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# THE EFFECT OF INTERMITTENT CRYOTHERAPY AND COMPRESSION ON MUSCLE INJURIES IN RATS: A MORPHOMETRIC ANALYSIS

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## ABSTRACT

Introduction: Although cryotherapy associated with compression has been recommended as an immediate treatment for muscle injuries, the effect of intermittent sessions of these procedures in the area of secondary muscle injuries has not been clearly established. Objective: To evaluate the effect of intermittently applying cryotherapy and compression (three 30-minute sessions at 90-minute intervals) on an injured area of the right tibialis anterior (RTA) muscle in rats. Method: An injury was induced in the RTA muscle by means of cryoinjury. Twenty-four Wistar rats (340 ± 20g) were divided into four experimental groups: a) Injury + Cryotherapy (I+C), which received intermittent cryotherapy and compression; b) Injury + Placebo (I+P), which received placebo treatment; c) Injury (I), which did not undergo any treatment protocols; and d) Cryotherapy, which remained intact and underwent cryotherapy and compression treatment. The animals were sacrificed 24 hours after the injury, and the muscles were sectioned in a cryostat. The histological sections were stained with toluidine blue for subsequent measurement of the area of the muscle injury (morphometry). The statistical analysis consisted of the ANOVA and Tukey tests ($p \leq 0.05$). Results: The morphometric analysis 24 hours after the injury indicated that there had been a significant reduction in the area of the muscle injury in the I+C group (35.87 ± 4.9%), in comparison with the I+P group (46.4 ± 3.9%; $p = 0.001$) and the I group (46.5 ± 4.1%; $p = 0.002$). Conclusion: Three sessions of cryotherapy and compression were efficient in preventing an increase in the injured area, while compression alone did not achieve such effectiveness.

*Key words*: cryotherapy; compression; muscle injury; morphometry; tibialis anterior muscle.

## RESUMO

### O Efeito da Crioterapia e Compressão Intermitente no Músculo Lesado de Ratos: Uma Análise Morfométrica

Introdução: Embora a crioterapia associada à compressão seja recomendada como tratamento imediato após lesão muscular, o efeito de sessões intermitentes desses procedimentos na área de lesão muscular secundária não é bem estabelecido. O objetivo deste estudo foi avaliar o efeito da aplicação intermitente de crioterapia e compressão (três sessões de 30 min a cada 1h30min) na área de lesão do músculo *tibialis* anterior direito (TAD) do rato. Metodologia: A lesão muscular foi induzida por criolesão no TAD. Vinte e quatro ratos Wistar (340 ± 20g) foram divididos em quatro grupos experimentais: a) O grupo Lesão + Crioterapia (L+C) recebeu tratamentos intermitentes de crioterapia e compressão; b) O grupo Lesão + Placebo (L+P) recebeu tratamento placebo; c) O grupo Lesão (L) não foi submetido a nenhum protocolo de tratamento; e d) o grupo Crioterapia (C) que permaneceu intacto e foi submetido a tratamentos de crioterapia e compressão. Os animais foram sacrificados 24h pós-lesão, sendo os músculos seccionados em criostato e os cortes histológicos corados com Azul de Toluidina para posterior mensuração da área muscular lesada (morfometria). A análise estatística constou da ANOVA e do teste Tukey ($p \leq 0,05$). Resultados: A morfometria aplicada 24 horas pós-lesão indicou redução significante da área de lesão muscular no grupo L+C (35,87 ± 4,9%) quando comparado aos grupos L+P (46,4 ± 3,9%; $p = 0,001$) e L (46,5 ± 4,1%; $p = 0,002$). Conclusão: Três sessões de crioterapia e compressão foram eficientes na prevenção do aumento da área de lesão, enquanto somente a compressão não apresentou a mesma efetividade.

*Palavras-chave:* crioterapia; compressão; lesão muscular; morfometria; *tibialis* anterior.

## INTRODUCTION

Cryotherapy is one of the most affordable and widely recommended therapies for the treatment of musculoskeletal injuries. The main objective of cryotherapy is to minimize sequelae related to the injury process (pain, edema, hemorrhage, muscle spasm) and, especially, to reduce secondary injury[1-3].

According to Knight[1], physiological responses to the primary injury can lead to a secondary injury through enzymatic and hypoxic mechanisms that affect the cells surrounding the initial injury. The secondary injury caused by post-traumatic hypoxia is a consequence of several factors, such as hemorrhaging from injured vessels, hemostasis, decreased blood flow due to increased blood viscosity and increased extra-vascular pressure. Additionally, edema caused by injury to the cell membrane can occlude small vessels, increasing the ischemic area[4]. The secondary injury caused by enzymatic mechanisms is due to the liberation of lysosomes of dead or dying cells[5]. Thus, in the first hours after the primary injury there will be an increase in the total injury area, which is a consequence of the secondary injury.

The physiopathology of soft tissue injury is characterized by increased cell metabolism, hemorrhage, hyperemia, edema and recruitment of leukocytes[6]. These characteristics justify the use of localized cooling for the immediate treatment of injuries to the soft tissues, including muscle contusion, joint sprains and dislocations[7].

Several procedures are described for the application of cryotherapy (gels, sprays, ice packs, immersion, etc). However, in clinics and hospitals and in sports medicine, ice packs are the most frequently used resource[8].

Some studies suggest that, during the acute inflammatory response in musculoskeletal injuries, perturbation in the capillaries and congestion caused by an edema can decrease oxygenation of healthy cells around the injured tissue, i.e. hypoxia that leads to the death of cells. However, cryotherapy reduces the metabolic rate of hypoxic tissues, allowing a better survival rate during this period, and thus decreasing the area of secondary injury[3,9-11].

A previous study demonstrated that a treatment consisting of cryotherapy for 5 consecutive hours after a crushing injury effectively decreased the injured area in the triceps surae of rats[10]. In another study, treatment consisted of 6 consecutive hours after trauma and it effectively reduced microcirculation deficits, localized inflammation and muscle necrosis[12]. However, the continuous application of cryotherapy is not common in humans due to the risk of skin burns[13]. Intermittent application of cryotherapy, e.g. applying icepacks for 30 minutes with 1 to 2-hour intervals, has been recommended after muscle injuries in humans[2] despite the scarcity of studies related to intermittent cryotherapy in animals which morphometrically analyze the muscle injury area[3].

In this context, there are no *in vivo* studies on the effect of intermittent cryotherapy applied during the first hours after injury, showing the immediate effects of preventing the development of long-term secondary injuries.

The objective of this study was to determine the effects of 3 sessions of intermittent cryotherapy and muscle compression applied immediately after muscle injury to the area of secondary injury in rat muscles.

## METHODS

### Experiment animals

Twenty-four Wistar rats ((340 ± 20g) were divided among four plastic cages. Six animals were kept in each cage. All animals had free access to water and standard pellet food and were kept under controlled lighting conditions (12:12-h light-dark cycle) at room temperature. This study was conducted according to the International Guide for the Care and Use of Laboratory Animals (National Research Council)[14]. The project (004/03) was approved by the Ethics Committee of Universidade Federal de São Carlos (UFSCar).

First, the animals were weighed (initial body mass) and anesthetized with an intraperitoneal injection of xylazine chlorhydrate (12mg/kg) and ketamine chlorhydrate (95mg/kg) during induction of muscle injury and application of treatment protocols. After that, they were sacrificed with an overdose of anesthetics and weighed again (final body mass). Muscles were then removed and weighed (muscle mass).

### Muscle injury protocol

The skin that covers the right tibialis anterior (RTA) muscle was shaved and cleaned. A transversal incision (1 cm) was then performed in the muscle belly area. The overlaying fascia had to be removed in order to adequately expose the muscle.

Tissue injury was induced by freezing (cryoinjury) the central area of the RTA muscle belly. This procedure is commonly used to induce muscle injury[15]. A 6 mm wide and 30 mm long iron stick previously immersed in liquid nitrogen was transversely pressed against the muscle belly for 10 seconds. After the stick was cooled again, the procedure was repeated and the skin was then sutured (3-0 Nylon thread – Shalon LTDA) and cleaned with iodized alcohol.

This procedure of muscle injury has been previously tested in our laboratory and was chosen because it can

produce a similar primary muscle injury area on the surface of the muscle belly of rats[3].

**Cryotherapy protocol**

The animals were placed in cages in the horizontal position so that the right limb could be fastened with adhesive tape to a wooden platform to allow the application of cryotherapy. The limb was carefully taped to maintain it in a horizontal position, with the metatarsal heads fastened to the wooden platform.

The cryotherapy technique consisted of the application of a plastic bag containing crushed ice (weighing 33g), attached with adhesive tape directly to the skin of the anterior limb of the animal so that it covered the entire surface of the TA.

Each application of cryotherapy lasted for 30 minutes. The first session always occurred immediately after induction of muscle injury. The animals were submitted to three sessions of cryotherapy at 90-minute intervals. In every session, the animals were anesthetized. It should be noted that the ice pack and/or the placebo was placed over the entire limb of the animal and attached with adhesive tape, generating compression of the limb.

The placebo protocol consisted of the application of a plastic bag filled with sand, weighing the same as the ice pack and positioned in the same way as described for the cryotherapy. In this case, only the effect of the compression on the muscle was analyzed.

**Experimental groups**

The RTA muscle belly was injured in three groups of animals (n= 18). Each group was submitted to one of the following procedures: a) 3 sessions of cryotherapy and compression, as previously described; b) 3 sessions of compression and c) no treatment. One of the experimental groups was not injured (n= 6) but underwent three sessions of cryotherapy and compression. This group was included to assess possible injuries related to cooling. It is important to point out that experimental procedures always took place immediately after muscle injury.

At the end of the cryotherapy and compression sessions, the animals were placed in plastic cages and sacrificed 24 hours after injury. The RTA and left *tibialis* anterior (LTA) muscles of all animals were carefully dissected to avoid mechanical injuries and then removed. After that, they were each carefully weighed (Denver Instruments Company, Model 100[A], USA) and frozen in isopentane, previously frozen in liquid nitrogen and placed in a freezer at –80 C (Forma Científica, USA).

**Muscle injury area**

Serial histological cross sections (10 μm) were obtained from the TA muscles in a cryostat (Microm hm 505E). Two histological cross sectional areas were obtained at every 100 μm of the entire length of the muscle belly.

The cross sectional areas were stained with toluidine blue for subsequent characterization of the general morphology of the fragment and identification of injured areas. Signs of injury were identified by observation of the histological cross sectional areas under a light microscope (Axiolab, Carl Zeiss, Germany). The injured area was characterized by hyper-contracted muscle fibers, large spaces between fibers, tissue infiltration of mononuclear cells and edema, as previously described[16-18].

A cross sectional area was obtained from the RTA muscle belly of each animal in the different experimental groups and stained with toluidine blue. A light microscope and morphometry software (Axiovision 3.0.6 SP4, Carl Zeiss, Germany) were used. In each cross sectional areas, photographs of the fields were taken in order to reconstruct the entire muscle area. This procedure allowed identification and quantification of injured and uninjured muscle areas of the different experimental groups. A double-blind procedure was utilized to select the injured muscles to be assessed.

**Statistical analysis**

Statistical analysis included the paired Student-t test (to compare results for RTA and LTA muscle mass of the same animals). The ANOVA and Tukey tests were used to compare the muscles between groups. Significance level was set at 5%.

## RESULTS

**Muscle mass**

Table 1 compares the injured RTA muscles to the intact contralateral LTA muscles 24 hours after induction of injury. The groups that underwent injury and cryotherapy (I+C) and injury and placebo (I+P) and the group that only underwent injury (I) had a significant increase in RTA muscle mass (0.7298 ± 0.05g vs 0.6384 ± 0.05g, p= 0.0003; 0.6916 ± 0.06g vs 0.6050 ± 0.07g, p= 0.002; 0.7451 ± 0.11g vs 0.6512 ± 0.08g, p= 0.001, respectively, paired Student-t test). In the control group (C), that did not suffer muscle injury but underwent the cryotherapy protocol, there was no difference in mass between the RTA and the contralateral LTA muscle (0.6497 ± 0.05 vs 0.6574 ± 0.05, p> 0.05).

**Cross-sectional area of the AT muscle**

In Table 2, the results demonstrate a significant reduction in the injured area of the I+C group compared to the I+P group (35.87 ± 4.98% vs 46.47 ± 3.93%; p= 0.001). The I+C group had a reduction in injured area

**Table 1.** Mean body mass and mass of the right and left tibialis anterior muscles of the animals 24 hours after the injury.

| | Initial Body Mass (g) | Final RTA Mass (g) | RTA Mass (g) | LTA Mass (g) |
|---|---|---|---|---|
| **I + C** | 335 ± 16.11 | 323 ± 14.25 | 0.7298 ± 0.05 | 0.6384 ± 0.05 |
| **I + P** | 338 ± 52.19 | 328 ± 42.64 | 0.6916 ± 0.06 | 0.6050 ± 0.07 |
| **I** | 339 ± 33.52 | 338 ± 34.17 | 0.7451 ± 0.11 | 0.6512 ± 0.08 |
| **C** | 348 ± 36.04 | 341 ± 36.05 | 0.6497 ± 0.05 | 0.6574 ± 0.05 |

* p= 0.0003; p= 0.002; p= 0.001 (paired Student's t test) when contralateral. Results are mean ± standard deviation (X ± SD).

**Table 2.** Total cross sectional area of the right tibialis anterior muscle, total injured area and non-injured area assessed at the muscle belly.

| | Injured Area | | Non-injured Area | |
|---|---|---|---|---|
| **I + C** | 20.55 ± 3.48 | 35.87 ± 4.98 | 36.54 ± 1.68 | 64.13 ± 4.98 |
| **I + P** | 24.04 ± 3.66 | 46.47 ± 3.93 | 27.69 ± 2.15 | 53.53 ± 3.93 |
| **I** | 26.70 ± 4.93 | 46.57 ± 4.17 | 30.47 ± 3.75 | 53.43 ± 4.17 |
| **C** | - | - | 40.20 ± 4.13 | 100 ± 0 |

* p= 0.001 and p= 0.002 (ANOVA-Tukey test) when compared to percentage of injured area with groups L+P and L, respectively; ( - ) absence of injury. Results are mean ± standard deviation (X ± SD).

when compared to the I group (35.87 ± 4.98% vs 46.57 ± 4.17%; p= 0.002). The approximate percentages of injured and normal areas were 35% and 65% for the I+C group, 46% and 54% for the I+P group, and 46% and 54% for the I group.

Intermittent cryotherapy alone did not to cause injury to the *tibialis* anterior muscle in the C group.

## DISCUSSION

The results of this study demonstrated that, although they did not effectively prevent the increase in muscle mass, the three intermittent sessions of cryotherapy associated with muscle compression immediately after primary muscle injury (cryoinjury) effectively reduced the secondary injury area. However, the compression sessions alone did not prevent a significant increase in the secondary injury area as was the case of the injured group that did not receive treatment.

According to a previous study developed in our laboratory[3], under the same experimental conditions, with the I+C, I+P and I groups sacrificed 4 hours and 30 minutes after muscle injury, the I+C group demonstrated a lower percentage of injury area of the RTA muscle (29.8 ± 6.6%) compared to the I+P (39.2 ± 2.8; p= 0.003) and I groups (41.7 ± 4.0; p= 0.0009). According to results, therefore, cryotherapy was more effective in preventing or reducing the injured area than compression alone, which did not have any effect on the injured area and maintained values similar to those of the I group.

It should be emphasized that the injured area of the 24h groups increased approximately 6% compared to the 4h30min groups. This result demonstrates that three sessions of cryotherapy immediately following cryoinjury were insufficient to completely prevent the increase of the injured area. Nevertheless, regarding the 24h groups, the I+C group demonstrated a smaller percentage of injured area (35.8 ± 4.9) than the I+P (46.4 ± 3.9; p= 0.001) and I (46.5 ± 4.1; p= 0.002) groups. Compression in the placebo group could not limit the extent of the injured area in relation to the I group.

Although the I+C group demonstrated greater effectiveness in reduction in or prevention of secondary injury, it is not possible to determine whether such prevention is related to the cells not initially injured by cryoinjury or whether the effectiveness is explained by delayed death of primarily injured but not destructed

cells.

Some studies that investigated the effects of cryotherapy on muscle injuries demonstrated that the decrease in tissue temperature leads to decreased metabolism and decreased cell demand for oxygen, thus avoiding tissue ischemia during reduction of capillary perfusion and consequently minimizing secondary injury[1,11,12].

The results of the present study suggest that the use of ice packs on injured muscles induced a physiological response that delayed muscle injury, therefore corroborating the use of cryotherapy for acute muscle injuries. The intermittent application of three sessions of cryotherapy associated with compression (30 minutes) during the first hours after injury (4h30min) differs from most clinical protocols for humans[1], which usually involve intermittent application of cryotherapy during the first 72 hours.

Additionally, although some studies have reported that compression limits development of edema because of mechanical reduction of localized blood flow[19], treatment with compression alone, implemented in this study under the same experimental conditions, did not produce the same effects as cryotherapy.

There was a decrease in the body mass of the animals during the 24-hour experiment, which may be related to different factors, such as decreased ingestion of food, anesthesia, immobilization or pain.

The different experimental groups assessed in the present study, with the exception of the I group, were submitted to cryotherapy and compression. According to Merrick et al.[10], the association of cryotherapy and compression delays secondary injury, with cryotherapy acting at tissue level in the treatment of musculoskeletal injuries. Such evidence corroborates results for the 4h30min groups, in which the I+C and I+P groups had similar RTA and LTA muscle mass values[3]. However, in the 24h groups, there was a significant increase in mass in all previously injured muscles (I+C, I+P and I), indicating that the injured area and/or inflammation increased with time, regardless of the kind of treatment (cryotherapy or compression). Therefore, it can be suggested that the benefits observed for the 4h30min groups are reduced a few hours later. This demonstrates that three sessions of cryotherapy are insufficient to maintain long-term benefits of hypothermia.

The significant difference between the RTA and LTA muscle mass of the injured groups suggests the presence of an inflammatory process in the RTA. This injury mechanism probably resulted in an increase in muscle mass, in accordance with previous reports by Jarvinen[20] and Crisco et al.[21].

The literature is not consistent in regard to muscle mass after injury and mechanisms related to this process. Crisco et al.[21] and Salvini et al.[18] reported that the increase in mass, immediately after injury in rat muscles, probably occurred due to the initial phases of the inflammatory process observed in the histological analysis of the muscle. Previously, Jarvinen[20] demonstrated that the increase in mass in this same muscle occurred only during the first two days after injury. After this period, there was a reduction in mass. Fisher et al.[4], on the contrary, investigated the effects of a single trauma and did not find any increase in mass in the gastrocnemius muscle 48h after injury, which suggests that post-traumatic protein depletion could have masked a possible increase in muscle mass caused by edema and hemorrhage. Despite the differences in injury mechanisms, subsequent adaptations and analyzed muscles, the same explanations could apply to the results of the present study.

In the 24h groups, there was an increase in RTA muscle mass compared to TLA for the I+C, I+P and I groups. This result indicates that the three sessions of cryotherapy immediately after injury were insufficient to block the inflammatory process during subsequent hours.

The vasoconstriction induced by cryotherapy is considered the main mechanism of reduction of edema and hemorrhage after injury[22,23]. Several researchers claim that cooling can decrease blood flow and thus reduce the amount of hemorrhage inside the injured tissue. Other studies, however, suggest that the beneficial effects of cryotherapy on acute injuries are more related to decreased metabolism than to circulatory alterations[24].

The main objective of cryotherapy in the acute phase is to prevent the development of edema and secondary hypoxia, as primary injury damage caused by trauma cannot be altered[25].

A recent study using magnetic resonance imaging demonstrated that cooling immediately after exercise minimized perfusion increase and prevented edema development in skeletal muscle[26]. Eston and Peters[27] also reported that immersion in cold water decreases the rate of muscle injury after extenuating eccentric exercise in humans.

Recent studies on microcirculatory dynamics after contusion and immediate application of cryotherapy suggest that this therapy does not alter arteriolar diameter but increases vein diameter. This would explain the observed increase in edema reabsorption as well as the leukocyte/endothelial reduction[28,29].

Previous studies examined the effects of localized tissue cooling to induce rat muscle contusion on leukocyte activity and on micro-vascular hemodynamics using real-time microscopy. Results suggested that localized tissue cooling, similarly to cryotherapy, decreases inflammatory response and edema without inhibiting blood flow during contusion[30]. There are also reports that cryotherapy reduces microvascular permeability

by means of a reduction in the number of leukocytes. This association suggests that the reduction of edema in injured muscles treated with cryotherapy may happen as a result of the reduction in the leukocyte-endothelium interaction[23].

The present study provides new information about the effects of a few sessions of cryotherapy and compression immediately after muscle injury. This information is useful for rehabilitation and sports activities. Complimentary studies will be necessary to assess injured muscles at different time periods after the treatment protocols implemented in the present study.

In conclusion, three intermittent sessions of cryotherapy (for 30 minutes at 90 minute intervals), applied immediately after induced muscle injury and assessed 24h later, were effective in reducing the secondary injury area. Intermittent muscle compression alone was not as effective in preventing the increase in the secondary injury area.

## REFERENCES


1. Knight KL. Crioterapia no tratamento das lesões esportivas. São Paulo: Manole; 2000.
2. Jarvinen TA, Jarvinen TLN, Kaariainen M, Kalimo H, Jarvinen M. Muscle injuries: biology and treatment. The Am J Sports Med. 2005;33(5):745-64.
3. Oliveira NML, Rainero EP, Salvini TF. Three intermittent sessions of cryotherapy reduce the secondary muscle injury in skeletal muscle of rat. Journal Sports Science & Medicine 2006;5:228-34.
4. Fisher BD, Baracos VE, Shnitka TK, Mendryk SW, Reid DC. Ultrastructural events following acute muscle trauma. Med Sci Sports Exerc. 1990;22(2):185-93.
5. Farges MC, Balcerzak D, Fischer BD, Attaix D, Béchet D, Ferrara M, et al. Increased muscle proteolysis after local trauma mainly reflects macrophage associated lysosomal proteolysis. Am J Physiol Endocrinol Metab. 2002;282(2):E326-31.
6. Olson JE, Stravino VD. A review of cryotherapy. Phys Ther. 1972;53:840-53.
7. Shelbourne KD, Wilckens JH. Current concepts in anterior cruciate ligament rehabilitation. Orthop Ver. 1990;19(11): 957-64.
8. Enwemeka CS, Allen C, Avila P, Bina J, Konrade J, Munnz S. Soft tissue thermodynamics before, during, and after cold pack therapy. Med Sci Sports Exerc. 2002;34(1):45-50.
9. Knight KL, Bryan KS, Halvorsen JM. Circulatory changes in the forearm in 1, 5, 10 and 15° C water. Int J Sports Med. 1981;4:281.
10. Merrick MA, Rankin JM, Andress FA, Hinman CL. A preliminary examination of cryotherapy and secondary injury in skeletal muscle. Med Sci Sports Exerc. 1999;31:1516-21.
11. Merrick MA. Secondary injury after musculoskeletal trauma: a review and update. J Athl Train. 2002;37(2):209-17.
12. Schaser KD, Disch AC, Stover JF, Lauffer A, Bail HJ, Mittlmeier T. Prolonged superficial local cryotherapy attenuates microcirculatory impairment, regional inflammation, and muscle necrosis after closed soft tissue injury in rats. Am J Sports Med. 2007;35(1):93-102.
13. Ilic S, Cernak I, Skaro-Milic A, Spasic P, Savic J, Milosavljevic I. Experimental study of the pathogenesis of frostbite. Part III. Significance of energy status os muscle tissue in the pathogenesis of frostbite. Vojnosanit Pregl. 1999;56(4):358-68.
14. National Research Council. Guide for the care and use of laboratory animals. Washington (DC): National Academy Press; 1996.
15. Miyabara EH, Martin JL, Giffin TM, Moriscot AS, Mestril R. Overexpression of inducible 70-kDa heat shock protein in mouse attenuates skeletal muscle damage induced by cryolesioning. Am J Physiol Cell Physiol. 2006;290(4): C1128-38.
16. Morini CC, Pereira EC, Selistre de Araujo HS, Ownby CL, Salvini TF. Injury and recovery of fast and slow skeletal muscle fibers affected by ACL myotoxin isolated from Agkistrodon contortrix laticinctus (Broad-Banded Copperhead) venom. Toxicon. 1998;36(7):1007-24.
17. Minamoto VB, Grazziano CR, Salvini TF. Effect of single and periodic contusion on the rat soleus muscle at different stages of regeneration. Anat Rec. 1999;254(2):281-7.
18. Salvini TF, Belluzzo SS, Selistre de Araujo HS, Souza DHS. Regeneration and change of muscle fiber types after injury induced by a hemorragic fraction isolated from Agkistrodon contortrix laticinctus venom. Toxicon. 2001;39:641-9.
19. Hedges JR, Anwar RAH. Management of ankle sprains. Ann Emerg Med. 1980;9:298-302.
20. Jarvinen M. Healing of a crush injury in rat striated muscle. Acta Chir Scand. 1976;142:47-56.
21. Crisco JJ, Jokl P, Heinen GT, Connell MD, Panjabi MM. A muscle contusion injury model - Biomechanics, physiology and histology. Am J Sports Med. 1994;22(5):702-10.
22. Curl WW, Smith BP, Marr A, Rosencrance E, Holden M, Smith TL. The effects of contusion and cryotherapy on skeletal muscle microcirculation. J Sports Med Phys Fitness. 1997;37(4):179-86.
23. Deal DN, Tipton J, Rosencrance E, Curl WW, Smith TL. Ice reduce edema. A study of microvascular permeability in rats. J Bone Joint Surg. 2002;84:1573-8.
24. Knight KL. Cryotherapy: theory, technique and physiology. Indiana: Chattonooga; 1985.
25. Iserhard AL, Weissheimer KV. Crioterapia: por que sua aplicação na fase aguda. Fisioter Mov. 1993;6(1):92-9.
26. Yanagisawa O, Kudo H, Takahashi N, Yoshioka H. The use of magnetic resonance imaging to evaluate the effects of cooling on skeletal muscle after strenous exercise. Eur J Appl Physiol. 2004;89(1):53-62.
27. Eston R, Peters D. Effects of cold water immersion on the symptoms of exercise induced muscle damage. J Sports Sci. 1999;17:231-8.

28. Menth-Chiari WA, Curl WW, Paterson-Smith B, Smith TL. Microcirculation of striated muscle in closed soft tissue injury: effect on tissue perfusion, inflamatory cellular response and mechanisms of cryotherapy. A study in rat by means of laser doppler flow measurementsand intravital microscopy. Unfallchirurg. 1999;102(9):691-9.

29. Schaser KD, Stover JF, Melcher I, Lauffer A, Haas NP, Hermann J. Local cooling restores microcirculatory hemodynamics after closed soft-tissue trauma in rats. J Trauma. 2006;61(3):642-9.

30. Lee H, Natsui H, Akimoto T, Yanagi K, Ohshima N, Kono I. Effects of cryotherapy after contusion using real time intravital microscopy. Med Sci Sports Exerc. 2005;37(7):1093-8.