Hyperbaric oxygen therapy treatment for the recovery of muscle injury induced in rats

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Abstract

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Introduction: We evaluated the effect of hyperbaric oxygen treatment (HBOT) in the recovery of muscle injury in rats.

Materials and methods: Twelve female Wistar rats, weighing 200–250 g, were submitted to contusion of the right gastrocnemius muscle. Animals were then randomly assigned to an untreated control group or an HBOT-treated group. The HBOT group was given three, 60-minute sessions of HBOT at 253 kPa pressure at 24, 48 and 72 hours post injury. After the last session all animals were sacrificed and both gastrocnemius muscles removed, the left muscle as an internal control. Blood samples were taken for creatine phosphokinase (CPK). Using a standard traction technique, the muscles were analysed for their biomechanical properties: hardness, maximum elongation and maximum weight.

Results: Significant differences were found between uninjured and injured muscles and between untreated and HBOT groups in maximum weight and hardness: maximum weight in the non-treated group 18.27± 2.99 N versus 26.18 ± 2.84 N in the HBOT group ($P = 0.007$); hardness in the non-treated group 2.24 ± 0.38 10^3 N m^{-1} versus 3.19 ± 0.32 10^3 N m^{-1} in the HBOT group ($P = 0.001$). The difference in maximum elongation was not significant ($P = 0.793$). CPK was significantly different between the two groups (non-treated 6,445 ± 387 i.u. L^{-1}; HBOT 4,551 ± 80 i.u. L^{-1}; $P = 0.009$).

Conclusions: HBOT seems to play a positive role in the recovery of induced muscle injury in rats. However relevant, these results cannot be extrapolated to humans, for whom further clinical studies are warranted.

Key words

Hyperbaric oxygen, hyperbaric oxygen therapy, musculo-skeletal, injuries, research

Introduction

Muscle injury presents a challenging problem in traumatology and is very common in sports medicine.[1] Such injury may be a consequence of direct mechanical deformation (e.g., contusions, lacerations and strain) or of indirect causes (e.g., ischaemia and neurological damage).[2] More than 90% of muscle injuries are caused either by excessive strain or by contusion of the muscle.[1] A muscle suffers a contusion when it is submitted to a sudden, heavy compressive force, such as a direct blow.[3] This can be classified as mild, a tear in a few muscle fibres with few symptoms that do not interfere with mobility; moderate, involving greater damage to the muscle affecting its function, or severe, a tear that extends across the entire cross-section of the muscle, preventing normal function.[3]

There is an emerging need for improved therapies that allow the injured athlete to return to competition faster and with a low risk of re-injury. The role of HBOT in the recovery of muscle injuries has been debated for several years, but remains poorly understood.[4] The mode of action of HBOT is complex, the result of a number of physiological and pharmacological mechanisms based on elevation of both the partial pressure of oxygen and of the hydrostatic pressure.[5] Considering the lack of consistent research on HBOT in soft-tissue injury, we aimed to evaluate the effect of HBOT in the recovery of muscle contusion inflicted to rats by measuring the biomechanical properties and haematological markers of muscle injury**.**

Materials and methods

ANIMALS

Twelve female rats, *Rattus norvegicus* albinos, Wistar-type, weighing 200–250 g, were studied. The animals were kept in the facilities of the Laboratory of the Faculty of Pharmacy, University of Porto, in collective cages with two animals per cage, at room temperature, receiving water and standard food ad libitum. All procedures were performed according to the FELASA recommendations for animal welfare and according to Portuguese legislation.

PROCEDURE FOR INDUCTION OF THE INJURY

Prior to the induction of the injury, the animals were anesthetized using 60 mg kg^{-1} ketamine and 8 mg kg^{-1} xylazine intraperitoneally. They were then positioned at the base of the lesion production equipment, in the ventral decubitus position, with their knee at maximal extension and ankle in neutral position (90^{O}). In order to cause the lesion, a 171 g weight was released from a height of 102 cm onto the belly of the right gastrocnemius muscle in accordance with previous studies.[6]

Animals were then randomly assigned to two groups using an online programme, (www.randomizer.org). The control group received no treatment and the treatment group received three, 60-minute sessions of HBOT at 253 kPa pressure at 24, 48 and 72 hours after the injury. After the third session, all the

animals were sacrificed and the injured right gastrocnemius and the uninjured left gastrocnemius, which was used as an internal control, were surgically removed.

Blood samples were taken for analysis of creatine phosphokinase (CPK), a marker of muscle injury.[7] Serum CPK was analyzed using a commercial enzymatic kit (Siemens, Flex Reagent Cartridge).

MECHANICAL TRACTION ASSAY

The removed muscle was biomechanically analysed using a standard traction machine (EMIC, DL 10000) to measure maximum elongation (ME), maximum weight (MW) and hardness (H). For the experiment, the machine was loaded with a charge of 50 kg force, and a pre-charge of 200 g was applied during 30 seconds for system accommodation. Afterwards, the assays were performed at a speed of 10 mm min^{-1}. ME corresponds to the maximum length of the muscle string before rupture; MW represents the maximum of imposed load before muscle rupture and H is a property obtained by the software (Tesc) that is determined by the slope of the line obtained in the elastic phase of the process.

STATISTICAL ANALYSIS

Statistical analysis was performed through the BioEstat® program v. 2.0. Normality was checked with the Kolmogorov -Smirnov test. ANOVA analysis combined with a Bonferroni post-hoc test was used to evaluate differences between the groups. A pre-established significance level of $P = 0.05$ was used.

Results

For hardness, the internal control muscles had 3.92 ± 0.41 10^3 N m^{-1}, the non-treated group 2.24 ± 0.38 10^3 N m^{-1} and the HBOT group 3.19 ± 0.32 10^3 N m^{-1} ($P = 0.001$, Figure 1).

For maximum elongation, the internal control muscles had 13.40 ± 1.61 10^{-3} m, the non-treated group 10.91 ± 2.20 10^{-3} m and the HBOT group 11.70 ± 2.32 10^{-3} m ($P = 0.793$, Figure 2).

For maximum weight, the results were 32.23 ± 3.12 N for the internal control muscles, 18.27 ± 2.99 N for the non-treated group and 26.18 ± 2.84 N for the HBOT group (Figure 3, $P = 0.007$, Figure 3).

For CPK, a significant difference ($P = 0.009$) was found between the non-treated (6,445 ± 387 IU L^{-1}) and the HBOT groups (4,551 ± 80 IU L^{-1}).

Discussion

Not surprisingly, the non-injured muscle always had better results than the injured muscle, independently of whether or

Figure 1
Mechanical properties of muscle for hardness (10^3 N m^{-1}) in a non-injured control group and injured, non-treated and HBOT-treated groups; * $P = 0.001$ HBOT vs. non-treated

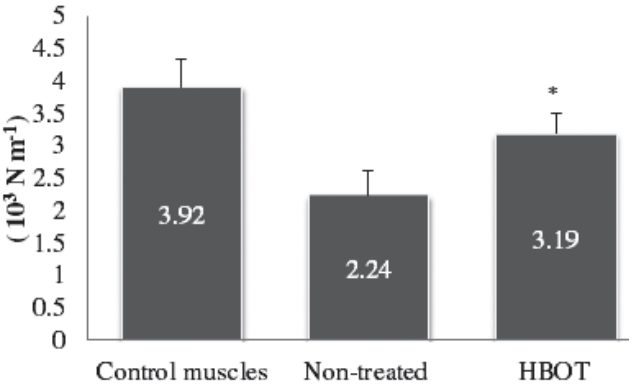


Figure 2
Mechanical properties of muscle for maximum elongation (10^{-3} m) in a non-injured control group and injured, non-treated and HBOT-treated groups; $P = 0.793$ HBOT vs. non-treated

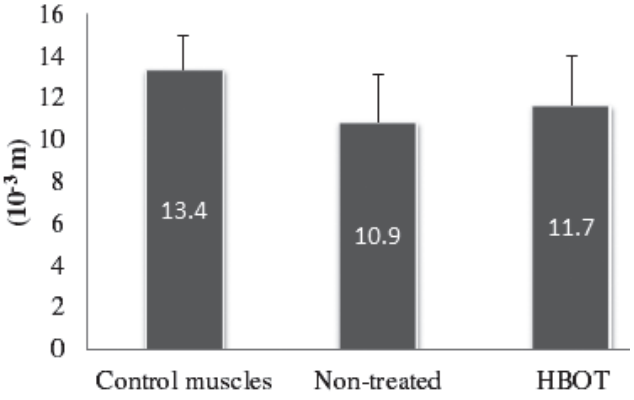
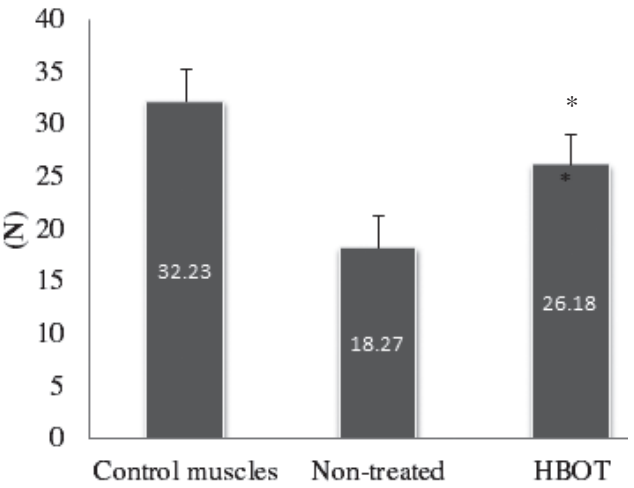


Figure 3
Mechanical properties of muscle for maximum weight (N) in in a non-injured control group and injured, non-treated and HBOT-treated groups; * $P = 0.007$ HBOT vs. non-treated



not the animal received HBOT. This indicates that the injury protocol used was effective. The significant differences in hardness and maximum weight between the non-treated and HBOT groups, combined with the greater elevation of CPK in the untreated compared to the HBOT group indicates that HBOT had a positive effect on muscle injury recovery.

There is a lack of studies on the effect of HBOT on biomechanical properties of muscle injuries, particularly with regard to contusion. Therefore, we need to look at other types of muscle injury and the parameters measured in other studies. In a study of acute stretch injury of the tibialis anterior muscle in 18 rabbits, the animals were randomly assigned to two groups.[8] One group received HBOT at 253 kPa for 60 minutes daily for five days starting 24 hours post injury, while the other had no treatment. Seven days after the injury, the deficit of ankle isometric torque in the HBOT group was less than that of the non-treated group and recovery was more complete, suggesting that HBOT may play a role in accelerating recovery after acute muscle stretch injury. Similarly in Sprague-Dawley rats, after 4 hours of ischaemia, the changes in levels of intracellular muscle compounds adenosine triphosphate, phosphocreatine and lactate were less in HBOT-treated rats than in untreated animals.[9]

In humans, a randomized double-blind trial studied 32 subjects with acute ankle sprains.[10] Treatment consisted of three HBOT sessions at 203 kPa. HBOT did not influence ankle oedema, subjective pain indices, passive motion indices or the time to recovery. In a randomized, placebo-controlled trial in 66 patients with muscle soreness of the quadriceps, a control group, and sham, immediate and delayed HBOT groups were studied.[11] Delayed treatment and delayed sham were done at three or five days following injury using 203 kPa . Immediate HBOT patients had a better recovery than those treated at three days, while a delay of five days to HBOT provided the best results. In all groups, pain was similar.

If HBOT is applied within eight hours after tissue injury, it has been reported that accelerated recovery from soft-tissue injury results.[12] This was attributed to the known actions of HBOT, such as reduction of local hypoxia and inflammation, promotion of vasoconstriction, reduction of neutrophil adhesion, extinction of free radicals, control of oedema, increased leukocyte activity and promotion of procedures for the synthesis of collagen and blood vessel growth.[5] In a Cochrane review examining the effect of HBOT on delayed-onset muscle soreness in untrained individuals, no conclusive evidence of benefit was found for HBOT on the speed of recovery in muscular pain.[6,13–17] This review stated that more and larger randomized trials were needed to determine the role of HBOT in the treatment of sport injuries. High inspired normobaric oxygen has been reported to have positive effects on lymphatic vessel metabolism and oedema reduction.[18] This has an interesting, potential clinical application to enhanced protein removal from the site of injury by the lymphatic system.

In the present study, we used rats not only as an established animal injury model but also because, according to some authors, they exhibit musculoskeletal structure similar to humans.[19] However, these results cannot be transferred to humans, but should give guidance to further research.

Conclusions

Using three biomechanical properties of injured muscle and CPK as a systemic marker of muscle injury, HBOT seems to play a positive role in the recovery of induced muscle contusion injury in rats. However, there is much we still need to understand about the use of HBOT in sports injury treatments. Therefore, more studies are warranted.

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