Quantitative MRI analysis of two icing techniques in skeletal muscle injury in a rat hind limb model

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Introduction:

Contusion injuries to muscle are common in the athletic population. They result from high energy impacts that rapidly compress local tissues. Following skeletal muscle injury, the tissue undergoes a sequential process of degeneration and regeneration [1]. Cryotherapy, the therapeutic use of cold, is widely used in humans with acute musculoskeletal trauma because it has the ability to not only alleviate some of the acute symptoms of the injury but also because it appears to reduce the magnitude of the injury itself [2,3]. In this study injury was induced in a rat hind limb model under controlled experimental conditions by a standard weighted free falling mass. Two different cooling regimes, continuous and intermittent, of the injured muscle were compared using volumetric and relaxation time MRI methods during the first six hours after injury.

Subjects & Methods:

Male Wistar rats (273 ± 30 g) were anesthetized throughout the experimental procedures via inhaled anesthetic (isoflurane/oxygen). The hind limbs were shaved and the mid-belly position of the gastrocnemius muscle was determined via palpations, and marked. Animals were then placed prone in the injury device with hind limb extended to ensure direct impact over the gastrocnemius muscle. A free falling weight (267g) was dropped from 60 cm to induce the contusion injury [4]. Force output, displacement, and energy were also recorded. Following injury, crushed ice packs were applied to the injured limb of the animal and secured with cloth tape. During the continuous treatment, ice packs were administered immediately following injury and replaced as needed. During the intermittent treatments ice packs were applied for 30 min and then removed for 60 min and then reapplied for 30 min until the 6 hr timeframe was completed. Each group consisted of nine rats. Animal body temperature was monitored throughout the entire treatment via a rectal temperature probe (SA Instruments inc., Stony Brook, NY, USA). All MRI experiments were conducted on a Varian 7 tesla horizontal system with Direct Drive technology using a 6 cm inner diameter birdcage resonator driven in quadrature mode. Five spin echo (SE) experiments (56 slices) with a resolution of (215 x 194 x 500) μm^3 and increasing echo times (11, 20, 30, 40, and 60 ms) were acquired to calculate T2 maps of the muscle in the hind limb 2, 4, and 6 hours after injury. An additional SE with a TE of 11 ms was acquired before the injury to serve as a control for the volume measurements. Segmentation using AMIRA (Mercury Computer Systems, Inc., Chelmsford, MA, USA) of 30 adjacent slices starting at the bottom of the knee joint for each animal were used to calculate the three-dimensional volume of the hind limb. Analysis of variance (ANOVA) was performed to determine the effects of cryotherapy on the extent of acute injury. Post hoc testing was conducted using the least significant difference (LSD) test.

Results:

Figure 1 shows representative axial slices at the same position for the injured hind limb before and 6 hours after injury, as well as the T2 maps at 6 hours. The upper row displays images from continuous cooling (see icepack on left hind limb) and the lower row shows those from intermittent cooling. The anatomical image at 6 hours shows a substantial increase in volume of the injured left hind limb compared to the reference ($p < 0.001$). Large localized increases in the T2 value are also apparent ($p < 0.001$) when compared to the non-injured limb. The effect of the different cooling methods is displayed in Figure 2. While the volume changes compared to the reference were significant, the difference in volume changes between continuous and intermittent cooling was not significant ($p = 0.517$). In terms of T2 values, there is a significant increase of T2 compared to the healthy limb ($p < 0.01$), and there was also a significant difference ($p=0.009$) between the two cooling methods. However the clinical difference between the two icing treatments as determined through effect size is relatively small (0.15).

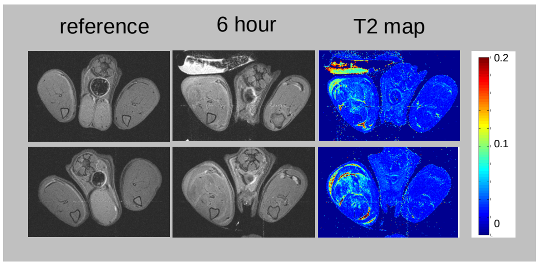

Figure 1: Examples of continuous (upper row) and intermittent icing. The images represent the reference scan, the scan at 6 hours after injury and the T2 map at 6 Hours.

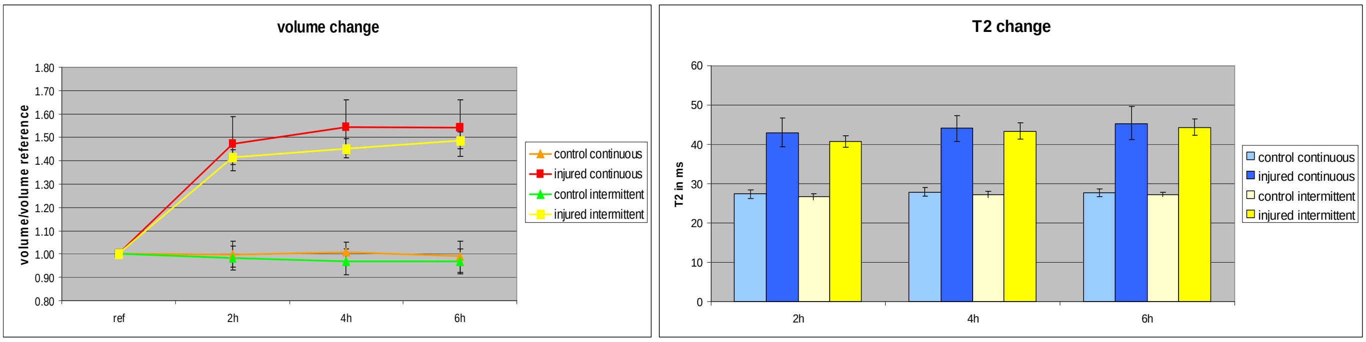

Figure 2: Diagrams of volume (left) and T2 (right) change of the injured and control limbs for both cooling methods. Error bars are the standard deviation of the nine limbs in each case. The volume is referenced to the control scan before the injury.

Discussion:

Cooling of acute contusion injuries is a widespread procedure and helps the healing process [2].This MRI based study compared two different methods of cooling, a continuous cooling approach and an intermittent cooling scheme. Statistically significnat differences were noted between cooling methods when comparing T2 values; intermittent has less of an increase compared to the control, but this difference is small as indicated by effect size. Limb volume changes were identical for both cooling methods. These data suggest that intermittent cooling for 30 minutes may be just as effective as continuous cooling.

References:

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