties assessed in this study to control levels. When NMES techniques added to passive stretching were employed, only the mechanical properties of stretching at maximum limit, stretching at proportionality limit and stiffness were re-established, while the properties of load at maximum limit, load at proportionality limit and resilience could not be re-established on the muscle.

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