The Effects of Icing on Muscle Regeneration

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Disclosures

• No disclosures
• No financial interest
• No solicitation for any product

Learning Outcomes

• Describe the inflammatory process
• Outline the phases of skeletal muscle regeneration
• Describe the effect of icing on
  • Inflammation
  • Skeletal muscle regeneration
  • Collagen levels
Traditional Treatment for Muscular Injuries

• Cryotherapy (ice) to decrease:
  • Pain
  • Swelling
  • Inflammation
  • Degeneration

*Sport* Training 1st edition
“The Trainers Bible” by Bilik 1917

“If a reduced supply of blood to the part is indicated, apply cold packs”

Knight, 1976

• Controls the size of hematoma formation
• Reduces secondary hypoxic injury by reducing need for oxygen in the tissues
Merrick, 1999

- Cold applied immediately after injury
- Reduces secondary injury

↓ degeneration → ↓ healing time

Hubbard, 2004

- Cryotherapy ↓ tissue temperature
- Slowing chemical reactions
- ↓ demand for ATP

↓ ATP demand → ↓ O₂ demand → ↓ Necrosis → ↓ Healing time

Hubbard, 2004

- Cryotherapy effective in ↓ pain
- The efficacy of cryotherapy has been questioned
 Cryotherapy & Muscle Regeneration

• Cryotherapy suppresses inflammation (Meeusen, 1996, Smith, 1997).
• May lead to inhibition of muscle regeneration (Takagi, 2011).

PICO Question

• Does cryotherapy after injury decrease muscle regeneration as compared to no cryotherapy treatment?

Phases of Healing

• Inflammation and Degeneration
• Repair
• Remodeling
Inflammation

- Inflammation is characterized by movement of
  - Plasma proteins
  - Fluid
  - Leucocytes
  into the tissue in response to injuries (MacIntyre, 1995)

Inflammation and Degeneration

Influenced by

- Cytokines
- Leucocytes
- Fluid movement – Swelling & Edema
- Necrosis
- Apoptosis

Cytokines

- Greek “to put cells into motion”
- Pro-inflammatory cytokines
  - Interleukins (IL) and tumor necrosis factor-α (TNF-α)
    - “Margination” of leucocytes
    - Attract leucocytes
    - Upregulate leucocyte adhesion (Butterfield, 2006)
Tumor Necrosis Factor-α (TNFα)

- ↑ Leucocyte accumulation
- ↓ Capillary perfusion
- ↑ Vascular permeability
- ↑ Cell death

Leucocyte Recruitment

- Rolling along vessel endothelium
- Firm adhesion
- Migration into interstitial space
  (Lawrence, 1991, Lindbom, 1992)

Leucocytes
Primarily Neutrophils and Macrophages

- Attack and breakdown debris (neutrophils & macrophages)
- Removal of cellular debris (macrophages)
- Regeneration of cells (macrophages) (Randall & Eisen, 2002)
- Attracted to injured muscle cell, by various chemotactic factors, resident leucocytes, and cytokines
Neutrophils

- Programmed to overkill (McCord, 1995)
- Little ability to distinguish between foreign and host
  - Destroying healthy as well as damaged cell and debris (Pyne, 1994)

Normal Fluid Movement

Injury
Capillary Pressure on Lymph Vessels

↑ Pressure on Lymph Vessels

Capillary Swelling

↑ Swelling

Edema ↑ interstitial pressure and ↓ capillary perfusion
**Necrosis**

- ↓ Capillary perfusion
- ↑ Oxygen demand
- Failure of blood supply to meet the cells demand for oxygen, leads to cell death

**Apoptosis**

Apoptosis (Programmed Cell Death)

- Trigger by physiological stimuli, physical injury or ischemia
- Distinct changes causing condensing and fragmentation of nuclear structures (Wyllie, 1991)
- TNFα provoked a 10-fold ↑ apoptosis
- Cryotherapy abolished the TNFα induced apoptosis (Westermann, 1999)

**Muscle Regeneration**

- Inflammatory and Degeneration Phase (previously presented)
- Repair Phase
  - Regeneration of myofibers
  - Formation of connective tissue scar
- Remodeling Phases
Formation of Connective Tissue Scar

• Hematoma
• Fibrin and fibronectin form early granulation tissue
  • Extracellular matrix acts a scaffold and anchorage site for fibroblast (Hurme, 1991)

Formation of Connective Tissue Scar

• New tissue provides initial strength to withstand contraction force (Hurme, 1991, Lehto, 1985a&b, 1986)
• First proteins form fibrils that are elastic
  • Provide early strength and elasticity (Jarvinen, 2000, 2003a&b)

Formation of Connective Tissue Scar

  • Weak, thin unorganized, but provide initial tensile strength (Houglum, 2016)
  • Stronger and more durable along lines of stress, cross-link develop
  • Add significant strength to injury site (Houglum, 2016)
Formation of Connective Tissue Scar

• Granulation tissue condenses to relatively small connective tissue mass of mainly Type I collagen (Hurme, 1991; Jarvinen, 1975 & 1993, Lehto, 1985a,b,c, 1986)
• Weakest point after trauma
  • Tensile strength ↑ with production of Type I collagen (Kaariainen, 1998, Lehto, 1985a,b)
  • Mechanical stability from formation of cross-links during maturation (Lehto, 1985)

Formation of Connective Tissue Scar

• ~10 days post trauma, scar is no longer the weakest link
• If loaded to failure, rupture usually occurs at myotendinous junction
• However, a relatively long time is still needed until strength is restored to preinjury level
  (Jarvinen, 1975, 1976, Kaariainen, 1998)

Regeneration of Muscle Fibers

Satellite Cells
• Under cell membrane of each muscle fiber
• In response to injury,
  • Proliferate
  • Differentiate into myoblast
  • Join to form multi-centrally nucleated myotubes (Hurme, 1992)
Regeneration of Myofibers

Myotubes
• Fuse with injured myofibers that survived the trauma
• As they mature, form cross-striations (actin and myosin)
• Peripherally located nuclei (Hurme, 1992)

Satellite Cells and Muscle Regeneration

• Satellite cells important in muscle regeneration (Grounds, 1993, Schultz, 1994, Collins, 2005)

Macrophages and Muscle Regeneration

• Regulate satellite cell proliferation and differentiation (McMullen, 1991, Specker, 1994)
• Stimulate satellite cells division (Jones, 1995)
• Important part of regeneration in terms of satellite cell proliferation (Grazini, 1995, Moft, 1995, Lewandows, 1999)
Macrophages and Muscle Regeneration

Lescaudron, 1999
• Macrophages play a role in muscle regeneration beyond phagocytosis
• Inhibition of macrophages slowed muscle repair

Effect of Cryotherapy on Muscle Regeneration

Outcome measures
• Inflammatory response
• Muscle regeneration
• Collagen levels

Takagi, 2011

Purpose
• To determine the effect of icing on muscle regeneration after crush injury
Takagi, 2011

Wistar rats
n=78

EDL muscle crushed

Control
Icing

EDL Muscle harvested
For analysis

Macrophages

<table>
<thead>
<tr>
<th>Number of ED1+ macrophages</th>
<th>After the injury (h: hours; d: days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6h/12h 1d 2d 3d 4d 5d 6d 7d 14d 28d</td>
</tr>
<tr>
<td>Icing</td>
<td>Non-icing</td>
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</tbody>
</table>

Icing group: number of macrophages delayed by day

6 hours post injury
Cross section of muscle fibers

Control
Icing

Control - degenerating muscle fibers swollen and round in shape
Icing - group fiber contour clearly defined
12 hours post injury
Cross section of muscle fibers
Control
Icing
Control - almost no cell membranes observable
Icing - some cell membranes still clearly defined

3 days post injury
Cross section of muscle fibers
Control
Icing
Control - regenerating muscle with centrally located nuclei
Icing - no muscle regeneration signs

4 days post injury
Cross section of muscle fibers
Control
Icing
Control - regenerating muscle fibers larger than icing group
Icing - some myotubes forming
Centrally located nuclei (immature muscle cells)

- 14 days
  - Icing group greater proportion (p<0.05)
- 28 days
  - No differences

Muscle cross sectional area

- 14 days
  - Control 9.6% > icing group
- 28 days
  - Control 64.5% > icing group (p<0.01)

Collagen fibers

- 14 and 28 days
  - More excessive in icing group
  - Control – collagen fibers only among bundles of muscle fibers
  - Icing – each muscle fiber surrounded by collagen fibers

**Graphs:**

A. Centrally located nuclei (immature muscle cells)
B. Muscle cross sectional area
C. Collagen fibers

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3/28/18
Takagi, 2011

Summary
• Delayed inflammatory response by 1 day
• Delayed proliferation and differentiation of satellite cells
• Impaired muscle regeneration with more collagen deposition around muscle fibers

Ramos, 2016

Purpose
• To determine the effect of cryotherapy on inflammation, regeneration of muscle and extracellular matrix

Ramos, 2016

Wistar rats n=42

Muscle Injury to TA - freezing

Control L 3d L+C 3d L 7d L+C 7d L 14d L+C 14d

TA muscle harvested for analysis
Control uninjured
Cross section of muscle fibers

- Uniform shape and size
- Nuclei on the periphery

Lesion

L - Necrotic fibers, intense macrophage infiltration
L+C - ↓ macrophage infiltration

3 day post injury

Regeneration of muscle fibers with centralized nucleus
L+C - ↓ macrophage infiltration
Lesion + Cryotherapy

No difference in regeneration process
No difference in macrophage infiltration

14 day post injury
Cross section of muscle fibers

Collagen mRNA Levels

Ramos, 2016

Summary
- Cryotherapy reduce inflammatory processes
- ↓ macrophage infiltration
- No affect on muscle regeneration
- No change in collagen I & III protein levels
- Cryotherapy did not enhance muscle repair and collagen content
Singh, 2017

- Hypothesized that icing would attenuate inflammation, which may slow myofibers regeneration.

Wistar rats
N=90

Contusion Muscle Injury
n=80

Icing n=40
Sham n=40

Control n=10
Uninjured, Untreated

Muscle biopsies

Control uninjured
Cross section of muscle fibers

- Uniform shape and size
- Nuclei on the periphery
Sham Icing

Necrotic fibers identified by enlarged myofibers without nuclei

1 day post injury
Cross section of muscle fibers
Sham Icing

Macrophages more abundant in sham group
At 1 day (p=0.035) and 3 days (p=0.001)

1 day post injury
Staining of macrophages
Sham Icing

Macrophages more abundant in icing group
At 7 days (p=0.045) and 28 days (p=0.020)
Singh, 2017

**Conclusion**
- Icing attenuated or delayed infiltration of inflammatory cells following injury
- However, these effects were not sufficient to prevent effective muscle regeneration

### Summary of Cryotherapy Effects on Muscle Regeneration

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<tr>
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<th>Muscle Regeneration</th>
<th>Collagen Levels</th>
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<tr>
<td>Takagi, 2011</td>
<td>↓</td>
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<td>Ramos, 2016</td>
<td>↓</td>
<td>↔</td>
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<tr>
<td>Singh, 2017</td>
<td>↓</td>
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Questions

Thank you
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