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Effects of therapeutic ultrasound on the mechanical properties of skeletal muscles after contusion

Efeitos do ultra-som terapêutico nas propriedades mecânicas do músculo esquelético após contusão

Matheus JPC, Oliveira FB, Gomide LB, Milani JGPO, Volpon JB, Shimano AC

Abstract

Background: Therapeutic ultrasound is a resource commonly applied to speed up tissue repair in muscle injuries. The absorption of the ultrasound waves is determined by their frequency and intensity. For a given intensity, the depth reached by 1MHz is greater than the depth reached by 3MHz. **Objective:** To analyze the mechanical properties of muscles subjected to acute impact injury treated with therapeutic ultrasound at the frequencies of 1 and 3MHz. **Methods:** Forty female Wistar rats (200.1±17.8g) were used, divided into four groups: (1) control; (2) muscle injury without treatment; (3) muscle injury treated with therapeutic ultrasound at the frequency of 1MHz (0.5W/cm^2); and (4) muscle injury treated with therapeutic ultrasound at the frequency of 3MHz (0.5W/cm^2). The injury was produced in the gastrocnemius muscle by means of an impact mechanism. The treatment consisted of a single five-minute session per day, for six consecutive days. The muscles were subjected to mechanical traction tests in a universal test machine. **Results:** Means and standard deviations for the mechanical properties of the injured groups that received therapeutic ultrasound were significantly greater than those of the injured group without treatment ($p<0.05$). The property of stiffness should be highlighted: the application of therapeutic ultrasound increased muscle stiffness by approximately 38%. **Conclusions:** Therapeutic ultrasound increased the mechanical properties of the injured muscles, and brought them to a level close to the control group. However, no significant difference in mechanical properties was observed between the groups treated with ultrasound at the frequencies of 1MHz and 3MHz.

Key words: therapeutic ultrasonic; muscle injury; biomechanical properties.

Resumo

Contextualização: O ultra-som terapêutico (UST) é um recurso comumente aplicado na aceleração do reparo tecidual de lesões musculares. A absorção das ondas ultra-sônicas é determinada pela freqüência e pela intensidade, sendo que, em uma mesma intensidade, a profundidade atingida por 1MHz é maior quando comparada a 3MHz. **Objetivo:** Analisar o comportamento das propriedades mecânicas de músculos submetidos à lesão aguda por impacto e tratados com UST, utilizando as freqüências de 1 e 3MHz. **Materiais e métodos:** Foram utilizadas 40 ratas Wistar (200,1±17,8g), divididas em quatro grupos: (1) controle; (2) lesão muscular sem tratamento; (3) lesão muscular tratada com UST de freqüência 1MHz (0,5W/cm^2) e (4) lesão muscular tratada com UST de freqüência 3MHz (0,5W/cm^2). A lesão foi provocada no músculo gastrocnêmio por mecanismo de impacto. O tratamento foi de cinco minutos diários durante seis dias consecutivos. Os músculos foram submetidos a ensaios mecânicos de tração em uma máquina universal de ensaios. **Resultados:** As médias e desvios-padrão das propriedades mecânicas dos grupos lesionados e tratados com UST foram significativamente maiores quando comparadas ao grupo lesionado sem tratamento ($p<0,05$). Em destaque, a propriedade de rigidez que, com a aplicação do UST, teve acréscimo de aproximadamente 38%. **Conclusões:** A intervenção, por meio do UST, promoveu aumento das propriedades mecânicas nos músculos lesionados aproximando-as do grupo controle. Entretanto, não foi observada diferença significativa entre as propriedades mecânicas dos grupos tratados com ultra-som de freqüências 1MHz e 3MHz.

Palavras-chave: ultra-som terapêutico; lesão muscular; propriedades biomecânicas.

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Bioengineering Laboratory, Departament of Biomechanics, Medicine and Rehabilitation of the Locomotor Systems, School of Medicine of Ribeirão Preto, Universidade de São Paulo (FMRP-USP) – São Paulo (SP), Brazil

Correspodence to: João Paulo Chieregato Matheus, Rua Afonso Rato, 852, Mercês, CEP 38060-040, Uberaba (MG), Brazil, e-mail: jpcmatheus@yahoo.com.br

Introduction

Muscular tissue is most affected by sport traumas[1] and the lesions are usually caused by direct impact mechanisms[2]. These lesions frequently cause pain and impairments, leading the individuals to look for treatment, very often at physical therapy outpatient clinics[3]. Therapeutic ultrasound (TU) is a commonly used resource for the treatment of disorders affecting the musculoskeletal system, as well as enhancing the tissue recovery in muscular lesions[4]. The possibility of using different frequencies between 1 and 3MHz is important insofar as the higher frequencies (3MHz) are more intensely absorbed, which makes them more specific for the treatment of superficial tissues, whereas the lower frequencies (1MHz) penetrate more deeply, aimed at the treatment of deeper tissues [5].

According to Ter Haar[6], the acoustic vibrations produced by the TU induce cellular changes that alter the gradient of molecule concentrations of calcium and potassium ions, which stimulate cellular activity. This phenomenon may result in various changes, such as increases of protein synthesis, and mastocite secretions changes in the mobility of the fibroblasts, among others. Although these mechanisms are not yet known, other research shows the influences of the TU in tissue repair through the modifications caused by the permeability of the cell membranes. Some authors suggest that these effects are due to the cavitation[7,8], while others state that ultrasound produces microvascular hemodynamic changes that increase the perfusion, the granulation tissue, tissue repair[8,9], fibroblastic proliferation and the growth of precursor cells[10].

As far as muscular tissue is concerned, Rantanen et al.[10] carried out a study regarding the effects of TU at 3MHz in the regeneration of muscle-skeletal fibers after lesion in the gastrocnemius muscle of rats. By using immunohistochemistry and morphometry techniques, they demonstrated a very significant proliferation (96%) of satellite cells through the use of the TU in a pulse mode, with an intensity of 1.5W/cm^2, during the first five stages of recovery. Karnes and Burton[11] analyzed the effects of TU in the muscular recovery of rats, using a 1MHz frequency with a modulated intensity of 0.5W/cm^2, for five minutes, over the course of seven consecutive days. They concluded that the treatment accelerated the tissue recovery and promoted an increase in the muscular contraction strength when compared to those that did not undergo the treatment.

Because these types of disorders are very frequently found in physical therapy clinical practice, and due to a shortage of data regarding this subject, the aim of the present paper was to analyze the behavior of the mechanical properties of muscles that suffered acute lesions by impact and treated with TU at frequencies ranging from 1 to 3MHz, with an intensity of 0.5W/cm^2.

Materials and methods

To develop this study, 40 Wistar adult rats of the *Rattus norvegicus albinus* species were used, with an average body mass of 200.1±17.8g. The animals, originally brought from the Central Biotherium of the Universidade de São Paulo (USP), *Campus* Administration at Ribeirão Preto. They were kept in the Biotherium of the Bioengineering Laboratory of the School of Medicine of Ribeirão Preto (FMRP) of the USP, during the experimental protocols. The rats were housed in collective standard cages, with three animals per cage, at a controlled room temperature of 25±1^{o}C, with a photoperiod of 12 hours light and 12 hours dark and receiving *ad libitum* standard water and food. All procedures to which the animals were submitted received the approval of the Commission of Ethics in the Use of Animals of the USP *campus* at Ribeirão Preto, which follows the International Guiding Principles for Biomedical Research Involving Animals, under protocol nº 03.1.529.53.7.

Experimental groups

The animals were randomly distributed into the following experimental groups of 10 individuals each:

- Group 1: control (C). The 10 animals were kept in cages for seven days;
- Group 2: muscular lesion without treatment (L): The ten animals had their right gastrocnemius muscle submitted to a lesion by impact, and were accompanied for seven days up to the moment of the assessment;
- Group 3: muscular lesion treated with 1MHz frequency TU (LT1). The ten animals had their right gastrocnemius muscle submitted to a lesion by impact and were then treated with TU at 1MHz, with an intensity of 0.5W/cm^2;
- Group 4: muscular lesion treated with a 3MHz frequency TU (LT3). The ten animals had their right gastrocnemius muscle submitted to a lesion by impact and treated with a 3MHz UST, with an intensity of 0.5W/cm^2.

Equipment for lesion production

The equipment was manufactured at the Precision Workshop of the USP *Campus* at Ribeirão Preto, adapted from the model initially proposed by Stratton[12], which consisted of two telescoped metallic poles, adjustable from 30 to 40cm (Figure 1A). These poles were fixed on a plastic base with an area of 272.5cm^2 (Figure 1B). A rectangular metallic surface measuring 12.25cm^2 was also placed upon this base (Figure 1C), serving as a support for the weight. Uniting both vertical poles, there was a horizontal pole with a pulley through which the guiding wire ran next to the weight to be released (Figure

1D). Between both poles there was a transparent acrylic rail, which lead a 200g weight (Figure 1E) during the free fall, thus avoiding interruptions.

Experimental muscular lesion

Despite the non-invasive nature of the experiment, some precautions were taken when it came to causing the lesion, in addition to routine techniques such as the asepsis of both the area and the equipment. Prior to the creation of the lesion, the animals were anesthesized with ketamin chloridrate (80mg/kg) and xylazine chloridrate (15mg/kg), in a dosage of 0.6mL of the mixture for every 100g of body mass and administered intramuscularly. After being anesthetized, they were submitted to the tricotomy in the posterous-lateral and medial region of the right leg; they were then positioned at the base of the lesion production equipment, in the ventral decubitus, with their knee at maximal extension and ankle in neutral position (90º). In order to cause the lesion, a 200g weight was released from a 30cm height onto the belly of the right gastrocnemius muscle (Figure 2). The animals in groups 2, 3 and 4 were submitted to a single trauma, and immediately after the lesion was produced, they were manually inspected for bone fractures.

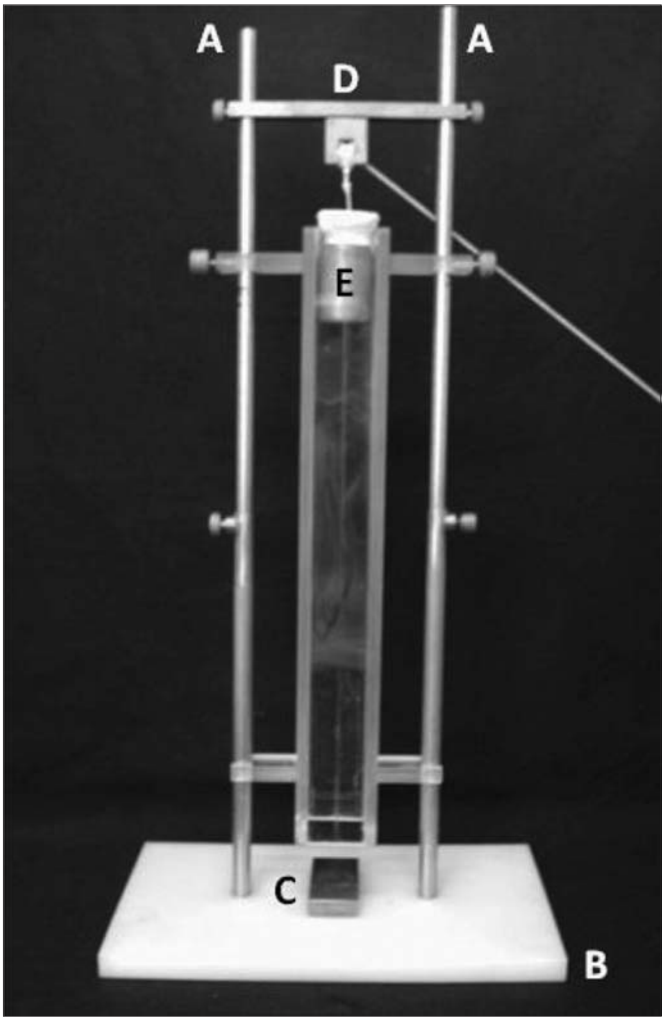


Figure 1. Equipment of production of the experimental lesion composed by two adjustable metallic stems from 30 to 40cm (A), plastic base with 272.5cm^2 of area (B), rectangular metallic surface with 12.25cm^2 of area (C) and metallic structure that supports the pulley (D) and a thread connect to a mass of 200g close to be liberated (E).

Ultra-sonic irradiation protocol

Before starting the treatments, the equipment was calibrated at the Bioengineering Laboratory of the Materials Department of the School of Engineering of São Carlos (EESC) of the USP, using a precision dosimeter (modelo UPM-DT-1, Ultrasonic Power Meter®). According to the correction curve of the intensity values emitted by the manufacturers, while the nominal intensity on the panel was 0.4W/cm^2, the emission on the adapted heads was 0.5W/cm^2 (Spatial Average Temporal Average, SATA).

For the treatment of group 3, a commercial ultrasonic generator (model Sonacel III, Bioset-Ibramed®) was set at a frequency of 1MHz. For the treatment of group 4, another commercial ultrasonic generator was used (model Sonacel Plus, Bioset®), set at a frequency of 3MHz. Custom made heads made by the manufacturer were adapted to the equipment, restricting the radiation area to 1.5cm^2, with a diameter of 14mm (Figure 3). Originally, both equipment had heads with an effective radiation area (ERA) of 3.5cm^2. The animals were submitted to daily sessions of TU exposure in the pulse mode with a modulated frequency of 100Hz, which according to the manufacturer has a work cycle 1:5 (2ms on and 8ms off, 20%), intensity of 0.5W/cm^2, frequency of 1 or 3MHz, depending on the group. The treatment consisted of daily five-minute exposure sessions, for six consecutive days. The treatments

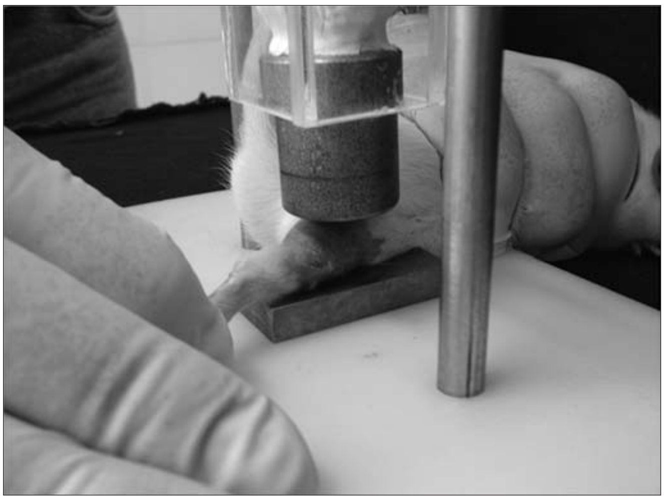


Figure 2. Equipment of production of the experimental lesion when the mass of 200g falls in the middle of gastrocnemius muscle.

with the ultrasound equipment began 24 hours after the lesion was induced, at the same period of the day for groups 3 and 4, using hydrosoluble gel as a contact medium and performing circular movements on the injured area. Considering the experimental lesion in the centermost area of the muscular belly, the treated area corresponded to the whole muscle region, which, after dissection, was 28mm long on average, with a perimeter of 27mm.

Preparation of the gastrocnemius muscle

After the experimental protocols were completed, the animals were submitted to euthanasia by an intraperitoneal administration of an excessive dosage of sodic tiopental anesthetic, so that their gastrocnemius muscles could be dissected and submitted to the mechanical traction test. The right upper limb gastrocnemius muscle of each animal was removed, by means of skin removal, as well as some soft parts, followed by the disarticulation of the ankle and hip. The precaution of keeping the muscle intact was taken, preserving its origin in the third femur distal and calcaneus insertions. The origin and bone insertions were maintained in order to facilitate its fixation to the testing machine. After dissection, the pieces were immersed in Ringer's lactate solution for 30 minutes, at room temperature, until the tests began.

Mechanical traction test

For the mechanical traction test of the gastrocnemius muscle, the universal testing machine (model DL10000, brand EMIC®) of the Bioengineering Laboratory FMRP-USP, was used equipped with a 50kgf capacity load cell. The machine used had a direct interface to a computer, containing the TESC® software, able to produce a load *versus* stretching for each test. Two accessories were manufactured for the fixation of the piece to be used, one being for the femoral fixation and the other for the calcaneus, while keeping the knee and the ankle at 90º. At the moment of the test, the muscle was attached to the machine and, according to the methodology established in the laboratory, a 200g pre-load was applied during the time of accommodation for 30 seconds, aiming at promoting the system's accommodation. All of the methodologies to run the mechanical tests had already been validated in a previous article[13].

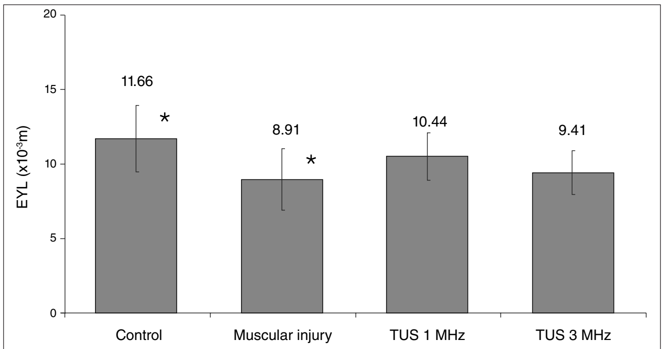
After the pre-loading, the test for eight minutes on average was carried out, with a pre-set speed of 10mm/minute. The software registered the load applied at regular stretching intervals up to the moment of muscle rupture. From the load *versus* stretching graphics of each test and analyzed the following mechanical properties were obtained:

- Stretching to a maximal limit (SML): it is a stretching value from the starting point up to the point of rupture, which is represented in meters ($x10^{-3}$m);
- load at a proportionality limit (LML): which is the maximal load value registered during the test and is represented in Newtons (N);
- stiffness (S): which is it is a biophysical property inherent to the muscle and to other materials, and corresponds to the elastic resistance to an applied force. It is obtained by the tangent of the angle (θ) and is represented in Newton/meters (N/m).

Figure 3. Reduced headstock of therapeutic ultrasound (14mm).

Statistical analyses

The statistical analyses were carried out by means of the BioEstat® v. 2.0 program. The Kolmogorov-Smirnov normality test was conducted. For the simultaneous analysis of the groups, an ANOVA test was used, and to compare the groups, the Turkey-Kramer test, both with pre-established significance levels of 5%.



m=meters/*significant difference between groups (p<0.05).

Figure 4. Mean values (±sd) for the elongation in the yield limit (EYL) of the groups.

Results

Forty muscles were tested, and the values expressed in means and standard deviations for each one of the properties in the four groups analyzed.

Stretching to a maximal limit (SML)

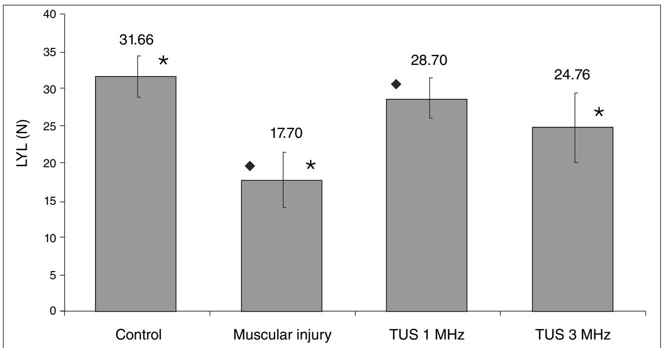
The means and standard deviations referent to the SML (stretching value observed until the moment when the muscle rupture took place) of the four groups are displayed in Figure 4. Considerable differences were observed ($p<0.05$) for the SML property, but only between groups 1 (control) and 2 (injured and non-treated).

Load at maximal limit

The means and standard deviations referent to the LML, or the maximal load supported by the muscle up to the moment of muscular rupture, of the four groups are presented in Figure 5. In regard to the LML, group 2 showed significant differences ($p<0.05$) in relation to groups 1, 3 and 4. There were also differences ($p<0.05$) between groups 1 and 4. There were no meaningful differences ($p<0.05$) between groups 1 and 3, or between groups 3 and 4.

Stiffness

The means and standard deviations referent to stiffness (which corresponds to the tangent of the angle of inclination of the load-curve *versus* stretching) of the four groups are shown in Figure 6. For the equivalent stiffness, group 2 showed a significant difference ($p<0.05$) when compared to groups 1, 3 and 4. No statistical differences ($p>0.05$) were observed between the control group and the groups treated with the TU at 1 and 3MHz, or even between those, treated with the TU.



N=Newtons/*◆significant difference between groups (p<0.05).

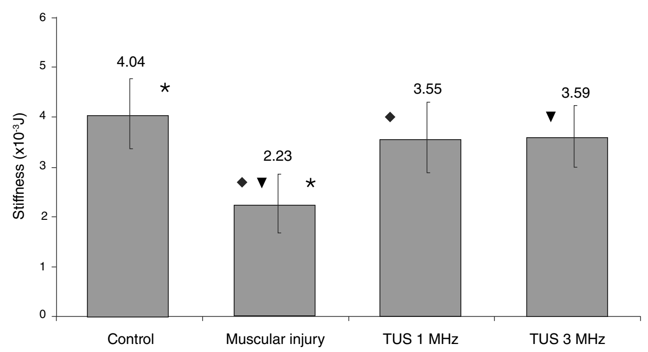
Figure 5. Mean values (±sd) for the load in the yield limit (LYL) of the groups.

Discussion

The muscle selected for the study was the gastrocnemius, due to its location and function. Besides, the gastrocnemius muscle works under extreme physical activity conditions and is susceptible to an increased risk of lesions and ruptures[14]. This muscle has been used by other researchers for studies involving muscle lesion and recovery [15].

The method of muscular lesion induction adopted in this study was based on the work of Stratton, Heckmann and Francis[12] and Minamoto, Grazziano and Salvini[16] who reported on its efficacy by means of histological studies. Some authors have recently used a similar methodology and described that a 170g mass was only capable of producing a minor lesion, or even no lesion whatsoever, which led them to adopt a 220g to produce an effective lesion[15]. After the pilot study was carried out, it was observed that a mass greater than 220g caused not only a muscular lesion, but also a tibia and fistula fracture. Thus, a 200g mass was adopted seeking to produce an effective muscular lesion without bone fractures. We were able to confirm the efficacy of the manufactured equipment and of the weight stimulated for the production of an experimental lesion by comparing the muscles submitted to the lesion and those of the control group, which remained intact. The injured muscles demonstrated a significant reduction in all analyzed properties, namely: 24% for SML, 44% for LML and 45% for stiffness.

The ultrasonic irradiation began 24 hours after the induction of the experimental lesion, a period which still encompasses the acute phase of tissue recovery[17-19]. During the physiological process of healing, the TU has a protective function by means of the neutralization of irritating chemical substances and by the precursor stimulus of the scar[4]. The TU treatment was administered in five-minutes daily sessions, following the recommendations for the acute phase of the lesion[20,21], besides taking into account the lesion area and the muscle length. Other authors suggest that the



J=joules/*◆▼significant difference between groups (p<0.05).

Figure 6. Mean values (±sd) for the stiffness of the groups.

beneficial effects of TU can be obtained with low intensities ($0.5W/cm^2$)[22,23], shorter exposures (five and six minutes)[22,23], work cycles of 1:5 (2ms on and 8ms off, 20%)[23] and pulse modes[24], with low heat production, as were the parameters used in this study. Furthermore, these parameters were chosen for being values typically used in clinical practice.

The effectiveness of TU is still very much discussed by many researchers. Whereas some authors have found positive results in the usage of TU to heal injured tendons, burns, and epicondylitis, others did not find it effective for the treatment of ulcers in burn patients or to treat muscular lesions[10,25,26]. Some factors, such as the type of evaluated tissue, the model of the experimental lesion, the intensity and the frequency of the treatment with the ultrasound[27] and particularly, the non-calibration of the equipment and the lack of a protocol to determine specific doses for each individual contribute to the discrepancies in the results. The effects of the ultrasound on morphological, physiological and biochemical aspects in the musculoskeletal system have been extensively examined[6,10,12,27]. On the other hand, very little research has found the behaviors of the mechanical properties of the biological tissues, *vis-à-vis* the therapeutic agents.

The skeletal muscles are continually being remodeled, and during this process there can be changes in their length, diameter, and resistance[28]. After a lesion, some authors recommend the use of external agents, aimed at accelerating the process of tissue healing[29]. In this way, TU has been employed in physical therapy treatment of many disorders of the musculoskeletal system[30] and, in spite of the conflicting conclusions on its efficacy, this study made it clear that the skeletal muscle does not remain inert to the effects of ultrasound. It was observed that its use on the injured muscles speeded up the process of recovery, increasing the stiffness and the load of muscle traction treated with therapeutic ultrasound. The 1MHz TU promoted increases of 38% in the LML and of 37% in stiffness, and the 3MHz TU did likewise with improvements of 29 and 38%, respectively. These results were not demonstrated in the injured muscles that did not undergo the treatment.

Some authors claim that stiffness is one of the fundamental muscular mechanical properties to be investigated[13]. Its reduction might be a sign that the muscle is stretching more when submitted to lighter loads, which in turn indicates a greater susceptibility to lesions[31]. In the present study, it was observed that significant improvements in the stiffness of the muscles treated with the ultrasound occurred, which suggests a better conditioning of these muscles when confronted to those that did not receive this treatment.

Considering the pulse mode used in this experiment, it would be suitable to suggest that improvements in the mechanical properties obtained in the muscles treated with ultrasound equipment was mainly attributed to mechanical or thermal effects. Nevertheless, some authors[32] hint that the divisions between thermal and mechanical effects are purely didactic, since in practice, these effects cannot be separated.

Conclusions

The therapeutic intervention by means of ultrasound increased the mechanical properties of the injured muscles, making them more similar to those of the control group, which indicated a partial muscular recovery. As far as the mechanical properties are concerned, no remarkable differences were observed between the group treated with a 1MHz frequency and the one that received a frequency of 3MHz. The thinness of the rat's muscles probably contributed to cause both frequencies to have a similar effect. Despite the positive results found in this study, it is fundamental to continue with research on therapeutic ultrasound, aiming to create protocols, as well as to determine more precise parameters for each individual, which will strengthen the scientific validity of this procedure.

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