

Morphological changes in distant muscle fibers following thermal injury in Wistar rats¹

Alterações morfológicas das fibras musculares de um músculo distante da lesão térmica em ratos Wistar

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ABSTRACT

Purpose: Thermal injury causes catabolic processes as the body attempts to repair the damaged area. This study evaluated the effects of a scald injury on the morphology of muscle fibers belonging to a muscle distant from the lesion. **Methods:** Thirty Wistar rats were divided into control (C) and scalded (S) groups. Group S was scalded over 45% of the body surface, standardized by body weight. Rats in both groups were euthanized at four, seven and 14 days following the injury. The middle portions of the medial gastrocnemius muscles were sectioned, stained with hematoxylin and eosin and Picrosirius, and submitted to histological analysis. **Results:** Control group sections exhibited equidistantly distributed polygonal muscle fibers with peripheral nuclei, characteristic of normal muscle. The injured group sections did not consistently show these characteristics; many fibers in these sections exhibited a rounded contour, variable stain intensities, and greater interfiber distances. A substantially increased amount of connective tissue was also observed on the injured group sections. **Conclusion:** This experimental model found a morphological change in muscle distant from the site of thermal injury covering 45% of the body surface.

Key words: Morphology. Muscle Fibers, Skeletal. Connective Tissue. Burns. Rats.

RESUMO

Objetivo: A lesão térmica conduz o organismo a processos catabólicos a fim de reparar a área lesada. O propósito do presente estudo foi avaliar os efeitos da lesão térmica por escaldadura na morfologia das fibras musculares de um músculo distante da lesão. **Métodos:** Foram utilizados 30 ratos Wistar, divididos em: Grupo submetido à lesão térmica por escaldadura (S) e Grupo Controle (C). A escaldadura foi provocada em 45% da superfície corporal dos animais e padronizada pelo peso corpóreo. A eutanásia ocorreu nos períodos pós-lesão de quatro, sete e 14 dias. Retirou-se a parte média dos músculos gastrocnêmio medial os quais foram submetidos à técnica histológica de Hematoxilina-Eosina e Picrosírius. **Resultados:** O aspecto poligonal das fibras musculares, com núcleos periféricos, bem como a distribuição equidistante das mesmas, característico das descrições da musculatura normal, verificado nos animais do grupo C não foi uma constante nas fibras do músculo dos animais do grupo S. Nestes, muitas fibras exibiram contorno arredondado, diferentes intensidades de marcação bem como maior distanciamento entre as mesmas. Observou-se ainda aumento substancial da quantidade de tecido conjuntivo no grupo S. **Conclusão:** Houve alteração da morfologia do músculo distante da lesão como resposta à lesão térmica de 45% em modelo experimental.

Descritores: Morfologia. Fibras Musculares Esqueléticas. Tecido Conjuntivo. Queimaduras. Ratos.

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Introduction

In Brazil, 70% of burns occur in the home; 50% of the victims are children, and most of them are of low socioeconomic status¹. Severe thermal injuries are typically followed by a catabolic state²⁻⁴. The catabolic response in skeletal muscle is the principle mechanism of protein loss in patients with thermal

injuries, resulting in muscle weakness⁵⁻⁷ that delays recovery and contributes to disease. Muscle loss additionally exposes these patients to neuromuscular complications that increase hospital stay and mortality.

Some authors have reported that the systemic consequences of changes due to thermal injury, such as tissue destruction can be attributed to the increased metabolic rate

that accompanies the body's attempts to repair the damaged regions^{8,9}.

Pereira *et al.*³ found that patients with lesions covering more than 40% of the body exhibited increased metabolism that persisted in most body tissues for at least one year after the trauma. The percentage of post-traumatic weight gain following injury indicated that thermal injury effectively promoted tissue loss due to systemic catabolism.

This study used hematoxylin and eosin (H&E) and Picrosirius staining to clarify the morphological changes in muscle fibers and connective tissue belonging to the gastrocnemius muscle (GM), located away from the site of thermal injury.

Methods

Animals and scald injury

Thirty 21 day-old male Wistar rats (*Rattus norvegicus*) were individually housed in wire-bottomed metabolic cages for five days in a temperature-controlled room (21-24°C) with a regular light-dark cycle. Water and food were available *ad libitum*. The use of metabolic cages facilitated the daily recording of daily body weights and cleaning of the cages to prevent contact with feces.

On the sixth day, 15 animals were anesthetized with an intraperitoneal (IP) injection of 40 mg/kg sodium pentobarbital (Hypnol 3%, Fontoveter, Cristalia, Brazil), and the hair on the dorsal and ventral surfaces of the body was removed. A nonlethal scald injury was administered to 15 animals constituting the scalded group – (S) by immersing 45% of each rat's body –in 87°C water, as described by Walker and Mason¹⁰. Each animal had 30% of its dorsal and 15% of its ventral area exposed to scald injury for 10 and 3 seconds, respectively¹¹. An additional 15 rats were untreated and comprised the control group (C). The rats in both groups were subcutaneously injected with the analgesic buprenorphine (0.2 mg/kg) immediately after sham or burn injury, and again 24h later.

The animals were returned to their cages. The nutritional status of each rat was monitored by recording daily body weight and food intake. Four, seven and 14 days following the scald injury, five animals from each group were euthanized with a lethal IP injection of 100 mg/kg sodium pentobarbital. All procedures were approved by the Committee of Ethics in Animal Experimentation (CEEA – ICB/USP), protocol number 014/03.

Histological analysis

The GMs of euthanized rats from Groups S and C were examined. The medial part of each GM was dissected and transversely sectioned into proximal, middle and distal parts. The middle part was mounted in a cork block with Tissue Tek® embedding medium, frozen by immersion in liquid nitrogen-cooled isopentane, and stored at -80°C. Serial transverse sections (10 µm thick) were cut at -25°C using a cryostat (CM 1850, Leica, Germany), and placed on coverlips.

The sections were stained with H&E to allow a complete histological analysis that identified the morphological characteristics of the muscle fibers. Histological analysis of the

collagen fibers was performed on sections stained with Picrosirius¹².

Picrosirius photomicrographs were made under normal and polarized light to differentiate type I (red and yellow) and III (green) collagen. Photomicrographs made at different magnifications for the morphological analysis were evaluated by light microscopy with an attached camera (Axioscope 40, AxioCam HRc, Zeiss, Germany).

Results

The group C sections showed equidistantly distributed polygonal muscle fibers with intensely stained peripheral nuclei (Figure 1A). These characteristics correspond to descriptions of normal muscle, and were not consistently present in the Group S muscle fibers (Figure 1 B-C and E-F). Many fibers in Group S sections exhibited rounded contours, variable stain intensities, weakly stained nuclei, and greater interfiber distances.

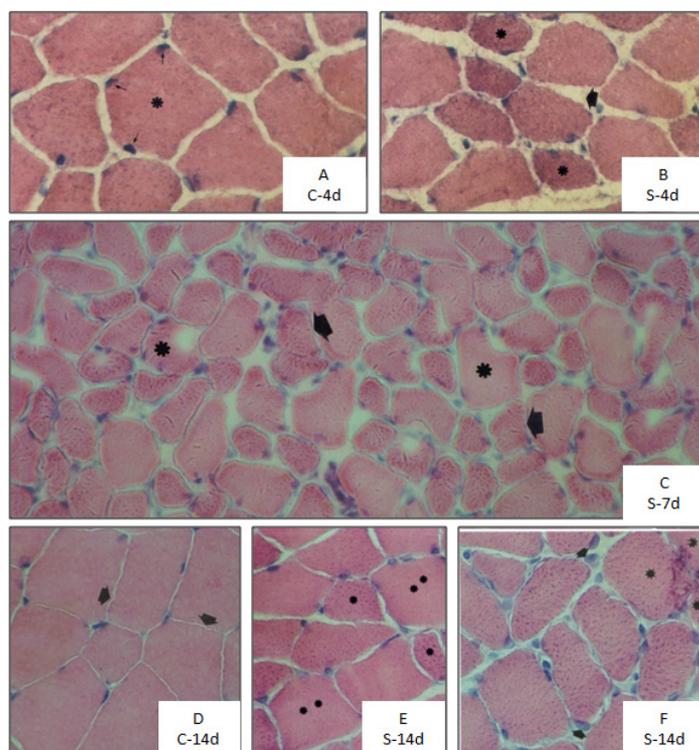


FIGURE 1 - Sections of rat gastrocnemius muscle stained with hematoxylin and eosin; panels show control group sections obtained at 4(A) and 14 (D) days, and scalded group sections obtained at 4(B), 7(C) and 14 (E and F) days after thermal injury. **A.** Normal fibers with polygonal aspects (*), peripheral nuclei (arrows) and slightly irregular contours. **B.** Muscle fibers with irregular granules of acidophilus aspect (*) and increased interfiber space (arrow). **C.** Disorganized muscle fibers of various shapes and sizes with irregular contours (*) and acidophilic granules (arrows). **D.** Muscle fibers of normal appearance, exhibiting some irregular contours (arrows) in certain areas of the muscle. **E.** Fibers containing acidophilic granules and splitting fibers (**) interspersed with fibers of normal appearance (**). **F.** Muscle fibers with acidophilic granules, markedly irregular outlines, a large number of macrophage-like round cells (arrows) in the increased intercellular space. Note that some cells are undergoing degeneration (*). (Magnification: A-B and D-F, 715x; C, 275x.)

The Group S sections also showed a substantial increase in the amount of connective tissue in the perimysia. This morphological alteration was more pronounced in sections obtained from rats euthanized 14 days after injury (Figure 2 A-B).

Analysis of the connective tissue under polarized light revealed the predominance of type I collagen. Collagen types did not differ between groups (Figure 2 C-D).

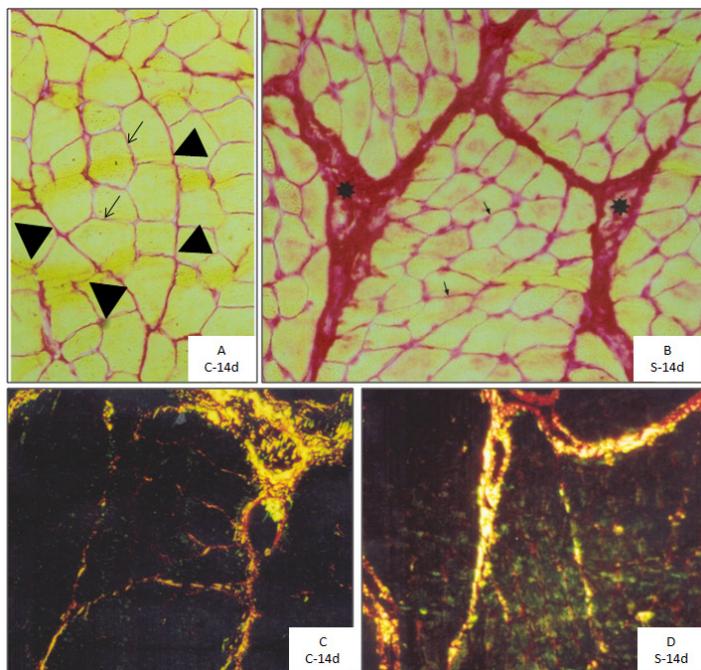


FIGURE 2 - Sections of rat gastrocnemius muscle stained with Picosirius under normal (A-B) and polarized (C-D) light; panels show control (A and C) and scalded (B and D) group sections obtained 14 days after thermal injury. **A.** Stained perimysium (arrow heads) and endomysium (small arrows). **B.** Substantially increased amount of connective tissue (*) and preserved endomysium (arrows). **C and D.** Type I collagen characterized by red and yellow muscle wraps in both groups. (Magnification A-D, 275x.)

Discussion

Given the consequences of thermal injury to distant organs, the treatment of burn patients should not solely address the acute injury. It should also be concerned with the delayed rehabilitation of the patient due to the alterations of other tissues, such as skeletal muscle. Protein catabolism persists for months after thermal injury⁶ and can cause up to 2 years of growth retardation in injured children¹³.

This study found that morphological changes in muscle altered fiber form, but did not observe central nuclei that would indicate regeneration. However, muscle cell fusion was observed on the Group S micrographs taken 14 days after injury. This characteristic indicates the beginning of muscle regeneration process. A longer follow-up period would allow better monitoring of the morphological changes that accompany the regeneration of damaged muscle fibers after thermal injury.

A large number of nuclei were present in the intercellular space within 14 days after injury. Apoptosis causes the elimination of the nuclear core through the cell membrane; a smaller number of cores increases the cytoplasmic volume per nucleus, and the reduction in volume to restore balance in the cellular cytoplasm decreases the size of the cell (i.e., atrophy)¹⁴. Although the present study did not measure cell volume, the thickening of tissue in the intercellular spaces indicated that it decreased.

A recent study¹⁵ examined the morphology of the mandibular condyle of rats and the associations of thermal injury with pre and post-natal malnutrition. The researchers found changes in condylar type collagen after 45% of the animals's body surfaces were scalded, despite the distance of the mandible from the site of injury. The present study did not obtain similar results for skeletal muscle type I collagen fibers were predominant in both groups. The only change observed in the Group S connective tissue was the filling of intercellular spaces caused by the apparent reduction in muscle fiber size, which was most pronounced 14 days after injury.

Another recent study⁷ analyzed the effects of a 12-week program of aerobic training combined with endurance exercise that was initiated seven weeks after thermal injury for children with lesions covering more than 40% of the body surface. The program was found to benefit these patients, as measured by clinical assessment of muscle loss. Future studies should use experimental models to investigate whether the morphological changes of cells distant from thermal damage are affected by exercise.

Conclusion

The present study found significant changes in rat muscle fibers after thermal injury by scalding in rats.

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