The Effect of Cryotherapy on Inflammation and Myofiber Regeneration following Acute Skeletal Muscle Injury: A Critically Appraised Topic

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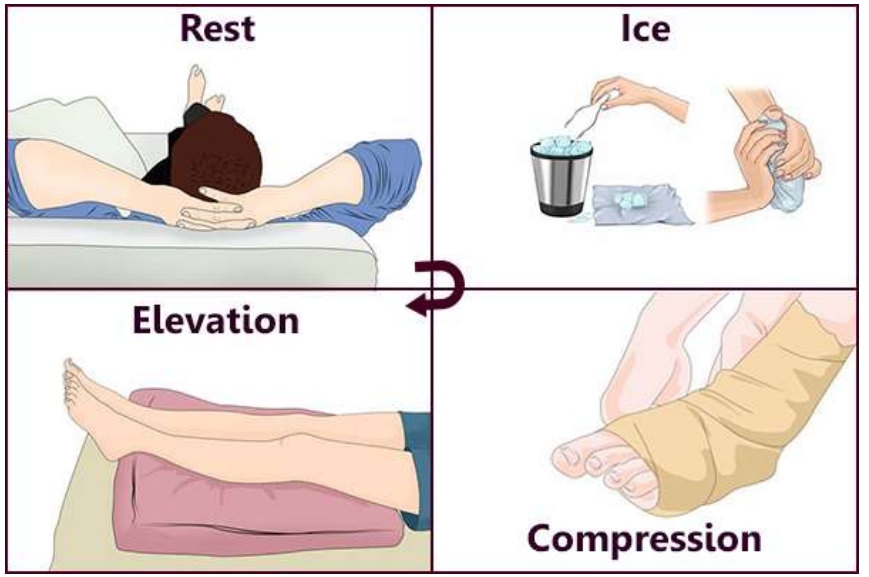
Introduction

- Goals of the presentation
  - Clinical Scenario
  - Search Strategy
  - Quality Assessment of the Evidence
  - Clinical Bottom Line
  - Implications for Practice, Education, and Future Research

Clinical Scenario

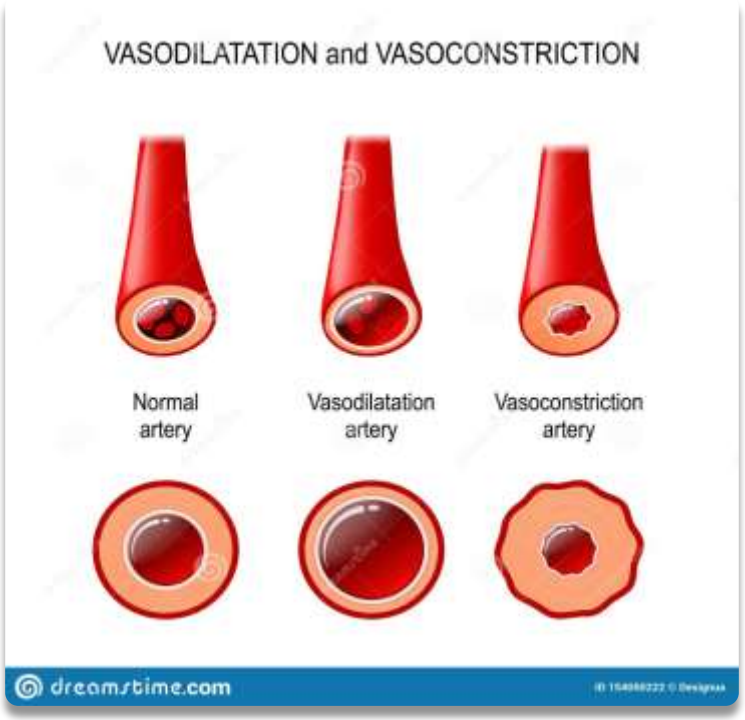
- Injuries involving damage to skeletal muscle are among the most common sports injuries, presenting at an incidence rate of between 10 - 55%
- Cryotherapy, R.I.C.E
- The effect of cryotherapy on the wound healing process

- Clinical Question: In patients with acute skeletal muscle injury, how does treatment with cryotherapy compare to no treatment effect the inflammation process and myofiber regeneration?



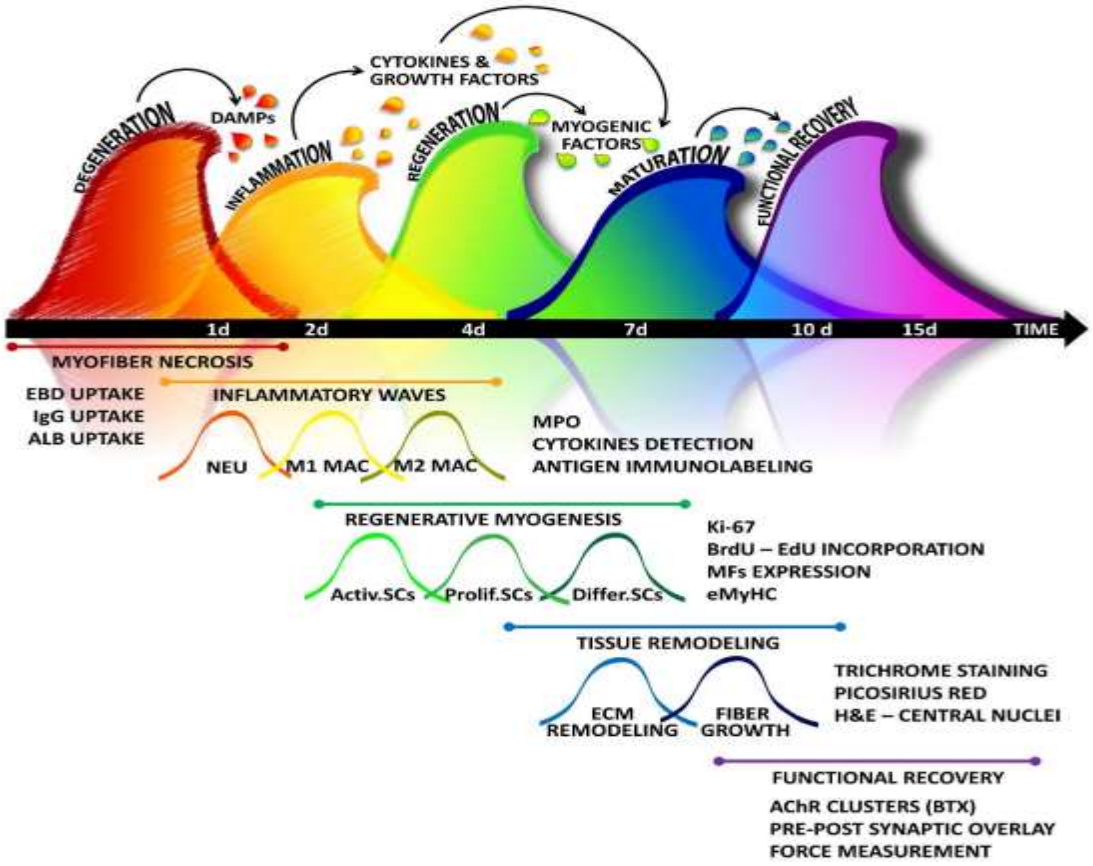
Cryotherapy

- Purpose:
  - Decrease pain
  - Decrease inflammation
  - Decrease secondary hypoxic injury
- Types
  - Ice bags/packs
  - Cold water immersion
  - Whole Body Cryotherapy



Wound healing process

- Skeletal muscles heal in 4 overlapping phases
  - Hemostasis
  - Inflammation
  - Proliferation
  - Remodeling
- Guided and regulated by:
  - Cytokines
  - Growth Factors



Search strategy

PICO

- Patient/Population
  - Patients with acute skeletal muscle injury
- Intervention-
  - Cryotherapy
- Comparison
  - No treatment (sham)
- Outcome
  - Decreased inflammatory markers, decreased growth factors, decreased myofiber regeneration

Sources/Search Terms

- MEDLINE
- Search Terms- Search included the keywords, "cryotherapy" or "Ice" or "cold therapy" and "muscle recovery" or "muscle damage" or "muscle regeneration" and "growth factor."

Inclusion/Exclusion Criteria

- **Inclusion**
- Articles that investigated direct comparison between cryotherapy and placebo for muscle recovery after muscle damage
- Articles with inflammatory markers and/or growth factors
- Evidence that is level 2 or higher
- Published after 2010
- **Exclusion**
- Articles published before 2009

Results of Search

- 4 Total Studies
  - 1 Randomized Control Trial
  - 3 Translational Rat Studies
- Key findings
  - All four of the studies showed a significant decrease in inflammatory cytokines and growth factors after the use of cryotherapy compared to a control group. Three of the four studies showed no significant difference in myofiber regeneration between the cryotherapy group and the group with no treatment. One of the four studies showed a decrease in myofiber regeneration in the cryotherapy group when compared to no treatment.

Evidence Quality Assessment

Article	Agnieska et al	Ramos et al	Singh et al	Takagi et al
Study design	Randomized controlled trial	Translational study	Translational study	Translational study
Participants	20 elite male wrestlers divided into a control and cryotherapy group (Control, n=9) (cryotherapy, n=11)	42, 3-month-old rats randomly assigned to one of 7 experimental groups (6 per group) 6 rats in the control group (no injury) L3= rats sacrificed at 3 days no ice. L3+C= rats sacrificed at 3 days with ice. L7=rats sacrificed at 7 days no ice. L7+C= rats sacrificed at 7 days with ice L14= rats sacrificed at 14 days no ice. L14+C= rats sacrificed at 14 days with ice	80 rats divided randomly into icing group or a sham group (n = 40 per group). The rats in each group were sacrificed at four separate time points: 1, 3, 7, and 28 d after injury (n = 10 per group at each time point). 10 rats used as a control group (uninjured n=10). Euthanized at 1, 3, 7, or 28 d after injury,	78, 8-wk-old male Wistar rats. Randomly divided into icing group and non-icing group. Measured at 6 hours, 12 hours, 1 day, 2 day, 3 day, 4 day, 5 day, 6 day, 7 day (n=3 in each group). At days 14 and 28 (n=6 in each group).
Intervention Investigated	Whole Body Cryotherapy (WBC)- exposed twice a day at 8 am and 6pm. Seven consecutive days for 3 minutes at -120 degrees Celsius. Control- sit at room temperature (23 degrees Celsius) for the same duration as WBC group (3 minutes)	Injury method- 30 seconds of frozen iron bar to Tibialis anterior muscle. Held for 10 seconds.Repeated 2 consecutive times with a 30 second time interval. Cryotherapy parameters- 3 sessions of 30 minutes ice application applied every 2 hours. First session immediately after injury. Second session at 24 hours. Third session at 48 h post-lesion. Cryotherapy applied with a plastic pack filled with crushed ice, taped directly over tibialis anterior	Injury method- A 400 gram weight used to perform contusion unjury on hind limb Specifically on the biceps femoris muscle. Cryotherapy parameters- 5 minutes after contusion injury, a ice block was placed over the injured area for 20 minutes. Massaged in a figure 8 direction. Sham- used the bottom of a beaker to repeat the same motions as cryotherapy group for 20 minutes	Injury method- rats were anesthetized and then the middle part of the extensor digitorum longus was crushed for 30 seconds using forceps which weighed 500 grams. Cryotherapy parameters- Ice pack placed over extensor digitorum longus for 20 minutes with minimal compression. Performed 5 minutes after injury.
Outcome measures	Exercise protocol- 14 day preparatory training for new season 50% distance work; 24% direct training (wrestling); & 26% special power training . Blood sampling taken 1 day before first WBC session and again on the 9th day Skeletal muscle damage: creatine kinase levels and myoglobin concentration Oxi-inflammatory mediators - H2O2 and NO levels, IL-B, TNFa, C-reactive protein Growth factors- HGF, IGF-1, PDGF, VEGF, BDNF Hematological and immunological variables- hemoglobin, red blood cells, hematocrit, mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and platelets and white blood cell counts leukocytes, lymphocytes, neutrophils, monocytes	Tibialis Anterior muscle is removed and measured using a histological and immunofluorescence analysis at days 3, 7 and 14. Measures conducted include muscle injury area (measurement in mm2 when animal was sacrificed) Muscle regeneration measured via MyoD and Desmin levels Extracellular matrix measured by Collagen I & Collagen III – mRNA levels and total amount of I & III by immunofluorescence. Also assessed by levels of MMP-2 & MMP-9 Inflammatory markers measured- TNF-α , macrophages (CD68), NF-κB Growth factors measured- CTGF, TGF-β & IGF-1	Biceps femoris muscle was removed and measured using histology and immunochemistry analysis at days 1, 3, 7, and 28 days. Vessel volume analyzed using microcomputed tomography (micro-CT) Inflammatory cells measured- neutrophils, macrophages Endothelial cells measured- CD 34, vWF, Nestin Growth factors measured- VEGF Myofiber regeneration measured by- Central Nucleus and myofiber cross sectional area.	Morpholical analysis, immunohistochemistry and in situ hybridization used. Measured growth factors- TGF-1, IGF-I, PAX7, ED1 (satellite cells) Inflamatory cells measured- Macrophages, ED1-positive macrophages was counted in 10 areas (1 area = .0625 mm2) at each period after the injury. Myofiber regenereration measured by- proportions of the muscle fibers containing central nucleus to total muscle fibers, and cross-sectional areas of 100 muscle fibers that have central nucleus at 14 and 28 days after the injury as indicators of the regenerating muscle fibers maturation.

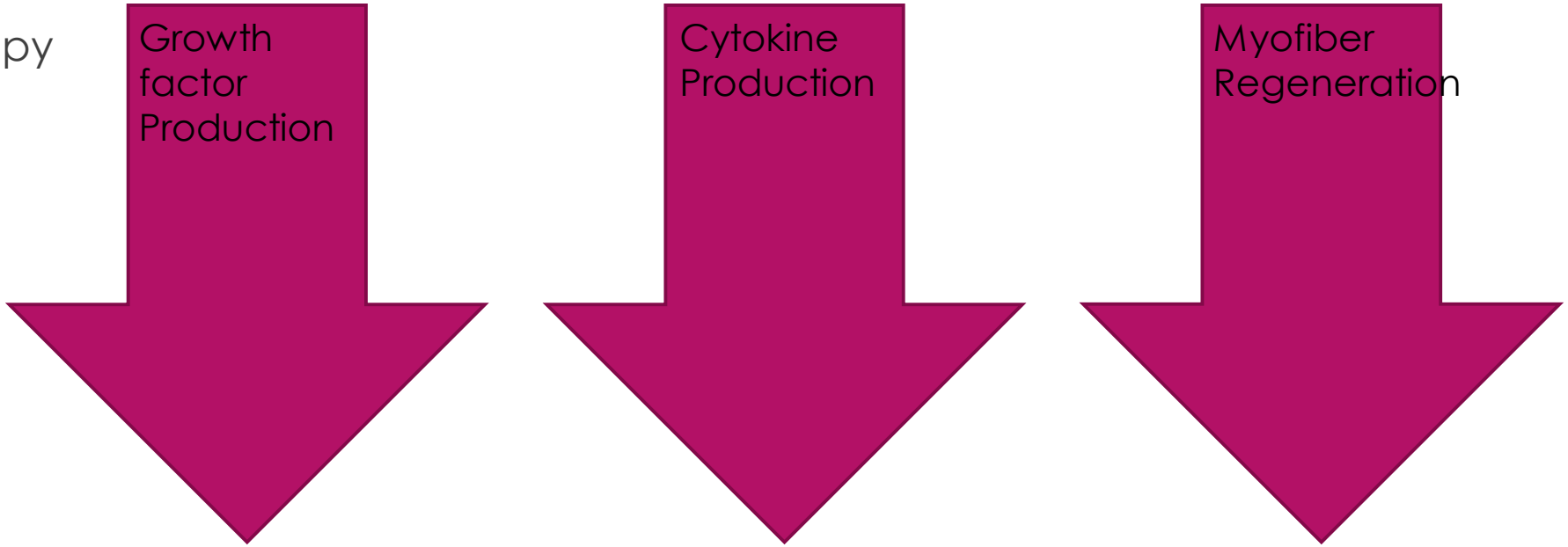
Evidence Quality Assessment

Article	Agnieska et al	Ramos et al	Singh et al	Takagi et al
Main findings	Both groups significantly increased levels of CK and Mb CK levels significantly increased in WBC group compared to control group Mb levels showed no sinificant difference betwen WBC and control group after 9 days NO and H2O2 significantly elevated in the control group compared to WBC group Significant decrease in IL-1B and hsCRP after 9 days in the WBC group compared to control TNF-A was unaffected Cryo group decreased levels of HGF, IGF-1, PDGF, VEGF in WBC group compared to control group No significant change for BDNF	Cryotherapy significantly decreased Collagen III @ days 3 & 7 compared to L3&L7 Cryotherapy significantly decreased Collagen 1 @ day 7 versus L7 group Cryotherapy treatment did not alter the percentage of collagen immunoreactivity fibers at any period Cryotherapy did not significantly alter the expression of Desmin Cryotherapy had no significant change on MyoD Cryotherapy did not limit the number of centralized nuclei at any period. No significant difference between control and cryotherapy on MMP-2 Cryotherapy significantly decreased MMP-9 on Day 3 compared to L3 CD68 significantly decreased in the cryotherapy group compared to L3 and L7 TNF-a is significantly decreased in the cryotherapy group at day 3 compared to L3 NF-kb is significantly decreased in the cryotherapy group at days 3 and 14 compared to L3 and L14 Cryotherapy group significantly reduced CTGF at day 3 compared to L3 TGF-B is significantly reduced in the cryotherapy group at day 3 compared to L3 IGF is significantly decreased at day 3 in the cryotherapy group compared to L3	Neutrophils increased significantly in muscle from the sham group compared to the icing group at 1 d after injury Neutrophils increased in muscle from the icing group compared to the sham group at 3 d after injury Macrophages increased in the muscle from the sham group compared to the icing group at 1 d and 3 days Macrophages significantly increased in muscle from the icing group compared to the sham group at 7 d Macrophage increased in icing group compared to sham group at day 28 CD34%- stained area significantly increased in muscle from the sham group compared to the icing group at 3 d Conversely, the percentage of CD34- stained area significantly increased in muscle from the icing group compared to the sham group at 28 d after injury vWF % increased in muscle from the sham group compared to the icing group at 3 and 7 days VEGF increased in the muscle from the sham group vs. the icing group at 3 d after injury The area of nestin staining increased in muscle from the sham group compared to the icing group at 3 d after injury Nestin increased in muscle from the icing group compared to the sham group at 7d Vessel volume increased in the sham group compared to the icing group at 3d and 7d No significant difference in vessel volume between the groups after 28 d At 7 d after injury, many centrally nucleated regenerating muscle fibers were present in the sham group. Few present in icing. No significant difference in percentage	TGFB1 began infiltrating faintly at 12 hours in non-icing. Invading the necrotic muscle fibers at 2 days after the injury in the non-icing group. At 2 days after the injury, the expression became the most pronounced in the non-icing group. Could be seen until day 3 in non-icing group TGFB1 began infiltrating at day 1 in icing group. Peaks at day 3 and can be seen until day 5. IGF1 started to be seen faintly in the necrotic muscle fibers at 1 day after the injury in the non-icing group. Peaks at 5 days and begins to decrease at day 7 IGF1 started to be seen at day 2 after injury in the cryotherapy group. Peaks at 5 days and begins to decrease at 7 days. Satellite cells were noted at 3 days after the injury in the non-icing group but in the icing group at the same period, they could be hardly seen At 4 days after the injury, a satellite cell which fused into the muscle fiber could be seen in the non-icing group, but in the icing group, satellite cells still remained near the muscle fibers. In the non-icing group 2 days after the injury, several macrophages were distributed within the muscle. In the icing group, macrophages were found in and among the necrotic muscle fibers but the number of macrophages was significantly less than in the non-icing group 4 days after the injury, in the non-icing group, macro- phages were observed mainly among the muscle fibers Both at 14 and 28 days after the injury, collagen deposition occurred more excessively in the icing group than in the non-icing group
Level of evidence	1b	2b	2b	2b
Validity Score (PEDRO)	6	n/a	n/a	n/a
Conclusion	Cryotherapy suppressed the cascade of injury–repair–regeneration of skeletal muscles which may cause a delayed skeletal muscle regeneration.	Cryotherapy suppressed the inflammatory processes thought to decrease macrophage infiltration as well as TNF-a, NF-B, TGF-ß and MMP-9 mRNA levels. However, cryotherapy did not change injury area, Desmin expression or Collagen I and III protein levels.Therefore, cryotherapy did not decrease myofiber regeneration.	Icing attenuated or delayed the infiltration of inflammatory cells, the eproduction of proangiogenic factors, and change in vessel volume in muscle following injury. However, there was no evidence that showed a redcution in capillary density or effective muscle regeneration.	Cryotherapy applied immediately after skeletal muscle injury may have suppressed proliferation and differentiation of satellite cells at the early stages of regeneration through a decrease of degeneration and macrophage migration, which play a crucial role in muscle regeneration. May have induced not only a delay in late stages of muscle regeneration but also impairment of muscle regeneration along with a thicker collagen deposition around the regenerating muscle fibers

Clinical Bottom Line

- Cryotherapy does not accelerate myofiber regeneration and even shows that it may be decreasing the wound healing process when compared to no treatment.

- Cryotherapy



Implications for Future Use

Different approach for the treatment of acute soft tissue injury is needed

- PEACE
  - Protection
  - Elevation
  - Avoid anti-inflammatories
  - Compression
  - Educate
- LOVE
  - Load
  - Optimism
  - Vascularization
  - Exercise

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