

Cryotherapy for Contusions

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1 Article

Singh et al., (2017)¹ Effects of Topical Icing on Inflammation, Angiogenesis, Revascularization, and Myofiber Regeneration in Skeletal Muscle Following Contusion Injury¹

2 Study Question

Does icing vs a sham treatment effect contusion recovery in rats?

3 Methods

3.1 Study Design

Item	Control group (sham)	Experimental group (ice)
n	40	40
Population	Rats	Rats
Intervention	Room temp + Figure 8 massage	20 min Ice + Figure 8 massage

- Testing process¹
 - Contusion event
 - 5 min period
 - Intervention applied¹

3.2 Dependent Variable

- Groups of 10 rats were then euthanized at day 1, 3, 7, 28 days
- Muscle biopsies were taken from biceps femoris mm to assess:
 - Neutrophils
 - Macrophages
 - Expression of CD34
 - Von willebrands factor (vWF)
 - Vascular endothelial growth factor (VEGF)
 - Nestin
 - Vessel volume
 - Capillary density
 - Skeletal myofiber regeneration

4 Results

4.1 Necrosis Results

By day 3, the sham group displayed less necrosis and more macrophages (a more advanced stage of healing) compared to the experimental (ice) group, but displayed no difference by day 7.

Table 2: Necrosis results table

Day	Control	Experimental
Day 1	Present	Present
Day 3	Nearly cleared necrosis with macrophage presence	Several necrotic areas with less macrophage presence
Day 7	Necrosis cleared, immature muscle fibers	Necrosis cleared, immature muscle fibers
Day 28	Normal tissue structure almost restored, inflammatory cell influx mostly resolved	Normal tissue structure almost restored, inflammatory cell influx mostly resolved

4.2 Neutrophil results

Neutrophils are pathogen fighting immune cells which are recruited to sites of infection and function to recognize and phagocytose microbes, and kill pathogens through cytotoxic mechanisms².

By day 1, the control group had more neutrophils than the ice group, indicating healing had begun earlier in the control group than the experimental group¹. By day 3, the control group had less neutrophils than the experimental group, which indicates that the control group had moved onto a more advanced stage of healing and was beginning to reuptake neutrophils. By day 7, both groups had no neutrophils, indicating they were both in advanced stages of healing.

Table 3: Neutrophil results table

Day	Control	Experimental
Day 1	More	Less
Day 3	Less	More
Day 7	None	None

Day	Control	Experimental
Day 28	None	None

4.3 Macrophage results

Macrophage count was higher in the control group in day 1 and 3, but was then less abundant in the control group in day 7 and 28¹. This indicates that the control group passed through the phases of healing faster than the experimental (ice) group.

Table 4: Macrophage results table

Day	Control	Experimental
Day 1	More	Less
Day 3	More	Less
Day 7	Less	More
Day 28	Less	More

4.4 CD34 Expression results

CD34 expression is a measure of capillary density.

Singh et al., found that by day 3 and 7, the control group had a greater percent of CD34 stained area in muscle¹. By day 28, the icing group had a greater percent of CD34 than the sham group¹.

4.5 Von Willebrand Factor (vWF) Results

vWF (indicates endothelial cell damage): percent of vWF stained area was greater in muscle from the sham group than icing at day 3 and 7 post injury.

4.6 Vascular endothelial growth factor (VEGF) Results

Vascular endothelial growth factor (VEGF) was used in this study as a marker of angiogenesis (new blood vessels)¹

Singh (2017) found that the percent of VEGF stained area in muscle was greater in the control group compared to the experimental (ice) group at 3 days post injury¹. Indicating that healing occurred earlier in the control group.

4.7 Nestin results

[Read more about Nestin](#)

Singh (2017) used Nestin count as a measure of maturing endothelial cells¹. Singh et al.¹ found that the area of nestin in the muscle was greater in the control group than the experimental (ice) group by day 3 post injury¹. By day 7 the experimental (ice) group had a greater amount of nestin in the muscle, indicating that the control group had progressed into the next stage of healing before the experimental group¹.

4.8 Vessel volume results

Micro-CT was used to measure vessel volume in mm. By day 3, the control group had greater vessel volume compared to the experimental (ice) group¹. No difference between groups by day 28¹.

4.9 Capillary density results

Number of capillaries per fiber and the number of capillaries per mm² at 28 day post injury did not differ between groups¹.

4.10 Skeletal myofiber regeneration results

Day 7: centrally nucleated regenerating mm fibers present in sham, only a few in icing group. Percentage of regenerating fibers relative to number of fibers was greater in the icing group than in sham 28 d post injury and no difference at 7 days.

4.11 Results Overview

Compared to the control group, Icing attenuated and/or delayed [neutrophil](#) and macrophage infiltration, expression of vWF, VEGF and nestin, and change in vessel volume within muscle 7 days after injury. Icing did not influence capillary density in mm 28 days post injury¹. The percentage of immature myofibers relative to the total number of fibers was greater in the icing group than sham 28 d after injury. Myofiber CSA did not differ between groups after 7 or 28 days¹.

5 Study Limitations

- Icing was applied once soon after injury, it is possible other effects could be seen if more frequent icing was applied¹
- This study only used male rats. Icing could have different effects in female rats due to estrogens that could affect muscle regeneration¹.

6 Conclusion

Icing may mildly suppress/disrupt inflammation, angiogenesis, and revascularization within the first few days. These effects do not stop or slow muscle regeneration/capillary density after contusion injury¹.

7 Clinical Significance

Icing does not seem to improve or be beneficial to the healing process post-contusion, however if a patient enjoys icing, it will not slow or stop muscle regeneration.

1. Singh DP, Barani Lonbani Z, Woodruff MA, Parker TJ, Steck R, Peake JM. Effects of Topical Icing on Inflammation, Angiogenesis, Revascularization, and Myofiber Regeneration in Skeletal Muscle Following Contusion Injury. *Frontiers in Physiology*. 2017;8. doi:[10.3389/fphys.2017.00093](https://doi.org/10.3389/fphys.2017.00093)
2. Mayadas TN, Cullere X, Lowell CA. The Multifaceted Functions of Neutrophils. *Annual Review of Pathology: Mechanisms of Disease*. 2014;9(1):181-218. doi:[10.1146/annurev-pathol-020712-164023](https://doi.org/10.1146/annurev-pathol-020712-164023)