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Original Article

Morphologic study of different treatments for gastrocnemius muscle contusion in rats☆

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ABSTRACT

Objective: Evaluate the effects of ultrasound and stretching in morphology after rat muscle contusion.

Methods: Male Wistar rats ($n = 35$, 8–9 weeks, 271 ± 14 g) were divided into five groups: control group (CG = 3); lesion group (LG = 8); lesion + ultrasound group (LUG = 8); lesion + stretching group (LSG = 8); lesion + ultrasound + stretching group (LUSG = 8). The ultrasound was applied in LUG and LUSG from the third to the seventh day, the dose used was 50% pulsed, 0.5 W/cm^2, 5 min. From the tenth until the twenty first day, passive stretching was performed, in four repetitions lasting 30 s each with 30 s of rest. Initial and final body weight, muscle weight and length, number and sarcomere length, muscle fiber cross-sectional area, and percentage of collagen were evaluated after 22 days.

Results: The final body weight was higher than the initial in all groups. The number of sarcomeres was statistically higher in LSG than LUG and higher in LUSG than LUS and CG; in sarcomere length was higher in LUG when compared with LSG ($p < 0.05$). The cross sectional area in LG was higher than LSG, and the percentage of collagen was higher in LG when compared with LSG and CG; in LUG when compared with LSG and CG; and in LUSG when compared with CG.

Conclusion: The passive stretching protocol induced sarcomerogenesis and antifibrotic effect over the muscle submitted to contusion. Ultrasound, even in association with stretching, was not sufficient to prevent fibrosis in the injured muscle.

☆ Study conducted at the Centro Universitário (UniBrasil), Pontifícia Universidade Católica do Paraná and Universidade Federal do Paraná, Curitiba, PR, Brazil.

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Estudo morfológico entre diferentes tratamentos da contusão muscular de gastrocnêmio em ratos

Palavras-chave:
Sistema musculoesquelético
Ferimentos e lesões
Exercícios de alongamento muscular
Terapia por ultrassom

RESUMO

Objetivo: Avaliar os efeitos do ultrassom terapêutico e/ou alongamento, na morfologia após contusão muscular em ratos.
Métodos: Ratos Wistar machos (n = 35, 8-9 semanas, 271 ± 14 g) foram divididos em cinco grupos: Grupo Controle (GC = 3); Grupo Lesão (GL = 8); Grupo Lesão + Ultrassom (GLUS = 8); Grupo Lesão + Alongamento (GLA = 8); Grupo Lesão + Ultrassom + Alongamento (GLUSA = 8). A aplicação do ultrassom no GLUS e GLUSA foi feita do terceiro ao sétimo dia, pulsado 50%, 0,5 W/cm^2, 5 min. Do décimo ao vigésimo primeiro dia foi feito o alongamento passivo no GLA e GLUSA, em quatro repetições de 30 s, com 30 s de intervalo, cada repetição. Após 22 dias, os ratos foram pesados e os músculos de ambas as patas foram retirados para análise do peso e comprimento muscular, número e comprimento dos sarcômeros, área de secção transversa e porcentagem de colágeno.
Resultados: O peso corporal final foi maior do que o inicial em todos os grupos. O número de sarcômeros foi maior no GLA em relação ao GLUS e no GLUSA em relação ao GLUS e ao GC; o comprimento dos sarcômeros foi maior no GLUS comparado com o GLA ($p < 0,05$). A área de secção transversa no GL foi maior do que no GLA e a porcentagem de colágeno foi maior no GL comparado com o GLA e o GC; no GLUS com o GLA e o GC; no GLUSA com o GC.
Conclusão: O protocolo de alongamento passivo induziu a sarcomerogênese e apresentou efeito antifibrótico em músculos submetidos a contusão. O ultrassom, independentemente da associação com o alongamento, não foi suficiente para impedir a fibrose nos músculos lesados.

Introduction

Muscle injury accounts for 60% of sports injuries.[1] Despite its high prevalence, there are few studies on its treatment, which hinders the standardization of physical therapy.[2] Among the different forms of treatment, the most commonly recommended are: rest; cryotherapy/compression/elevation; early mobilization; laser; ultrasound; and exercises of active mobilization, passive stretching, and concentric and eccentric strengthening.[2–5]

Although its biological effects in acute and chronic inflammation are not well understood,[2,6–8] therapeutic ultrasound (TUS) has been recommended. Piedade et al.[7] assessed the effects of pulsed application on lacerated gastrocnemius muscle of rats, and observed a significant increase in the number of myotubes in the regeneration zone 14 days after treatment. However, despite such results, the appropriate modulation of the TUS parameters (frequency, intensity, mode, treatment time) is still controversial.

In addition to treatment with electrothermal and phototherapeutic resources, exercise is recommended for the treatment of muscle injury. The most commonly prescribed exercise is muscle stretching.[1,5] As with TUS, its benefits on the injured fiber are unclear and it still has a low level of evidence. Nevertheless, Hwang et al.,[9] in experimental studies (rats), observed that the passive stretching done during the inflammatory, proliferative, and regenerative phases reduced the muscle fibrosis area by 50%, increasing strength and the number of myofibers when compared with the control group, demonstrating a benefit for muscle regeneration. The best results obtained in the study occurred when the stretching was initiated on the 14th day after injury.

It is important to emphasize that both TUS and muscle stretching are indicated for the treatment of muscle injury,[2,5–9] but there is still no consensus on when and how to prescribe them. Moreover, it is unclear whether the association between these therapeutic modalities can benefit the muscle regeneration mechanism. Thus, this study aimed to evaluate the effects of TUS and/or stretching on morphology after muscle contusion in rats.

Methods

Sample

This was an experimental, prospective, randomized study. After approval by the Ethics Committee for Animal Experimentation[10] (EAEC) of the UFPR (Certificate No. 491/2010), 35 young Wistar rats were selected (8–9 weeks, 271 ± 14 g). The animals were grouped in four per standard plastic cage, in an environment of controlled light (12 h light/dark cycle) and temperature, with free access to water and pelleted feed.

Inclusion criteria

The study included male rats weighing between 250 and 300 g, which underwent muscle contusion without the presence of fracture.

Sample distribution

The animals included ($n = 35$) were randomized into five groups: control group (CG = 3); lesion group (LG = 8); lesion + ultrasound group (LUG = 8); lesion + stretching group (LSG = 8); lesion + ultrasound + stretching group (LUSG = 8).

The animals were randomized by drawing. The CG Group was placed three times and the LUG, LSG, LG, and LUSG were placed eight times into a plastic bag for drawing. After the animals were weighed, the drawing was conducted, and the animals were assigned into each group and separated into the cages (Fig. 1).

Protocol for promoting muscle contusion

The animals were anesthetized with ketamine (95 mg/kg) and xylazine (12 mg/kg) intraperitoneally and kept in a prone position with the right paw manually immobilized in knee extension and 90° dorsiflexion of the ankle joint. The lesion was produced in the right gastrocnemius muscle (RGM) using a device consisting of a wooden platform with a hollow aluminum tube, graduated at 5 cm, placed perpendicularly to the platform, as previously described by Minamoto et al.[11] (Fig. 2). After the lesion, the absence of tibial fracture was assessed by means of palpation and handling.[1]

Ultrasound protocol

For the application of TUS, a researcher immobilized the mouse while another applied the device. To apply TUS (calibrated), the posterior region of the right paw was shaved and positioned in knee extension and 90° dorsiflexion of the ankle joint, and TUS was applied on the medial belly of the RGM. Gel was the contact medium. The effective radiation area was 1

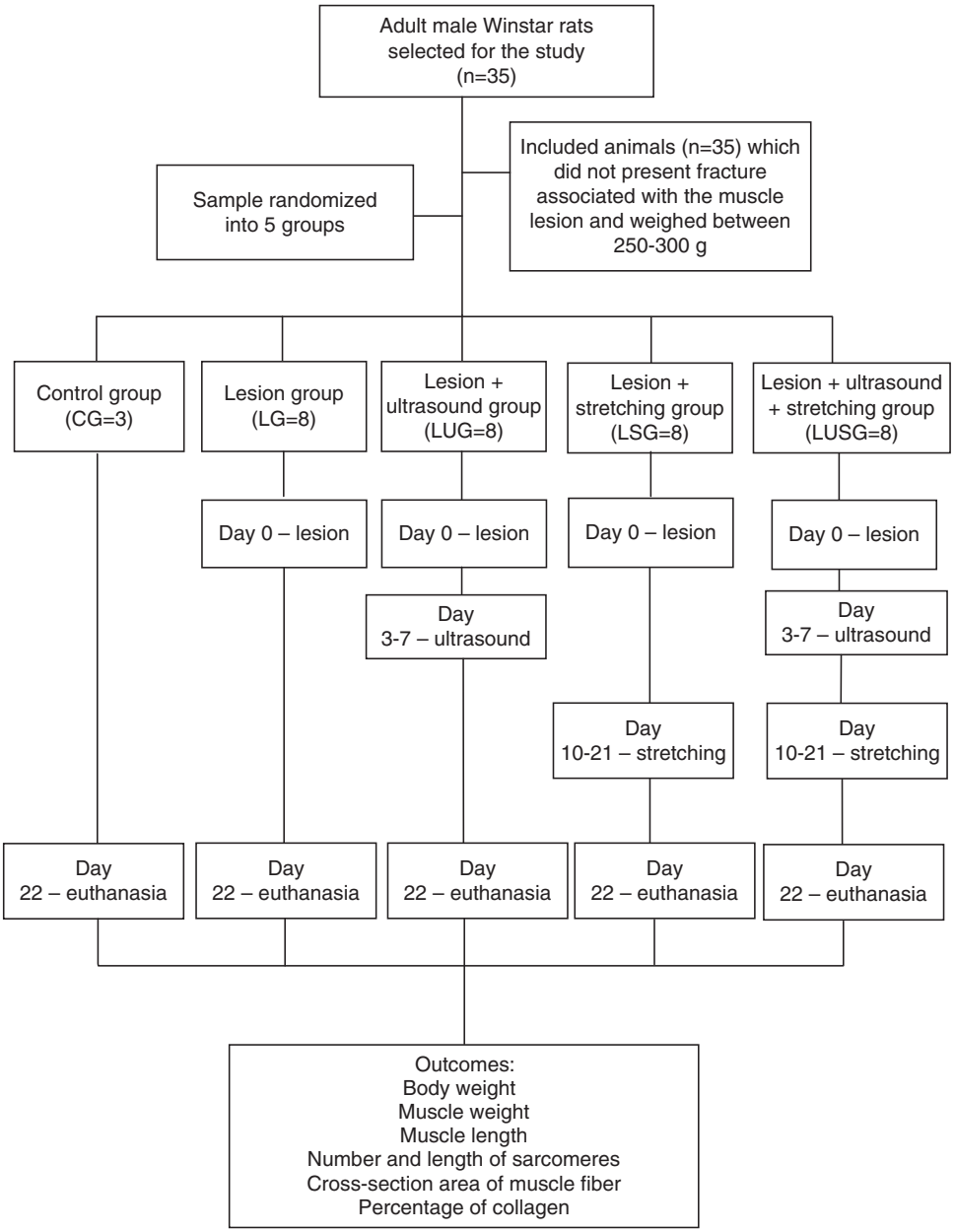


Fig. 1 – Study design.

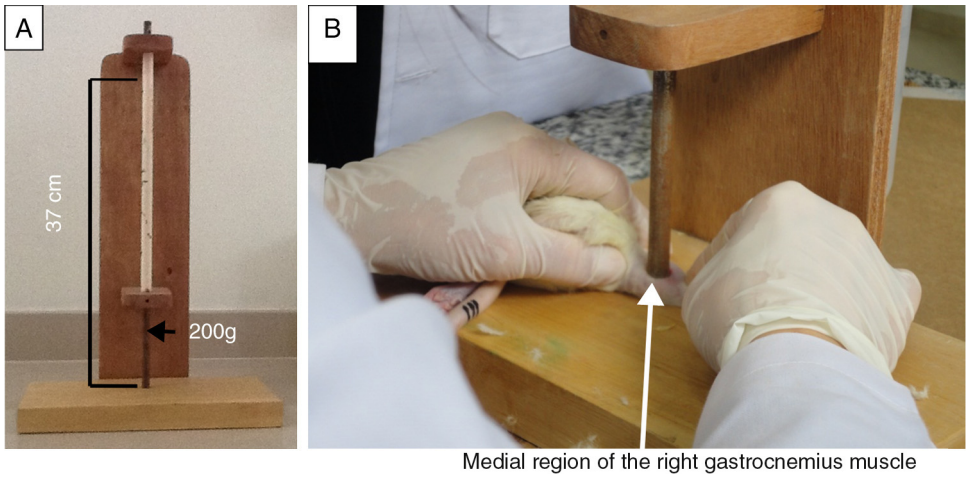


Fig. 2 – Apparatus for performing muscle lesion.
A, apparatus for induction of muscle lesion, which indicates the mass of the 200-g projectile (arrow), which is slid from a height of 37 cm; B, arrow indicates the projectile on the ventral region of the gastrocnemius muscle during the lesion procedure.

cm^2. The apparatus used was the *Sonopulse especial* (Ibramed), 1 MHz, pulsed at 100 Hz, 50% cycle, intensity[7] of 0.5 W/cm^2, and application time of 5 min. TUS was started 72 h after the lesion.

Protocol for stretching the gastrocnemius muscle

Animals were placed in the supine position with the front legs immobilized by a researcher, so that another researcher could perform passive stretching. To this end, the maximum dorsal flexion of the ankle joint was done manually with the knee extended, as described by Mattiello-Sverzut et al.[12] The protocol was maintained for 30 s[13] during each repetition, with an interval of 30 s, four repetitions,[14] five times a week (from Monday to Friday). Stretching was initiated on the 10th day after the lesion.

Chronological presentation of the interventions in the groups

Table 1 shows the sequence of interventions in each experimental group. As pulsed ultrasound has anti-inflammatory effects (among others), it was decided to use this feature during the inflammatory phase of the lesion. In turn, stretching was started in the fibroblast proliferation phase.[9]

Euthanasia and muscle collection

The animals of all groups were anesthetized with ketamine (95 mg/kg) and xylazine (12 mg/kg) intraperitoneally for removal of the right and left gastrocnemius muscles using scissors and tweezers. Subsequently, the animals were euthanized with an anesthetic overdose (ketamine and xylazine on the 22th day of the study).

Each muscle was separately weighed on an analytical balance and their length was measured by caliper. The muscles were longitudinally divided in half using a scalpel blade. The lateral portion was discarded and the medial portion was longitudinally divided in half. Half of the samples (lateral) had their distal ends fixed in the resting position with ultrafine needles and were maintained for 3 h in glutaraldehyde (2.5%), then nitric acid (30%) for 48 h, and subsequently stored in glycerol (50%). The lateral half was subjected to routine procedures for assessing the number of sarcomeres in series, as described by Williams and Goldspink.[15] The medial half of the RGM was fixed in 10% formalin for morphological analysis of cross-sectional area of muscle fibers (CSAMF) and collagen percentage. Left gastrocnemius muscle (LGM) was used as control.

The number of sarcomeres was assessed to verify whether the combination of ultrasound and/or stretching could

Table 1 – Sequence of interventions of the experiment.

Group intervention	LG	LUG	LSG	LUSG	CG
Contusion	Day 0	Day 0	Day 0	Day 0	
Ultrasound		3rd to 7th day		3rd to 7th day	
Stretching			10th to 21st day	10th to 21st day	
Euthanasia	22nd day	22nd day	22nd day	22nd day	22nd day

LG, lesion group; LUG, lesion + ultrasound group; LSG, lesion + stretching group; LUSG, lesion + ultrasound + stretching group; CG, control group.

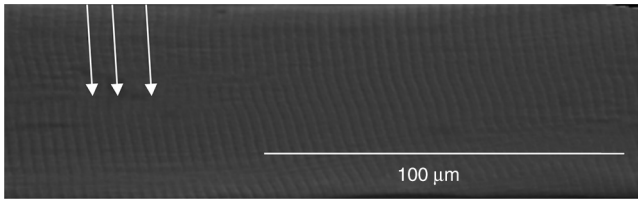


Fig. 3 – Photomicrograph of an isolated muscle fiber (100× objective). Arrow: each strip in the arrow head represents a sarcomere that was counted along 100 μm.

potentiate the increase in the number of sarcomeres in series after lesion. In turn, the cross sectional area and the percentage of collagen were assessed to verify whether the combination of ultrasound and/or stretching could prevent muscle atrophy and reduce the percentage of connective tissue after muscle lesion. All of these mechanisms may be affected after muscle lesion.

Identifying the number of sarcomeres in series

For preparation of histological slides, five fibers were isolated (using ultrafine tip forceps) of each muscle. The fibers were photographed under a light microscope (100× objective, immersion). On each fiber, the number of sarcomeres in series was identified in 100 μm; three fields of different different photomicrographs were used along each muscle fiber, totaling 300 μm.

After counting the number of sarcomeres in the 300 μm area, the total number of sarcomeres was estimated and correlated with the total length of the muscle, measured with calipers at the day of euthanasia (Fig. 3). Thus, the following equation was used: number of sarcomeres in 300 μm, multiplied by muscle length, divided by 300.

Therefore, the total sarcomere number and length in each isolated fiber were estimated by correlation between the number of identified sarcomeres and the total length of the muscle.[15–18]

CSAMF and percentage of type I *and* III COLLAGEN

For analysis of the CSAMF, the material was stained with hematoxylin and eosin. The photomicrographs were made using a light microscope at 20× magnification. To measure CSAMF, 100 muscle fibers were randomly selected in the central histological section, using cross-sections of 8 μm.[19] CSAMF was measured with the image program Pro Plus 4.0, and the unit of measurement adopted was the square micrometer (μm^2). The final arithmetic mean regarding the 100 measured fibers was calculated (Fig. 4).

For analysis of collagen percentage, the material was stained with sirius red. Photomicrographs were made in a light microscope at 20x magnification for histological slides, using cross-sections of 8 μm. The images were analyzed using the software Image Pro Plus 4.0. After calibration, a previously polarized image was chosen by the program to establish a pattern of red and green, which was quantified in all images. This image was considered as a "mask", which was superimposed upon any other images for identification of certain

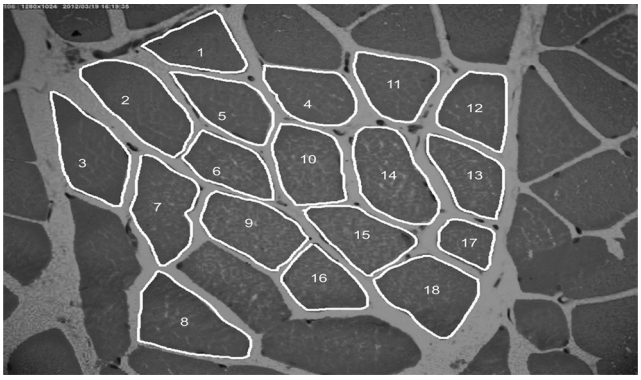


Fig. 4 – Photomicrograph of a histological cross-section of the gastrocnemius muscle to measure CSAMF using the *Image Pro Plus* 4.0 software. Outline in white: outline to measure the cross-sectional area of muscle fiber (CSAMF).

colors. After the "mask" was overlaid on each image, the program calculated the percentage of red and green colors, which correspond to the mature (type I) and immature (type III) collagen of the endomysium and perimysial, respectively.[20] The results were expressed as percentage of mature collagen (I, red) and immature collagen (III, green), and the sum of both is equal to 100% (Fig. 5).

Analysis of results

To assess normality and homoscedasticity, the Shapiro–Wilk and Levene tests were performed, respectively. Inter- and intra-group comparisons were made by one-way ANOVA *post hoc* Tukey unequal HSD for parametric values; for non-parametric values, the Kruskal–Wallis test was used. Values were considered significant when $p \leq 0.05$. Statistica 7 was used for the statistical analyses.

Results

Body weight

A significant increase was observed between the initial and final weight in all groups. Regarding absolute weight, the final body weight in the LG was higher than in the LUSG; the value in the LUG was higher than in the LUSG; and the value in the CG was higher than that in the LSG and in the LUSG. In relative difference, a significant increase was noted between LG and LSG; LG and LUSG; LUG and LSG; and LUG and LUSG (Table 2).

Weight and length of the gastrocnemius muscle

There was no statistically significant difference in the comparison of weight and muscle length between the RGM and the LGM and among the groups (Fig. 6A and B).

Estimated number of sarcomeres in series (ENSS)

In the group comparison, a significant increase was observed in the LUSG between the right and the left side. In the

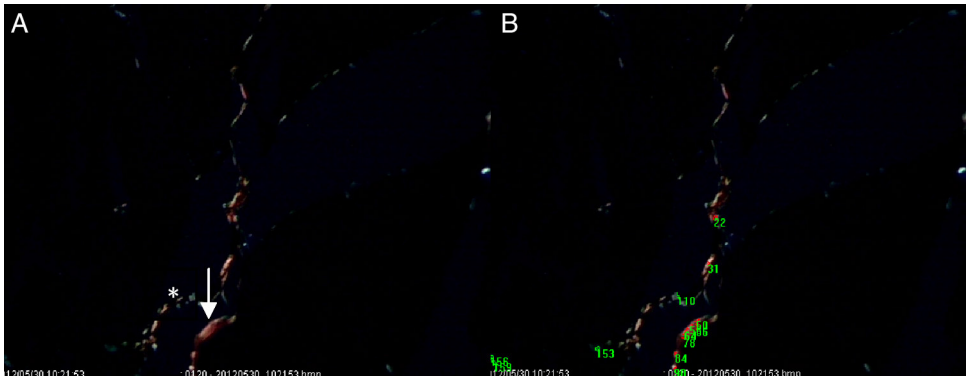


Fig. 5 – Photomicrographs of blades with transverse histological sections of the gastrocnemius muscle stained with sirius red.
A, image shows the cross section of the gastrocnemius muscle stained with sirius red; white arrow shows the area stained in red, identifying type I collagen (mature) and the asterisk the area in green corresponds to type III collagen (immature); B, image shows a photomicrograph of the cross-section of the gastrocnemius muscle overlapped by the "mask". The numbers represent the values of the areas marked in red and green colors, identified by the software.

intergroup comparison, a significant increase of the ENSS in the RGM was observed between the LSG and LUG; LUSG and LUG; and LUSG and CG (Fig. 6C).

Sarcomere length and CSAMF

In the comparison of the right and left sides in the LSG, it was found that sarcomere length was greater in the LGM than in the RGM. In the comparison between groups, an increased length of the sarcomeres of the RGM was observed between LUG and LSG (Fig. 6D). CSAMF of the RGM was greater in the LG when compared with LSG (Fig. 7A).

Analysis of type I *(mature) and* III *(immature) collagens*

There was no statistically significant difference in the comparison between RGM and LGM in each experimental group ($p > 0.05$). In the intergroup analysis of RGM, a significant increase ($p < 0.05$) was found in the percentages of mature collagen (type I) in LG and LSG (40 ± 12% *vs.* 24 ± 14%); LG and CG (40 ± 12% *vs.* 21 ± 9%); LUG and LSG (37 ± 10% *vs.* 24 ± 14%); LUG and CG (38 ± 10% *vs.* 21%); and LUSG and CG (36 ± 12% *vs.* 21 ± 9%) (Fig. 7B).

Discussion

Passive manual stretching increased the number of sarcomeres in series and prevented the increase in type I collagen in injured muscles. However, the ultrasound protocol, regardless of association with stretching, showed no antifibrotic effect in these muscles.

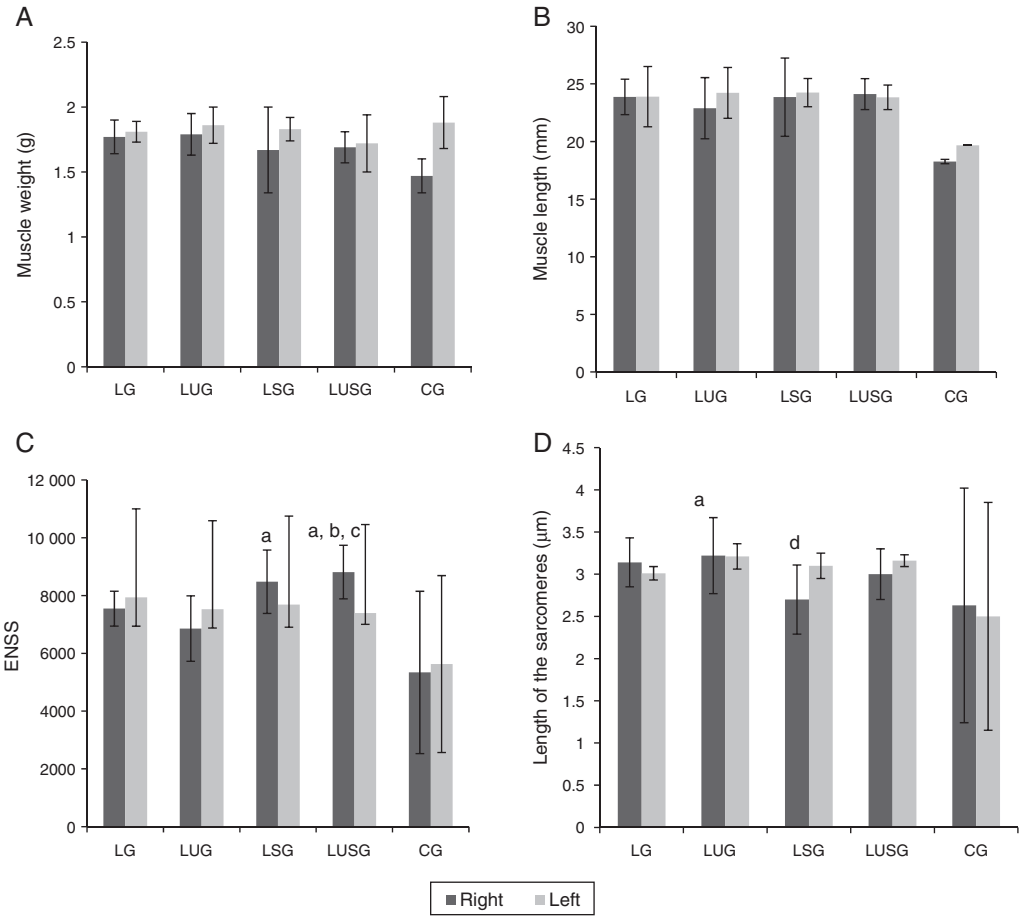
In relation to body weight, all groups showed an increase when comparing the initial with the final weight. The absolute final body weight was higher in the CG than in the LSG and in the LUSG; the LG and LUG had greater weight than the LUSG. Therefore, the groups that underwent the highest amount of stimulation (contusion, ultrasound, and stretching) may have presented a negative interference in absolute body weight gain, since the CG had the highest final body weight.

There were no significant differences in muscle weight and length, perhaps due to the short intervention period and the

Table 2 – Effects of ultrasound and/or stretching on the body weight of mice.

Groups	Initial weight (g)	Final weight (g)	Relative difference (%)	*p* ANOVA
LG	267 ± 11	350 ± 18[a,b]	31 ± 7[e]	0.02
LUG	266 ± 10	352 ± 19[a,c]	32 ± 10[f]	0.01
LSG	271 ± 11	323 ± 18[a]	19 ± 8	0.00
LUSG	270 ± 14	318 ± 16[a]	17 ± 3	0.00
CG	299 ± 16	383 ± 24[a,d]	28 ± 6	0.00

LG, lesion group; LUG, lesion + ultrasound group; LSG, lesion + stretching group; LUSG, lesion + ultrasound + stretching group; CG, control group.
[a] Compared with the initial body weight.
[b] Compared with LUSG ($p = 0.02$).
[c] Compared with LUSG ($p = 0.01$).
[d] Compared with LSG ($p = 0.00$) and LUSG (0.001).
[e] Compared with LSG ($p = 0.00$) and LUSG.
[f] ($p = 0.000$) compared with LSG and LUSG.
Mean ± SD.



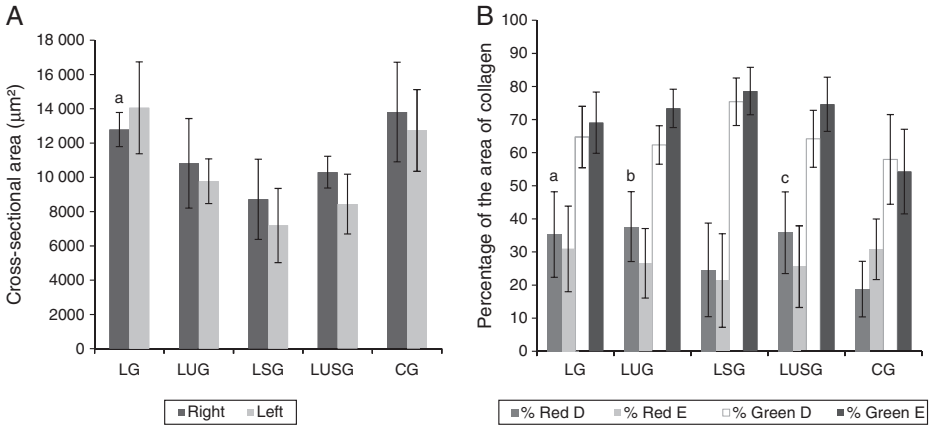
[a] Compared with the right gastrocnemius muscle of the LUG (P=.001).
[b] Compared with the right gastrocnemius muscle of the CG (P=.04).
[c] Compared with the left gastrocnemius muscle (P=.001).
[d] Compared with the right gastrocnemius muscle of the LSG (P=.001).
[e] Compared with the left gastrocnemius muscle of the LSG (P=.001).
Mean ± standard deviation.

Fig. 6 – Effects of ultrasound and/or stretching in weight and muscle length, number, and length of sarcomeres. A, muscle weight; B, muscle length; C, the number of sarcomeres in series; D, length of the sarcomeres. ENSS, estimate of the number of sarcomeres in series; LG, lesion group; LUG, lesion + ultrasound group; LSG, lesion + stretching group; LUSG, lesion + ultrasound + stretching group; CG, control group.

volume of the stretching protocol, that is, only two weeks, for two minutes each session. Coutinho et al.[19] observed an increase muscle length after 40 min stretching for three weeks. Therefore, the intervention period and the volume of stretching contribute to longitudinal muscle growth. However, the measurement of the cross-sectional area of muscle fibers and the number of sarcomeres in series count are the most accurate methods for assessing muscle trophism in parallel and in series, respectively.[21]

Regarding the number of sarcomeres in series, the LSG presented a higher number, demonstrating the effect of stretching on the sarcomerogenesis of injured muscles. This difference may also be due to the volume of stimulus, as the LUG received only five TUS applications and the LSG received ten stretching sessions.[19] The combination of TUS with stretching (LUSG) contributed to the increase in sarcomeres in series when compared with the LUG, demonstrating once again the stretching-stimulated sarcomerogenesis, which was also crucial to the growth of sarcomeres in injured muscles. Furthermore, this outcome was confirmed through the observation that the number of sarcomeres in series in the LUSG was higher than in the CG. Thus, this is the first confirmation of an increase in the number of sarcomeres in series in previously injured muscles. This suggests that the adaptation resulting from the association of TUS with stretching was not only a change in the passive tension of the length of the muscle-tendon unit, being sufficient to induce morphological adaptation of the longitudinal muscle.[22]

In the LUG, the length of the sarcomeres in the RGM was lower than in the LGM; comparing RGMs, the length of sarcomeres was lower in the LUG than in the LUSG. These outcomes confirm the sarcomerogenesis observed in the LSG, since, according to Koh,[23] skeletal muscle can increase or decrease sarcomeres in series to adjust the optimum length of the



[a] Compared with the right gastrocnemius muscle of the LUG (P=.01).
[b] Compared with the LSG (p =.00) and CG (P=.00).
[c] Compared with the LSG (p = 0,00) and CG (P=.00).
Mean ± standard deviation

Fig. 7 – Ultrasound and/or stretching effects on CSAMF (A) and on the percentage of the area of type I collagen (mature, red) and type III collagen (immature, green) (B).
R, right; L, left; LG, lesion group; LUG, lesion + ultrasound group; LSG, lesion + stretching group; LUSG, lesion + ultrasound + stretching group; CG, control group; Red R (dark gray column), type I collagen of the R gastrocnemius; Red L (gray column), type I collagen of the L gastrocnemius; Green R (white column), type III collagen of the R gastrocnemius; Green L (black column), type III collagen of the L gastrocnemius.

sarcomere in which maximum muscle strength will be produced during contraction. Thus, this mechanism might have occurred in the intragroup LSG and in the comparison with the LUG, since, as increased numbers of sarcomeres in series were observed in the LSG, there was a decrease of the sarcomere length in this group; the reverse pattern was identified in the LUG.

Regarding CSAMF, it was observed that this value was higher in the LG than in the LSG; however, no difference was observed when comparing with the CG. The body weight of the LG increased by 31%, *vs.* 19% in the LSG, which may have influenced the CSAMF, despite the fact that no increase in muscle weight was observed. In the present study, ultrasound and stretching did not influence CSAMF. Market et al.[6] assessed the effects of TUS and low-intensity walking exercise for 20 min on the treadmill (speed 14 m/min) for four days, initiated 24 h after mechanical lesion of the gastrocnemius, and also did not observe an increase in CSAMF. Coutinho et al.[19] found that 40-min stretching increased CSAMF in the immobilized and stretched group when compared with the group that was only immobilized; those authors concluded that stretching can prevent muscle atrophy, and its application is relevant in immobilized muscles. As the LG presented a higher CSAMF than the LSG, stretching may have interfered in reducing edema and regenerating the sarcolemma, but it was not sufficient to stimulate radial (transverse) muscle growth, as reported in other studies.[24,25] However, to further elucidate the mechanisms involved in changes in muscle trophism, studies analyzing the muscle ultrastructure and regulatory genes are suggested.

The injured muscles and those that did not undergo intervention (ultrasound and/or stretching) showed an increased percentage of type I collagen, demonstrating the fibrotic effect of the lesion. This effect was also observed when ultrasound was applied in isolation and in association with stretching. Conversely, when stretching was performed alone, the proliferation of collagen was lower than that observed in muscles that underwent TUS alone. Therefore, it can be concluded that stretching showed an antifibrotic effect when compared with isolated or combined ultrasound.

Williams et al.[26] stated that daily stretching of muscles shortened due to immobilization prevents the deposition of connective tissue in the muscle tissue. The study by Hwang et al.[9] corroborated these results. They assessed the effects of stretching on injured muscles and observed its antifibrotic effect. These authors indicated that stretching should be started on the 14th day after lesion. However, in the present study stretching was initiated on the 10th day; despite the early initiation, the fibrinolytic effect was still observed. Jarvinen et al.[27] have suggested that the increase intramuscular connective tissue contributes to functional deficits, producing tensile strength, compliance, and muscle stiffness. Stretching programs may contribute to increase muscle extensibility, due to the reorganization of intramuscular connective tissue.[28]

The LUG had a higher percentage of mature collagen in relation to the LSG and the CG. The increased density of connective tissue, associated with a decreased numbers of sarcomeres, which was found in LUG relative to LSG, is characteristic of shortened skeletal muscle under fibrosis process.[9,19,21,26] This demonstrates that ultrasound used alone for only five days has no fibrinolytic effect. Rantanen et al.[29] used 3 MHz pulsed TUS at 20%, intensity of 1.5 W/cm^2, in stationary technique for 6 min; application was initiated in one group 72 h after the lesion and in the other group, 6 h after the

lesion. The authors found an increased production of fibroblasts after muscle contusion in rats from the groups that had undergone at least five sessions of pulsed TUS. Piedade et al.,[7] started daily applications of 1 MHz pulsed TUS at 50%, intensity of 0.57 W/cm^2, for 5 min, through the sliding technique, and observed the early onset of type I collagen fibers four days after gastrocnemius muscle lesion in rats; a better structural arrangement and better alignment of myotubes in formation was observed in the group treated with TUS. These authors suggested that TUS can stimulate early aggregation of this type of collagen.

Although no increase in the percentage of type I collagen (mature) was observed in the present study, the authors did not assess the arrangement, that is, the orientation of the collagen fibers. Thus, it is not possible to predict whether this increase in type I collagen may generate functional deficits. Therefore, future studies should use polarizing microscopy for analysis of birefringence of intramuscular connective tissue[28] and range of motion.

The outcomes of this study indicate important clinical perspectives, demonstrating that a passive stretching protocol performed ten days after muscle lesion induced sarcomerogenesis and presented an antifibrotic effect, indicating a possible improvement of skeletal muscle extensibility. Thus, respecting the limitations regarding direct extrapolations of the present results humans, it can be hypothesized that stretching exercises could be prescribed for the treatment of muscles injured by contusion.

Study limitations

This study was limited by the use of an animal model, which produces a lower level of scientific evidence. Therefore, randomized controlled clinical trials should be performed to assess the effects of ultrasound, whether or not associated with stretching, in injured skeletal muscle of humans. The authors recommend the use of electron microscopy to assess sarcomere length in future studies. Furthermore, analyses with molecular biology techniques should be used to investigate the genes involved in the processes of repair and tropism of skeletal muscle, especially resulting from the application of the two therapeutic modalities, ultrasound and stretching. Biomechanical assays for analysis of muscle-tendon unit tension are also important to elucidate the viscoelastic properties in response to ultrasound and/or stretching.

Conclusion

The passive stretching protocol induced sarcomerogenesis in injured muscles. Furthermore, stretching alone had an antifibrotic effect, while ultrasound, regardless of association the stretching, was not sufficient to prevent the increase of collagen I in injured muscles.

Conflicts of interest

The authors declare no conflicts of interest.

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