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Effects of GaAs laser and stretching on muscle contusion in rats

Efeitos do laser GaAs e alongamento na contusão muscular em ratos

Efectos del láser GaAs y estiramiento en la contusión muscular en ratones

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ABSTRACT | Laser and stretching are used to treat skeletal muscle injuries. This study aimed to evaluate the effects of GaAs laser and stretching in the morphology of the tibialis anterior (TA) muscle after contusion. Thirty-six male rats (349±23g) were divided into six groups (n=6): control group (CG); lesion group (LG); lesion and laser group (LLG); lesion and stretching group (LSG); lesion, laser and stretching group (LLSG); and stretching group (SG). TA was wounded by a contusion apparatus. We used GaAs laser 4.5 J/cm^2 dose for 32 s each, beginning 48 h after lesion, for 7 days, once a day. Manual passive stretching was applied by 10 repetitions for 1 minute, initiating on the 8th day, once a day, 3 times a week, during 3 weeks. After 4 weeks, rats were euthanized and we analyzed: muscle weight and length, cross sectional area of muscle fibers (CSAMF), serial sarcomere number (SSN), sarcomere length, and percentage of connective tissue. Comparisons among groups were made by ANOVA and *post hoc* Tukey tests, with the significance level set at ≤ 0.05. The serial sarcomere number of LLSG was higher than LSG. The sarcomere length of LSG was superior to LLG, LLSG, and SG. SG increased SSN compared to CG, while the percentage of connective tissue of SG decreased in comparison to LLSG. Thus, the sarcomerogenesis of injured muscles was enhanced by laser therapy, stretching, and association of both. The stretching protocol was enough to increase SSN of intact muscles.

Keywords | Skeletal Muscle; Muscle Stretching Exercises; Wounds and Injuries; Low-Level Light Therapy; Rats.

RESUMO | Laser e alongamento são usados para tratar lesões musculares. Este estudo objetivou avaliar os efeitos do laser GaAs e alongamento na morfologia do músculo Tibial anterior (TA) após contusão. Trinta e seis ratos (349±23 g) foram divididos em seis grupos (n=6): grupo controle (GC); grupo lesão e laser (GLL); grupo lesão e alongamento (GLA); grupo lesão, laser e alongamento (GLLA) e grupo alongamento (GA). Foi realizada lesão no TA por meio de um aparato de contusão. O tratamento com laser GAAS foi usado com dose de 4,5 J/cm^2 durante 32 s, iniciando 48 h após lesão, por 7 dias. Alongamento passivo manual consistiu de 10 repetições de 1 minuto de duração, iniciando no 8º dia, 3 vezes por semana, durante 3 semanas. Após 4 semanas, os ratos foram eutanasiados para retirada do TA para análise de: peso e comprimento musculares, área de secção transversa das fibras musculares (ASTFM), número de sarcômeros em série (NSS), comprimento dos sarcômeros e porcentagem de tecido conjuntivo. A comparação entre os grupos deu-se por meio da ANOVA e *post hoc Tukey,* com nível de significância ≤0,05. O número de sarcômeros em série do GLLS foi maior que o GLS. O comprimento dos sarcômeros no GLA foi superior ao GLL, GLLA e GA. No GA houve aumento do NSS comparado

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com o GC, enquanto a porcentagem de tecido conjuntivo do GA diminuiu em comparação com o GLLA. Assim, a sarcomerogênese dos músculos lesionados foi aumentada pelo uso do laser, alongamento e pela associação destes. O alongamento foi suficiente para aumentar o NSS em músculos intactos.
Descritores | Músculo Esquelético; Exercícios de Alongamento Muscular; Ferimentos e Lesões; Terapia com Luz de Baixa Intensidade; Ratos.

RESUMEN | Láser y estiramiento son utilizados para sanar lesiones musculares. Este estudio tuvo como objetivo evaluar los efectos del láser GaAs y del estiramiento en la morfología del músculo tibial anterior (TA) después de contusión. Treinta y seis ratones (349±23 g) fueron divididos en seis grupos (n=6): grupo control (GC); grupo lesión y láser (GLL); grupo lesión y estiramiento (GLE); grupo lesión, laser y estiramiento (GLLA) y grupo estiramiento (GA). Se realizó lesión en el TA mediante un aparato de contusión. Se utilizó el tratamiento con láser GaAs con dosis de 4,5 J/cm^2 durante 32 s, iniciado 48 h después de la lesión, durante 7 días. El estiramiento pasivo manual consistió de 10 repeticiones de 1 minuto de duración, iniciado en el 8º día, 3 veces por semana, durante 3 semanas. Después de 4 semanas, los ratones sufrieron eutanasia para la retirada del TA para análisis de: peso y longitud musculares, área de sección trasversa de las fibras musculares (ASTFM), número de sarcómeros en serie (NSS), longitud de los sarcómeros y porcentaje de tejido conjuntivo. La comparación entre los grupos ocurrió mediante la Anova y *post hoc Tukey*, con nivel de significancia de ≤0,05. El número de sarcómeros en serie del GLLS fue mayor que el GLS. La longitud de los sarcómeros en el GLA fue superior al GLL, GLLA y GA. En el GA hubo aumento del NSS en comparación con el GC, mientras el porcentaje del tejido conjuntivo del GA disminuyó en comparación al del GLLA. Así, la sarcomerogénesis de los músculos lesionados fue aumentada por el uso del láser, estiramiento y por su asociación. El estiramiento fue suficiente para aumentar el NSS en músculos intactos.
Palabras clave | Músculo Esquelético; Ejercicios de Estiramiento Muscular; Heridas y Lesiones; Terapia por Luz de Baja Intensidad; Ratones.

INTRODUCTION

The most common skeletal muscle injury in contact sports is contusion[1]. Different physiotherapeutic intervention techniques are used to accelerate the muscle repair process and reestablish function, such as: cryotherapy[2]; therapeutic ultrasound[3]; laser[4]; and early mobilization and exercises[5]. However, the skeletal muscle adaptations to these techniques are not entirely elucidated[5].

Studies have shown that muscle regeneration, in vivo as well as in vitro, becomes more effective when treated with low intensity laser[6,7]. It has been demonstrated[8,9] that lasers operating at different wavelengths activate the macrophages to release factors that stimulate fibroblast proliferation. Low-intensity laser acts on the synthesis and collagen remodeling, fibroblast number, diameter, and tensile strength of the treated wounds[7].

Oxygen presence is also a critical step in fibroblastic proliferation and maturation, improved by laser action with increasing microcirculation[10]. Still, laser favors the regeneration of muscle tissue through the activation of satellite cells by introducing them in the cell cycle, which promotes its proliferation and progression to the status of new muscle fibers[11].

To our knowledge, only two studies have described the effect of stretching in injured muscle, which showed new myofibrils and also sarcomerogenesis after 14 days of performance of a stretching protocol[16,17].

Stretching skeletal muscle is very important not only to increase the joint range of motion, but also to prevent and treat injuries, thus being a tool of therapeutic intervention for physical rehabilitation and sports medicine[12]. Static stretch of skeletal muscle cells in vitro or in vivo results in well-defined changes in cellular growth, morphology, and metabolism[13], which includes muscle hypertrophy, prevents muscle atrophy and implicates in the modulation of muscle fiber behavior[14]. Koh et al.[15] found that a single bout of stretching protected skeletal muscle from contraction-induced injury. It has been shown that early mobilization induces more rapid and intensive capillary ingrowth into the injured area, better regeneration of muscle fibers, and more parallel orientation of the regenerating myofibers in comparison to immobilization, the previously preferred treatment for injured muscle[5].

It is recommended that treatment of the injured muscle be started gradually; local application of laser can be used, accompanied by careful passive and active stretching. However, it is not yet known exactly how this therapy combination affect muscle repair[4,5,7,17,18].

The use of therapeutic laser and the prescription of stretching exercises stand out among the interventions for skeletal muscle injuries, but both show a low level

of scientific evidence regarding their efficacy. Only limited evidence was found to support the use of laser or stretching[7,17,19]. Thus, this study aimed to investigate the rat's tibialis anterior muscle morphology submitted to contusion and treated with GaAs laser and/or stretching.

METHODOLOGY

Animal care and experimental groups

Thirty-six adult male rats, *Rattus norvergicus*, weighing 349±23g, aged around 10-12 weeks were used, as young adult animals have high potential for muscle regeneration[20]. The animals were housed in groups in standard plastic cages in an animal room with controlled environmental conditions (luminosity: 12 hour light/dark cycle), and had free access to standard food and water at the vivarium.

The study was conducted according to international standards of ethics for animal experiments[21] and approved by the Ethics Committee, protocol 1096/05. The animals were pre-weighed, anesthetized with ketamine (95 mg/kg body weight) and Xylazine (12 mg/kg body weight) and submitted to the following procedures: muscle contusion, passive stretching, laser therapy, tibialis anterior (TA) dissection, and euthanasia.

The animals were randomly divided into six groups: 1) Control group (CG, n=6): animals were kept intact during 4 weeks; 2) Lesion group (LG) (n=6): rats were submitted to the left TA muscle contusion with an apparatus as described by Minamoto et al.[22] and kept at the vivarium for 4 weeks[22,23]; 3) Lesion and laser group (LLG, n=6): the left TA underwent contusion and the treatment was started 48 hours later with GaAs Laser. On 8th day the animals were euthanized; 4) Contusion and Stretching group (LSG, n=6): the left TA muscle underwent contusion and on the 8th day (after 1 week), a stretching protocol was started on the TA muscle; 5) Contusion, Laser Therapy and Stretching group (LLSG, n=6): the left TA underwent contusion, laser therapy and stretching protocols; 6) Stretching group (SG, n=6): only the stretching protocol was performed, and 4 weeks later the animals were euthanized. There was no sample loss during the experiment.

The animals of all groups had the TA muscles of both hind limbs carefully removed and processed for morphology evaluation, measurement of muscle length, morphometry of muscle fiber cross sectional area, counting of serial sarcomere number, and estimation of the sarcomere length.

All experimental procedures were conducted starting at 1:30 p.m. each day, in ascending order of rats, 1 to 6.

Muscular contusion protocol

The animals were anesthetized and kept in dorsal position, in which their posterior left limb was immobilized in a knee extension and ankle plantar flexion. The belly region of the TA muscle was shaved, demarcated with a pen marker and positioned, and then the projectile of the contusion apparatus slid perpendicularly to the middle belly of the left TA muscle[22,23].

The projectile of the contusion apparatus was constituted by a metal mass that fell through a metal guide tube to produce the injury, as previously described[22,20]. The mass (200 g) was dropped from a height of 37 cm in the middle belly of left TA muscle. Right muscles were not injured and were also used as control[22, 23].

Laser therapy protocol

The animals were anesthetized and immobilized and then the Laser Infra-Red (IR) Gallium Arsenide (GaAs) was applied (KLD Biosistemas Electronics Equipment LTDA, UMDNS - numbering 12-299, identification Factory-IR1H805, LIV 9707, Amparo, SP – Brazil), with a peak power of 45 W, wavelength of 904 nm pulsed with 1110 Hz of frequency, pulse duration of 200 ns, invisible light, average power of 10 mW, spot 0.07 cm^2, power density of 142.85 mW/cm^2 for 32 s, time stipulated by the laser device, and a point application technique with only one point applied, with fluency of 4.5 J/cm^2, and 0.32 J of energy per point. The area was predetermined and limited with a pen marker to guarantee that all applications were performed in the same place, once a day, for seven consecutive days, with 24-hours intervals between the applications. At the end of the 7 applications, the total energy received by the injured TA muscle was 2.24 J for each animal in the LLG and LLSG groups[25].

After the end of each experimental group, the left TA was removed and weighed on a scale (Mark Bell Engineering, Italy) in isolation. Afterwards, the tendons of the muscle were clamped with the muscle in a resting position[26] and muscle length was determined by a digital

caliper (Vonder). Then, TA was divided longitudinally into two equal parts, one part was submitted to routine procedures to evaluate the serial sarcomere number and the other was fixed in Zenker for further morphological and morphometric analysis.

Muscle stretching protocol

The animals were anesthetized and immobilized for the application of the passive stretch. To stretch the TA, the left ankle joint was held manually in full plantar flexion. The stretching protocol consisted of 10 repetitions, 1 min for each, with a rest of 45 s between each repetition, once a day, three times a week (Mondays, Wednesdays and Fridays), for three consecutive weeks.

Serial Sarcomere Number Identification

The muscle fibers were isolated and fixed as described by Coutinho et al.[27] In each muscle fiber, the serial sarcomere number was identified over 300 μm in a light microscope (Nikon, Tokyo, Japan) at 100x immersion objective. The quantification was performed on a video monitor (14") with video-image system (Adler CCTV) coupled to the microscope (Nikon, Tokyo, Japan) with the help of a counter (Veeder - Root, Washington, USA).

The total serial sarcomere number and the sarcomere length in each isolated muscle fiber were estimated by the correlation between the number of sarcomeres identified along 300μm of each fiber and total muscle length[28].

Morphologic analysis procedures

A fragment was removed from the medial half belly of each TA muscle and fixed with Zenker (12 h), included in paraffin (Labsynth, Diadema, Sao Paulo Brazil), sectioned (8 μm) transversely in microtome (Instrument Company, Huntingdon, England). Then, the sections were stained with Harris Hematoxylin and Eosin (HE) for histomorphometric analysis of the cross-sectional area of muscle fibers (CSAMF). In other histological slides, other cuts were stained with Mallory Trichrome to evaluate connective tissue percentage (perimysium and endomysium).

The photomicrographs of the histological slices were performed under a light microscope (Axyophot, Carl Zeiss, Oberkochen, Germany) and captured on a video-image system (Applied Spectral Imaging, Migdal Ha'emek, Israel) through the Case Data Manager Expo Program (Applied Spectral Imaging, Migdal Ha'emek, Israel, version 4.0) in a laboratory of the Cellular and Molecular Biology Department of the Federal University of Paraná (UFPR), in Curitiba, Brazil. In each muscle, we measured the CSAMF and the connective tissue percentage, using the UTHSCSA Image Tool 3.0 software (developed at the University of Texas Health Science Center at San Antonio, http://ddsdx.uthscsa.edu/ dig/itdesc.html).

We measured the cross-sectional area of 100 muscle fibers of each muscle, chosen randomly from the area of the muscle belly of the histological section[27].

The percentage analysis of the connective tissue area was performed first through the selection of the whole area of the slice stained with Mallory Trichrome, and photographed using the 10x objective equivalent to 100%. After that, the cross-sectional area of all muscle fibers was excluded, and only the connective tissue, i.e., the perimysium and endomysium, was isolated. After such reading, results were expressed as percentage[29].

Statistical analysis

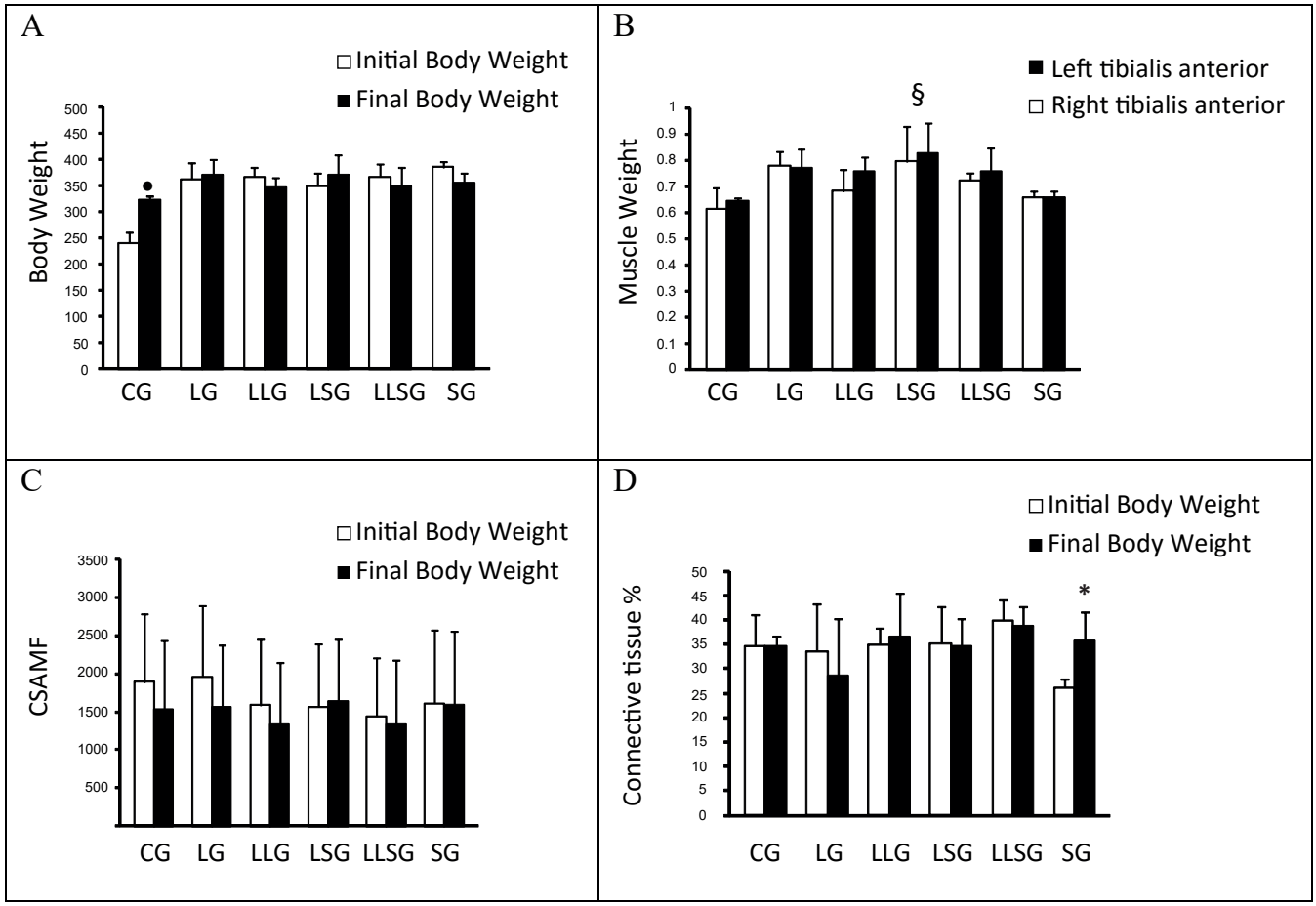
Shapiro Wilk's test was used to check normality and Levene for homogeneity of the results. As all the data showed normal and homogeneous distributions, the one-way ANOVA, *post hoc* Tukey test was used to compare the groups, using the following variables: body weight; muscle weight; muscle length; serial sarcomere number and length; cross-sectional area of the muscle fibers; and connective tissue percentage. For all tests, the significance level was set at 5% ($p \leq 0.05$).

RESULTS

Body and muscle weights

The final body weight of all groups showed normal ($p=0.29$) and homogeneous distributions ($p=0.13$). The final body weight of LG (370±30 g *vs.* 322±6 g, $p = 0.02$) and LSG (370±38 g *vs.* 322±6 g, $p=0.02$) were higher than the GC. The results for body weight are shown in Figure 1A.

The weight of the left TA that was injured and stretched (LSG) increased compared to the only stretched TA (SG) (0.83±0.11 g *vs.* 0.66±0.03 g, $p=0.003$). Muscle weight values are shown in Figure 1B.



A: Final Body weight: p= 0.02 (ANOVA) when compared to LG and LSG; **B:** Muscle Weight: §p=0.003 (ANOVA) when compared to SG. **C:** Cross Sectional Area of Muscle fibers (CSAMF); **D:** Connective Tissue Percentage: *p=0.004 (ANOVA) in comparison with LLSG. The results are mean±standard deviation. Control Group (CG); Lesion Group (LG); Lesion and Laser group (LLG); Lesion and Stretching Group (LSG); Lesion, Laser and Stretching Group (LLSG); Stretching Group (SG)

Figure 1. Effects of laser and stretching on radial muscle morphology

Cross-sectional area of muscle fibers (CSAMF)

The results showed normal and homogeneous distributions in all groups. There were no statistically significant differences in cross-sectional area of muscle fibers among the groups, as can be seen in Figure 1C.

Connective tissue percentage

We found normal and homogeneous distributions in the connective tissue percentage of all evaluated groups. We observed a decrease in the percentage of connective tissue in SG compared to the LLSG (35±5% vs. 38±4%, p=0.004), as presented in Figure 1D.

Muscle length

Mean values of muscle length were normal and homogeneous in all groups. The length of the TA muscle group that underwent muscle contusion (LG) was higher compared to the LSG (26±1 mm *vs.* 22±2.34 mm, p=0.008) and SG (26 mm ± 1 mm *vs.* 21 mm ± 1.28 mm, p=0.003). We also noted that the length of the muscles of the LG (17±1 *vs.* 26±1, p=0.00002); LLG (17±1 mm *vs.* 23±2.6mm, p=0.00006); LSG (17±1mm *vs.* 22±2.34mm; p=0.0001); LLSG (17±1mm *vs.* 25±1.87 mm, p=0.00003); and SG (17±1mm *vs.* 21±2.1mm, p=0.0003) was higher than the CG (17±1mm). We noted a decrease in muscle length of LSG, when compared to LLSG (23.4±2.2mm *vs.* 25±1.87 mm, p=0.04), and an increase, when compared to SG (23.4±2.2mm *vs.*

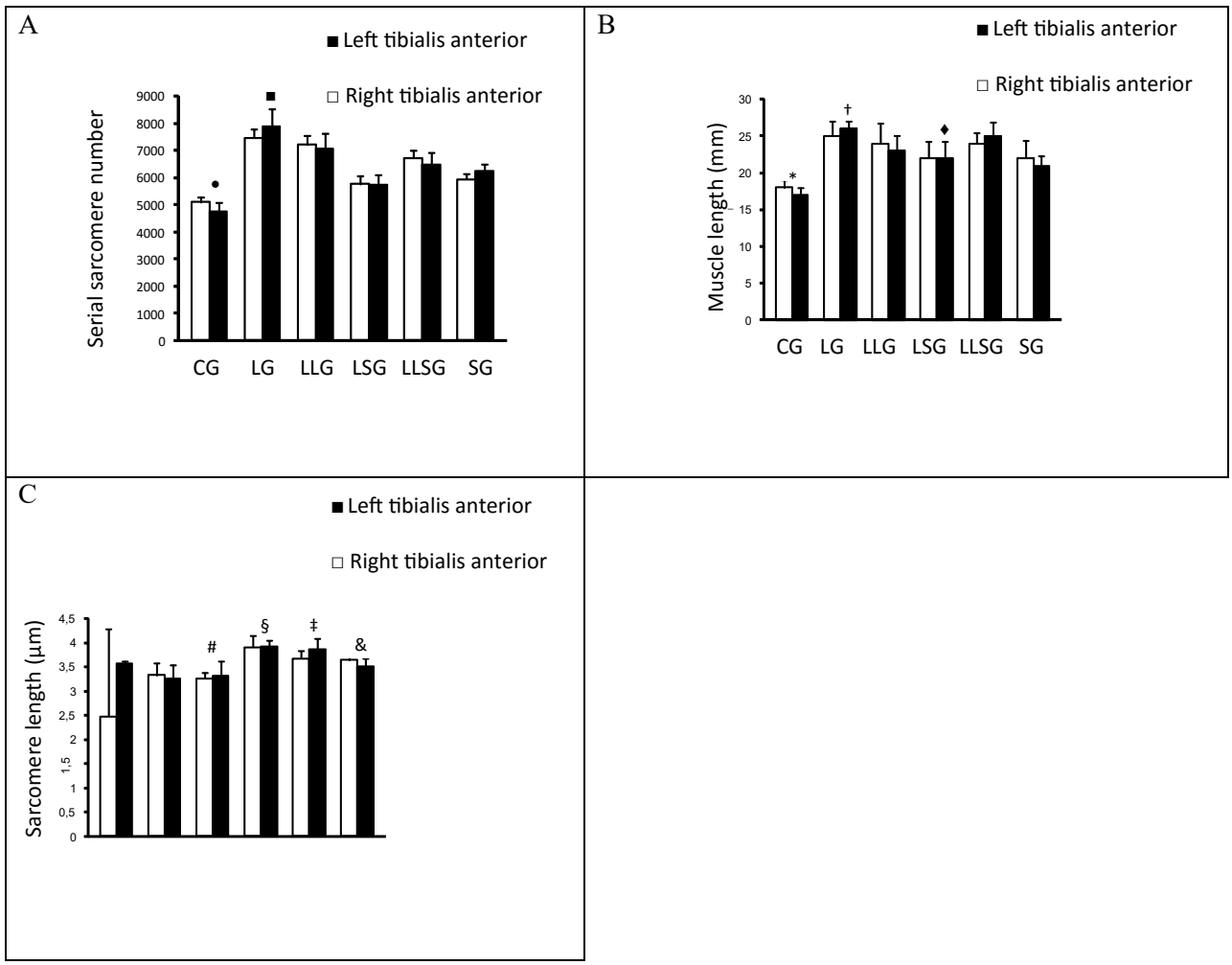
21±1.28mm, p=0.01). The results of the final length of the TA muscle are shown in Figure 2B.

Serial sarcomere number and sarcomere length

The average number of sarcomeres in series of all groups showed normal and homogeneous distribution. We noted an increase in the serial sarcomere number of the lesion group (LG), when compared to LSG (7191±510 *vs*. 5713±371, p=0.002) and SG (7191±510 *vs*. 6242±260, p=0.004). There was also an increase in LLG when compared to LSG (7078±530 *vs*. 5713±371, p=0.005).

In LLSG, we verified decrease in the number of sarcomeres in series in relation to the LG (6483±439 vs. 7191±510, p=0.004). We also observed less sarcomere number in the control group (4749±330) compared to LG, LSG, LLSG, LLG and SG (4749±330 *vs*. 7191±510, p=0.00003; 4749±330 *vs*. 5713±371, p=0.02; 4749±330 *vs*. 6483±439, p=0.001; 4749±330 *vs*. 7080±530, p=0.00004; 4749±330 *vs*. 6242±260, p=0.001, respectively), as shown in Figure 2A.

Sarcomere length of LLG decreased compared to LSG (3.31±0.30µm *vs*. 3.93±0.12µm, p=0.001) and LLSG (3.31±0.30µm *vs*. 3.87±0.22µm, p=0.03). We noticed a reduction in sarcomere length of SG when compared to LSG (3.52±0.15µm *vs*. 3.93±0.12µm, p=0.03), and also LLSG (3.52±0.15µm *vs*. 3.87±0.22µm, p=0.02). We found an increase in LSG compared to CG (3.93±0.12µm *vs*. 3.57±0.05µm, p=0.04) and LG (3.93±0.12µm *vs*. 3.63±0.37µm, p=0.04). The LLSG sarcomere length was larger than CG (3.87±0.22µm *vs*. 3.57±0.05µm, p=0.0006). These results are shown in Figure 2C.



A: Serial sarcomere number, ▪when compared to LSG (p= 0.002); SG (p=0.004) and LLSG (p=0.004); •p=0.005 compared to LSG (p=0.02); to LG (p= 0.00003); to LLG (p= 0.0004); to LLSG (p=0.001); to LSG (p=0.02) and to SG (p=0.001). **B:** Muscle Length, †when compared to SG (p=0.003) and LSG (p=0.008); ♦ when compared to LLSG (p=0.04) and SG (p=0.01); *p=0.00002 (LG); p=0.00006 (LLG); p= 0.001(LSG); p= 0.00003 (LLSG) and p=0.0003 (SG) when compared to CG. **C:** Sarcomere Length, §p=0.04 (CG and LG) when compared to LSG, ‡p=0.0006 (LLSG) compared to CG; #p=0.001 (LSG) and p=0.03 (LLSG) when compared to LLG; &p=0.03 (LSG) and p=0.02 (LLSG) when compared to SG. The results are mean±standard deviation. Control Group (CG); Lesion Group (LG); Lesion and Laser group (LLG); Lesion and Stretching Group (LSG); Lesion, Laser and Stretching Group (LLSG); Stretching Group (SG). mm: millimeter;µm: micrometer

Figure 2. Effects of laser and stretching on longitudinal muscular morphology

DISCUSSION

To minimize disability and enhance full functional recovery after skeletal muscle injuries, the current conservative treatment includes limiting the bleeding with compression, elevation, and local cooling, nonsteroidal anti-inflammatory drugs, and physical therapy, which include the use of laser therapy and muscle stretching[30]. The findings of this study showed that laser therapy and/or stretching protocols were enough to induce sarcomerogenesis even in injured muscles. Stretching protocol applied in isolation was sufficient to increase serial sarcomere number in intact TA.

In this study, the injury, laser therapy, and stretching protocols did not affect body weight gain. We observed a gain in final body weight in the lesion group compared to the control group. This outcome indicated that the muscle contusion induced with the apparatus did not prevent normal body weight gain of the animals, also observed by other authors[23]. However, Amaral et al.[31] found no statistical difference in the body weight of animals subjected to muscle injury and myotoxin treated by laser. Coutinho et al.[27] reported increased body weight in animals subjected to stretching in rat soleus muscle.

The group of animals in which the muscles underwent lesion, laser, and stretching showed muscle weight higher than those found in the control group. The combination of injury and eccentric stress of stretching may have contributed to weight muscle gain[32]. However, muscle fiber area is a better measurement than absolute muscle weight for the evaluation of skeletal muscle trophism and we found no difference in the muscle fiber across the section area. Other authors also observed no difference in the muscle weight of animals subjected to injury and treated with laser or just stretched[31,27]. Therefore, it can be suggested that the combination of the laser and stretching interferes differently in muscle mass, when compared to the isolated action of these resources, which require more investigation.

The lesion group presented no alteration in the CSAMF and connective tissue. The rats were euthanized 4 weeks after contusion. Thus, probably these muscles were already restored, accordingly to the duration of the skeletal muscle repair, i.e., approximately 21 days[5]. Also, in the group subjected only to stretching we did not observe any difference in the CSAMF, but we found a decrease in the percentage of connective tissue when compared to the group injured and treated with laser and stretching.

The data of this study showed that in a 3 times a week frequency, the stretching protocol applied isolated in the intact muscle was sufficient to prevent connective tissue proliferation compared to muscles injured and submitted to laser and stretching, corroborated with Coutinho et al.[33] Endorsing the effect of the stretching protocol applied in the intact muscles (SG), we found an increase in the serial sarcomere number compared to control group (CG). Other authors observed an addition in the serial sarcomere number of intact soleus muscle, applied 3 times a week, however, it was maintained for 40 min[27]. Nevertheless, in this study, the manual stretching over TA was held in a total of 10 min per session, showing that, even in a short duration, sarcomerogenesis could be observed.

Morrone et al.[34] reported that muscles injured and treated with laser Ga-Al-As showed an improvement in the regeneration process by accelerating cellular metabolism. This data could explain in part the increase in the muscle length confirmed with the sarcomerogenesis observed in injured muscles submitted to laser and/or stretching. Thus, this outcome demonstrated a new effect of laser, probably stimulating longitudinal muscle growth.

However, it was a surprise to detect an increase in the muscle length and serial sarcomere number of muscles subjected only to LG compared to CG, LSG, LLSG and SG. It has been suggested that the muscle length and serial sarcomere number (SSN) increase according to the animal growth, i.e, if the functional demand and the body weight increase it might raise the muscle length and serial sarcomere number due to the overload[35-38]. Thus, these aspects may possibly have caused the augment in the muscle length and SSN of LG because of the body weight gain more pronounced in this group[35].

It has been reported that changes in the SSN induce modifications in sarcomere length for an optimal overlap of actin and myosin, i.e., while increase the SSN decrease the sarcomere length[(27,39)]. This mechanism was confirmed in LLG and SG that showed an increase in the SSN and a decrease in the sarcomere length. On the other hand, we verified an increase in the sarcomere length in LSG and LLSG compared to CG, instead of the SSN increase in these groups. Therefore, the mechanisms of sarcomere adaptation in intact and injured muscles, also when stretching is applied in association with laser should be different.

This study presents some limitations as absence of ultra-structural analysis to detect sarcomere modifications;

qualitative and quantitative analysis of collagen type; functional assessments; molecular biology techniques to investigate the pathways involved in the injured skeletal muscle response to stretching and laser therapies.

The outcomes of this study confirmed the hypothesis that laser and stretching enhance skeletal muscle repair showing more serial sarcomere number in the lesion, laser, and stretching group (LLSG) compared to the lesion and stretching group (LSG) and the percentage of connective tissue of SG less than LLSG. Thus, the sarcomerogenesis of injured muscles was enhanced by laser therapy, stretching and by the association of both.

However, the present data are not sufficient to indicate laser application to the muscle lesions without restriction, since the striated muscle optimal repair requires not only morphologic aspects but also a functional assessment, which was not investigated.

The results of this study indicated an improvement in the muscle regeneration process in response to laser and/or stretching therapies. Thus, laser or stretching applied isolated or in combination could enhance range of motion and regeneration of injured muscle. Nevertheless, only stretching applied isolated prevented the deposition of connective tissue and improved the longitudinal growth in intact muscles. Despite laser and/or stretching therapies having improved the regeneration skeletal muscle process, randomized clinical trials investigating these therapies in humans should be performed to test their efficacy in a high level of evidence.

CONCLUSION

Laser infrared GaAs pulsed for seven consecutive days and/or manual passive stretching, 3 times a week, for three weeks improved sarcomerogenesis of muscles submitted to contusion. Ten bouts of 1 minute muscle stretching with 45 s rest, once a day, three times a week for three consecutive weeks was sufficient to increase the serial sarcomere number of intact TA.

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