**INTRODUCTION**

Currently, different therapeutic alternatives are being suggested targeting the full recovery of patients with muscle injuries within the shortest possible time, thus providing them back with a normal functional physical status and allowing a better physical performance. TUS has been used for over six decades to treat soft tissues, being currently a resource strongly employed in physiotherapeutic practice, and the most used one for treating soft tissues’ injuries. The effects of the TUS on muscular repair process in experimental injuries have been studied under different aspects. Stratton, Heckmann and Francis used different therapeutic ultrasound powers for the histochemical evaluation of its effects for the repair process of blunt muscle injuries, regarding it as beneficial. Rantanen et al. concluded that therapeutic ultrasound accelerates muscle repair after contusion promoting significant proliferation of satellite cells to the injury site. Menezes et al. applied therapeutic ultrasound in an experimental muscle injury model by smashing, acquired their results by means of mechanical assays and concluded that it seems to have an improvement of the injury repair quality. Injured muscles have not been previously treated with immobilization.

Kames and Burton found a significant improvement of the muscle strength degree, in an injury caused by repeated eccentric contraction, when the injury was stimulated with therapeutic ultrasound. Järvinen and Järvinen et al. showed the benefits of early immobilization as a part of the treatment of an injured muscle. Järvinen et al. recommended rest as a prompt treatment approach in muscle injuries, accompanied by local ice, compression and injured limb lifting, this method being widely employed in daily clinical practice. We didn’t find in literature studies correlating the effects of therapeutic ultrasound with plastered immobilization on muscle repair and the corresponding mechanical properties. Our objective was to assess the influence of TUS, added by plastered immobilization or not, after immediate trauma, on the process of muscle repair by assessing the mechanical properties of muscular fibers of gastrocnemius muscle.

**MATERIALS AND METHODS**

**Experimental animals**

Seventy female albino Wistar rats weighting 204 ± 15g and 10-12 weeks old were used. The animals were kept in separate plastic restrain cages, with water and food ad libitum, being exposed to bright/ dark environments of 12-h each until the experimental injury was produced.

**METHODS**

Seventy female rats were employed in the study, and the animals were divided into 7 groups: Group 1- Control; Group 2- Untreated; Group 3- CI for 24 hours; Group 4- CI for 72 hours; Group 5- TUS without CI; Group 6- CI for 24 hours combined with TUS; Group 7- CI for 72 hours combined with TUS. Results: Loads at proportionality limit and maximum limit showed that the group receiving TUS behaved similarly to control group. The property of stretching at proportionality limit was not different from one group to another; the maximum stretching of the group receiving TUS and of the groups immobilized for 72 hours was comparable to control group. Conclusion: The group receiving TUS showed similar stiffness levels compared to control group and superior resiliency compared to all remaining groups. The standalone use of TUS provided similar results to those regarded as normal, but these were not noticed when TUS was combined to CI.

**SUMMARY**

Introduction: We assessed the effects of therapeutic ultrasound (TUS), either added to cast immobilization (CI) as a treatment alternative to muscular injuries caused by impact by assessing the mechanical properties of stretching and load at proportionality and maximum limit, stiffness (S) and gastrocnemius muscle resiliency. Methods: 70 female rats were employed in the study, and the animals were divided into 7 groups: Group 1- Control; Group 2- Untreated; Group 3- CI for 24 hours; Group 4- CI for 72 hours; Group 5- TUS without CI; Group 6- CI for 24 hours combined with TUS; Group 7- CI for 72 hours combined with TUS. Results: Loads at proportionality limit and maximum limit showed that the group receiving TUS behaved similarly to control group. The property of stretching at proportionality limit was not different from one group to another; the maximum stretching of the group receiving TUS and of the groups immobilized for 72 hours was comparable to control group. Conclusion: The group receiving TUS showed similar stiffness levels compared to control group and superior resiliency compared to all remaining groups. The standalone use of TUS provided similar results to those regarded as normal, but these were not noticed when TUS was combined to CI.

**Keywords:** Muscle injury. Immobilization. Therapeutic ultrasound. Biomechanics.
described by Stratton et al. 4 and Minamoto et al. 12

All animals were previously anesthetized with Thiopental® – sodium used by Oliveira et al.11 consisting of an adaptation of the models Laboratory of the University of São Paulo, the same as the one on tool’s base. This tool was developed at the Bioengineering muscle, with the animals properly positioned on a metal surface caused by a 200g load release 30 cm over the gastrocnemius A tool able to produce a muscle injury by mechanism of impact Experimental contusion

The animals included in this group were not submitted to any injury, remaining in restraint cages for a period of 7 days.

Group 2 – Untreated (N=10)

These animals had their gastrocnemius muscle submitted to acute experimental injury by mechanism of impact; however, following trauma, no therapeutic resource was applied, being kept in their restraint cages for 7 days and free active mobilization.

Group 3 – Immobilized for 24 hours (N=10)

After the acute experimental injury was produced, this group of animals was immobilized for 24 hours by means of plastered device including hip, knee and ankle joints of the right limb. When the period was completed, plastered immobilization was removed, and the animals were kept in their restraint cages for additional 6 days.

Group 4 – Immobilized for 72 hours (N=10)

In this experimental group, the animals also had their gastrocnemius muscle submitted to injury production, being immobilized according to the same protocol as described for group 3, but for a 72-hour period. When this period was completed, the plastered immobilization was removed and the animals were kept in their restraint cages for additional 4 days.

Group 5 – Stimulation with TUS (N=10)

After the muscle injury was produced, the animals remained in their restraint cages for 24 hours, and then stimulated with pulsed therapeutic ultrasound (TUS) for 5 minutes for 6 consecutive days.

Group 6 – Immobilized for 24 hours and stimulation with TUS (N=10)

The animals in this group were submitted to muscle injury production process, being immediately submitted to immobilization with plastered device. After that period, the plastered device was removed and the animals were stimulated with pulsed therapeutic ultrasound (TUS) for 5 minutes for 6 consecutive days.

Group 7 – Immobilized for 72 hours and stimulation with TUS (N=10)

The animals were submitted to the experimental injury production, being immediately immobilized. After 24 hours of immobilization, stimulation on the injured area was initiated, the access to which was achieved by a window produced on the plaster cast, with pulsed therapeutic ultrasound (TUS) for 5 minutes for 6 consecutive days.

Experimental contusion

A tool able to produce a muscle injury by mechanism of impact caused by a 200g load release 30 cm over the gastrocnemius muscle, with the animals properly positioned on a metal surface on tool’s base. This tool was developed at the Bioengineering Laboratory of the University of São Paulo, the same as the one used by Oliveira et al.11 consisting of an adaptation of the models described by Stratton et al.4 and Minamoto et al.12 All animals were previously anesthetized with Thiopental® – sodium Thiopental– at a dosage of 4 mg/100g – administered intraperitoneally. The animals were manually immobilized, being positioned in pronation, with the thigh-femur joint extended and directly touching the metal surface, taking care to keep the maximum knee extension and dorsiflexion at 90° from the ankle (Figure 1 – A and B).

Mechanical assays

The mechanical properties of the specimens were identified by longitudinal traction assays at a Universal Assay Machine owned by the Bioengineering Laboratory at USP Medical School, Ribeirão Preto campus. A 50kgf load cell was employed, which presents a direct interface with a PC with mechanical assay automation software, allowing for accurate comparisons of loads and stretching achieved at each mechanical assay.
A 200g pre-load was applied with an accommodation time of 30 seconds and load application speed determined as 10mm/minute. (Figure 2 - A and B)

Graphs for load versus stretching were built from the results of each assay, enabling the determination of mechanical properties of load and stretching at the proportionality limit, load and stretching at maximum limit, stiffness and resiliency for each specimen. (Figure 3)

The data were processed by means of the Instat Graph-Pad® software v.3.00 to provide a statistical analysis of the results found between the different groups.

**RESULTS**

The results found for each specimen were summed and the arithmetic mean values and standard deviations were calculated by using a Microsoft® Excel® 2000 application for each experimental group.

The data achieved on the assays were assessed by the variance analysis test – ANOVA, and by the Student-Newman-Keuls’ test for comparison between groups, both with significance levels established as 5%, and processed by the Instat Graphpad® software, v.3.00.

**Stretching at proportionality limit**

The mean values found for stretching at the proportionality limit for the control group were (4.48 ± 0.88) x10^{-3} m; for group 2, (6.36 ± 2.28) x10^{-3} m; for group 3, (4.78 ± 1.22) x10^{-3} m; group 4, (4.91±1.46) x10^{-3} m; group 5, (5.60 ± 0.65) x10^{-3} m; group 6, (5.14 ± 1.72) x10^{-3} m, and; group 7, (4.73±1.10) x10^{-3} m. No statistically significant differences were found on the simultaneous analysis of the experimental groups (p>0.05).

**Load at proportionality limit**

The mean values found for load at proportionality limit for the control group were (17.77 ± 2.10) N; group 2, (13.50 ± 3.80) N; group 3, (13.86 ± 2.66) N; group 4, (14.21 ± 5.03) N; group 5, (19.60 ± 3.60) N; group 6, (14.10 ± 2.86) N, and; group 7, (14.08 ± 3.05) N. The results found in the assays showed a statistically significant difference in the simultaneous analysis of experimental groups, with p<0.0001. No statistically significant difference was found for the comparison of groups 1 and 6. In the comparisons between groups 1 and 5 with the other experimental groups, statistically significant differences were found.

**Maximum stretching**

The mean values found for maximum stretching on the control group were (11.66 ± 2.23) x10^{-3} m; group 2, (8.91 ± 2.04) x10^{-3} m; group 3, (8.83 ± 1.04) x10^{-3} m; group 4, (10.43 ± 1.45) x10^{-3} m; group 5, (10.44 ± 1.58) x10^{-3} m; group 6, (9.82 ± 3.21) x10^{-3} m, and; group 7, (16.66 ± 1.49) x10^{-3} m. The comparison of the results found for this property showed a statistically significant difference, with p<0.0001. No statistically significant differences were found when the groups were compared to each other, except for groups 7.

**Maximum load**

The mean values found for maximum load on the control group were (31.6 ± 2.7) N; group 2, (17.7 ± 3.7) N, group 3, (22.0 ± 2.9) N; group 4, (21.5 ± 3.1) N; group 5, (28.7 ± 2.7) N; group 6, (18.2 ± 5.0) N, and; group 7, (22.5 ± 2.3) N. In the statistical analysis of the mean values obtained for maximum load, a statistically significant difference was found in the simultaneous analysis of the experimental groups, with p<0.0001. No statistically significant difference was found when comparing groups 1 and 5 or between groups 2,3,4,6 and 7.

**Stiffness**

The mean value found for stiffness on the control group was (4.047 ± 0.707) x10^{3} N/m; on group 2, (2.239 ± 0.584) x10^{3} N/m; group 3, (2.990 ± 0.547) x10^{3} N/m; group 4, (2.808 ± 0.306) x10^{3} N/m; group 5, (3.658 ± 0.676) x10^{3} N/m; group 6, (2.880 ± 0.503) x10^{3} N/m, and; group 7, (3.205 ± 0.492) x10^{3} N/m. The stiffness comparison evidenced the presence of a statistically significant difference for the simultaneous analysis, with p<0.0001. No statistically significant differences were found in the comparison between groups 1 and 5 and on the other comparisons between groups 2, 3, 4, 6 and 7.

**Resiliency**

The mean value found for resiliency on the control group was (40.3 ± 11.7) x10^{3} J; on group 2, (37.1 ± 16.8) x10^{3} J; group 3, (34.2 ± 14.4) x10^{3} J; group 4, (37.9 ± 24.4) x10^{3} J; group 5, (57.2 ± 13.5)
The property of load at proportionality limit showed favorable results to the use of therapeutic ultrasound both in our study and in the one conducted by Menezes et al. However, we had favorable results to the use of ultrasound also for maximum load, which was not found on their study. Also concerning stretching, our results are controversial, once our tests did not differentiate our groups regarding stretching at the proportionality limit, as did Menezes et al. group. The maximum stretching did not differentiate the groups from these authors, but, in our study, the group submitted to ultrasound stimulation and also the one immobilized for 72 hours provided comparable results between these groups and the control group. The results found in our study are similar to those reported by Menezes, et al. for resiliency (energy absorbed at the elastic phase) and different for stiffness. For muscle rehabilitation and repair processes, stiffness is essential, because muscles with less stiffness stretch more when lighter loads are present. It is difficult to justify the discrepancy of results, which can be attributed to the fact that we have used different muscles, different ways to fixate on the assay machine and different follow-up periods after the injury. However, in our study, there is a trend of the results to favor the groups treated with ultrasound, and the same trend is noticed on the study by Menezes et al., although not in the same mechanical properties assessed. We expected that early immobilization after trauma, for a short period, could favor the results of mechanical resistance recovery for these muscles when associated to therapeutic ultrasound, but this did not occur.

CONCLUSION
The standalone use of TUS provided similar results to those regarded as normal on assays for load at proportionality limit and maximum load. The association of TUS with plastered immobilization for 72 hours provided comparable results to control group only for the maximum stretching property. Our results suggest that the combination of immobilization and ultrasound as a adjuvant treatment does not bring benefits for muscle repair by mechanical assays. These results achieved in laboratory animals should not be directly extrapolated to clinical practice, serving as a primary basis for further research.