In Vitro Measurement of Distal Limb Cooling in Horses Using the Game Ready Equine System

Klaske Brongers

Department of Equine Sciences

Faculty of Veterinary Medicine

University of Utrecht

Index

Abstract

***Objective*:** To measure the temperature decrease in the equine distal limb using the Game Ready Equine System in an in vitro setting and to investigate the influence of the application of compression during cooling. It has been hypothesized that the application of compression during cooling would result in a greater temperature decrease.

Methods: Eight cadaveric horse limbs (four fore limbs and four hind limbs) were used for this study. A copper tube was used as a heating element and was implanted in the limb in order to maintain a temperature of 37°C for at least 30 minutes. Measurement of the temperature was performed using a thermocouple. The Game Ready Equine System was tested at four settings: no compression, low compression, medium compression and high compression.

Hypothesis: Applying compression will result in a greater temperature decrease compared to no compression.

Results: The limb temperature decrease of the low compression setting was significantly lower compared to the setting with no compression. The temperature decrease of both the medium and high compression settings were significantly greater compared to the setting with no compression.

Conclusion: Cooling the limb with compression to at least 50 mm Hg (≈ medium compression) resulted in a greater temperature decrease of the distal limb in horses in an in vitro set-up.

***Potential Relevance*:** Application of compression during distal limb cooling of horses has a measurable effect *in vitro*. Whether this would also be the case *in vivo* is yet to be investigated, as are the clinical implications of this finding.

Key words: Game Ready Equine System, horse, cooling, distal limb, compression, in vitro.

Introduction

Distal limb cooling (i.e., 'cryotherapy') is widely used and is commonly recommended by veterinarians to prevent or to treat various musculo-skeletal injuries in horses.[1] However, the exact functions of distal limb cooling are poorly understood. Cooling has a local anesthetic effect and inhibits reflexive muscle spasms that can significantly reduce pain.[2,3,4] It can also reduce primary bleeding through its vasoconstrictive effect, and it will thus inhibit swelling, which may ultimately result in quicker and more effective healing and injury recovery.[2,3,4] In vivo, it has been demonstrated that during distal limb cooling in horses, a temperature decrease of 14 degrees can be reached in the digital laminae by using a water boot or an ice bag for 120 minutes.[5]

Complications such as frost bite and nerve palsy have been reported with the application of cryotherapy in human medicine.[6,7] In Equine medicine, frost bite has also been reported as a complication.[8] Furthermore, tissue damage caused by cryotherapy can delay the efficacy of other treatments.[8] Cold-induced pain was observed in human patients when low temperatures (5˚C) were used continuously for 48 hours.[6] The study from Politt et al. (2004) showed no cold-induced injury or any clinical signs that were attributable to cold-induced pain in horses despite extremely low ice boot and tissue temperatures (<5˚C).[6]

After an injury, cooling should be started as soon as possible within at least 20 minutes to be effective.[9] The application of ice is a superior method of cooling deep structures compared to other commonly used limb cooling modalities such as the cryocuff or commercial gel packs.[6,10]

Distal limb compression in combination with distal limb cooling has been hypothesized to be superior in terms of the cooling effect.[2] During cooling , the ultimate decrease of temperature is time-dependent and also depends on the depth of the injured anatomic structure relative to the overlying skin.[2] The advantage of compression during cooling has been hypothesized to have an immediate effect on the reduction of bleeding within the injured horse.[2] With this background knowledge, the Game Ready Equine System has been developed for use in horses to treat a variety of orthopedic problems in the distal limb.[1,9,11] It was designed with the help of veterinarians and the United States Equestrian Team (USET) and by using proprietary NASA spacesuit technology.[11] This system is able to deliver cold in combination with active compression of the soft tissues and is partially based on the RICE principle (Rest, Ice, Compression and Elevation) in human sports medicine.[11] Although limb elevation is practically not feasible in horses, the other components of RICE (i.e. Rest, Ice and Compression) are applicable in horses and they will help to attain the ultimate goal to reduce the potential severity of the injury and enable quicker recovery of the injury.[11] Compression provided by the Game Ready Equine System mimic the way in which muscles naturally contract and relax in order to push fluids and cellular debris (edema) into and along the lymphatic drainage system, as well as to help drive cold therapy deeper for longer lasting effects.[2,11] The compression is cyclic and adjustable, with different settings that vary from low (increase from 5 to 15 mm Hg in 4-5 minutes, followed by decrease to 5 mm Hg in 1 minute[11]) to high (increase from 5 to 75 mm Hg in 2-3 minutes followed by decrease to 5 mm Hg in 1 minute).[11]

The aim of the present study was to test the Game Ready Equine System in the distal limbs of horses using an in vitro system. It was hypothesized that the application of compression during cooling would result in a higher temperature decrease measured in the core of the limb, i.e. at the interface

of the palmar/ plantar cortex of the midmetacarpal/ midmetatarsal region and the suspensory ligament.

Methods

Development of the in vitro measurement system

Eight cadaveric horse limbs (four fore limbs and four hind limbs) were used for this study. After euthanasia, the fore limbs were resected at the elbow joint and the hind limbs at the stifle joint. A self-made heating system was implanted in the limbs. To achieve this, an incision was made between the carpal joint and the metacarpo-phalangeal joint at the palmar/ plantar section of the limb. The skin was dissected from the underlying tissue. A copper tube with a diameter of 1 cm was inserted after which the skin was sutured with a continuous suture using Vicryl 3-0. (See figure 1, and annex for full description)

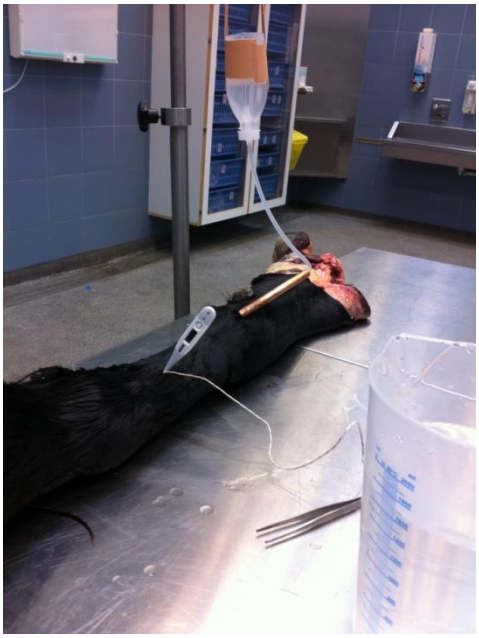


Figure 1: Test set-up.

Temperature measurements

The Game Ready Equine System has four settings: no compression, low compression, medium compression and high compression. The 'no compression' setting does not have cyclical pneumatic (air) compression. The other settings have the following characteristics: low compression: 4 to 5 minutes of increasing compression from 5 to 15 mm Hg and 1 minute release of compression from 15 to 5 mm Hg.[11] Medium compression: 2 to 3 minutes of increasing compression from 5 to 50 mm Hg and 1 minute release of compression from 50 to 5 mm Hg.[11] High compression: 2 to 3 minutes of increasing compression from 5 to 75 mm Hg and 1 minute release of compression from 75 to 5 mm Hg.[11] All four settings were evaluated according to the protocol that is described in the annex. The limb temperature during the first 3 minutes after the initiation of limb cooling were measured in intervals of 30 seconds, followed by measurements in intervals of 1 minute until a total time period of 30 minutes elapsed. Refer to the annex for a more detailed description of the temperature measurements.

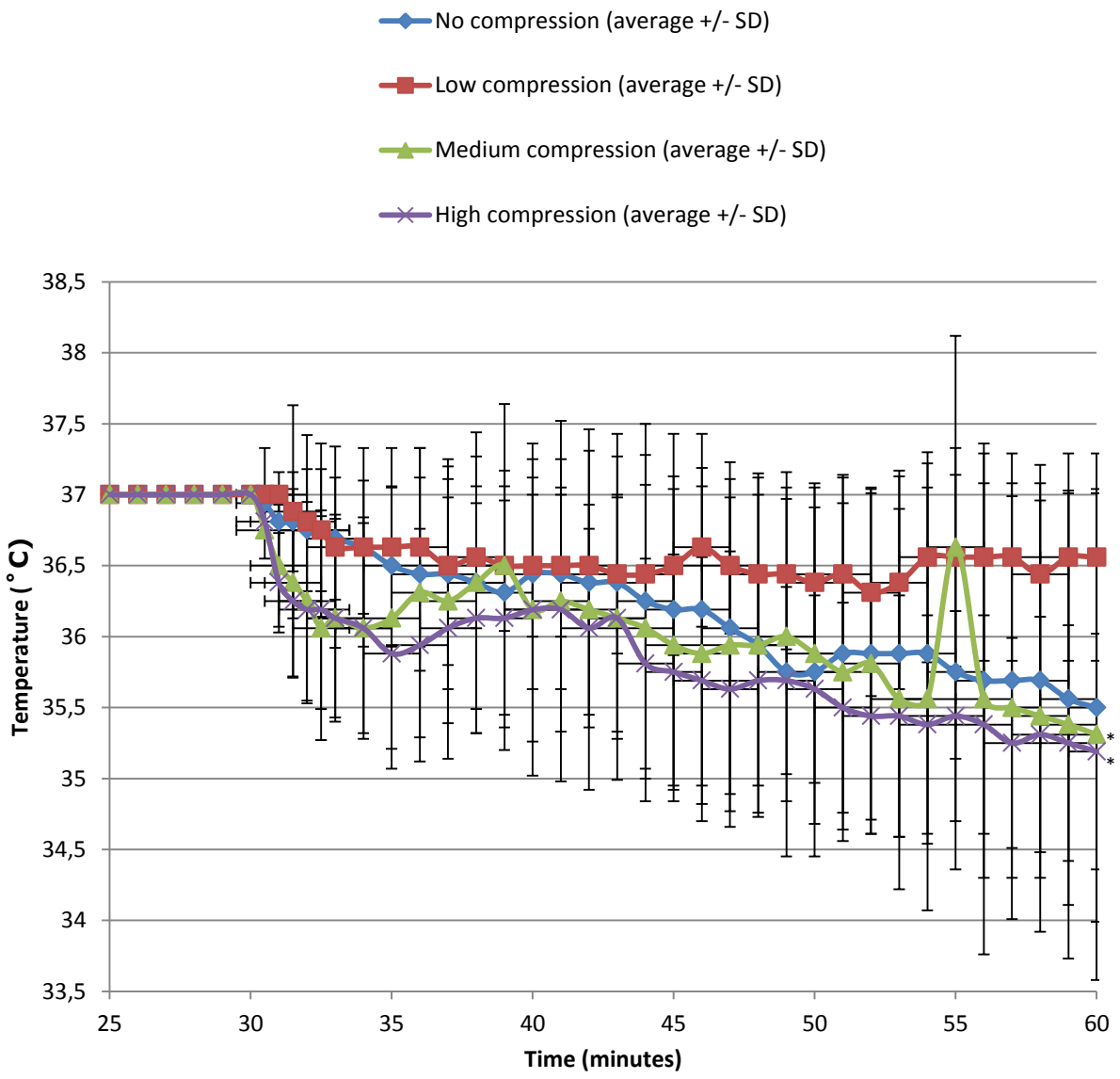
Statistics

From the measurements of the 8 limbs, the mean $\pm$ SD of the temperature for each time measure point was calculated. Because the numbers of fore limbs and hind limbs were relatively small (both n = 4), potential differences in the cooling effect between fore limbs and hind limbs were not taken into consideration. 'No compression' was used as reference and differences in temperature drops during the entire cooling period using compression according to the three settings (low, medium, and high compression) were statistically analyzed using a mixed model (SPSS 20) in which values of $P < 0.05$ were considered significant.

Results

The average (± SD) temperature decrease of the eight limbs after using the different settings of the Game Ready Equine System is shown in graph 1. All settings showed a temperature drop after 30 minutes. Relative to no compression, medium compression and high compression showed a significantly higher temperature decrease than after using the low compression setting for 30 minutes. Low compression did lead to a significantly lower decrease in limb temperature than the 'no compression' setting.

The measurements in the first 25 minutes are not shown in graph 1, but remained a temperature that was also constant at 37 ± 0˚C.



Graph 1: No compression (average ± SD), low compression (average ± SD), medium compression (average ± SD) and high compression (average ± SD). Time in minutes (starting at 25 minutes), temperature in °C.

*=p<0.05

The low compression setting reached a plateau at 36.6˚C $\pm$ 0.73 after 30 minutes, medium compression and high compression resulted in a temperature decrease to 35.3 $\pm$ 1.80 and 35.2 $\pm$ 0.86 after 30 minutes, respectively.

Medium compression showed a temperature 'peak' of +1˚C at 25 minutes, which was probably due to a measurement error.

The temperature decrease with the low compression setting was significantly *lower* compared to the no compression setting ($p<0.0001$). The temperature decrease of the medium compression setting was significantly *higher* compared to the no compression setting ($p=0.003$). The temperature decrease with the high compression setting was significantly *higher* compared to the no compression setting ($p<0.0001$).

Discussion

Discussion of the development of the in vitro system

Normal heating of the limb by blood flow through the vasculature in an in vivo situation was initially mimicked by using smaller tubes of an infusion system. However, this resulted in limbs that could not be maintained at a steady temperature of 37˚C for 30 minutes. To solve this problem, a copper pipe was used which helped the limbs to maintain a temperature of 37˚C for 30 minutes. However, the use of a rigid tube instead of a 'flexible' system had several disadvantages. First, a copper pipe gives more local heat instead of more dissipated heat. This could have influenced the temperature measurements, especially when the Game Ready Equine System was used with compression. This was because the temperature measurement system could be pressed closer to the heating element. Second, the temperature in a live animal is steady because of the constant heating by the blood flow through the vessels. Through vasodilatation or vasoconstriction, a blood vessel regulates the body temperature. The copper pipe is rigid and could not adapt to temperature changes, so the copper pipe was not able to precisely mimic an in vivo vascular system.

A direct consequence of using an in vitro system is that a cadaveric limb does not react the same as a limb from a living animal. The limb used in this research was only heated locally; the entire limb was not heated. This could also have influenced the temperature changes found in the limbs used in this research.

Additionally, the thermocouple was not perfectly accurate in the way that it measured the temperature (not in decimals). This could explain the large standard deviations that were found. The thermometer that was used before the measurements were started with the Game Ready Equine System was more accurate than the thermocouple but it could not be used while the Game Ready Equine System was used, because a regular thermometer was not compatible with the compression wrap of the Game Ready Equine System.

To obtain more reliable measurement results, an adaptation of the in vitro system could be considered in order to create a heating system that would more closely mimic an in vivo vascular system. This might be possible by using a less rigid heating element that would still provide enough heat to maintain a constant limb temperature of 37˚C for over 30 minutes. Alternatively, an in vivo system could be considered, but using live animals for this kind of study would definitely lead to ethical discussions. Finally, to further improve the measurement system and the reliability of the temperature measurements, using a more accurate thermocouple is recommended.

Discussion of the results

Low compression resulted in a significantly *lower* temperature decrease compared to no compression. There is no clear and satisfactory explanation for this outcome.

Medium and high compression resulted in a significantly *higher* temperature decrease compared to no compression. This could possibly be explained by the fact that there was a volume decrease of the limb due to the compression that was applied, with a consequence that after compressing the limb to a certain level, a constant level of cold was applied to less tissue mass that caused the tissue to receive relatively more cold. Whether there is a linear relationship between the level of compression and the level of temperature decrease is not clear and is a topic for further research.

Conclusion

The temperature decrease with the low compression setting was significantly *lower* compared to the no compression setting ($p<0.0001$). The temperature decrease with the medium compression setting was significantly *higher* compared to the no compression setting ($p=0.003$). The temperature decrease with the high compression setting was significantly *higher* compared to the no compression setting ($p<0.0001$).

This in vitro study has shown that compression applied to limb cooling causes a significant reduction of the core temperature measured at the core of the midmetacarpal/ midmetatarsal region, but only when a cyclic compression rate was used with a gradual increase from 5-50 mm Hg for 2-3 minutes and relief from 50-5 mm Hg for 1 minute. Compression of a lower magnitude had a contrary effect, i.e. there was less temperature decrease compared to the non-compression mode. However, it should be mentioned that the reliability of the findings should be interpreted with care because the in vitro system that was developed for this study had characteristics that did not mimic the in vivo situation in entirety. Therefore, whether the findings could be extrapolated to the in vivo situation and the clinical implications of these findings must still be investigated.

References

1. Petroc R. Influence of topically applied cold treatment on core temperature and cell viability in equine superficial digital flexor tendons. *Am J Vet Res.* **64**(7), p 835-844 (2003)

2. Knobloch, K., Grasemann, R., Spies, M. & Vogt, P. M. Mid portion achilles tendon microcirculation after intermittent combined cryotherapy and compression compared with cryotherapy alone: a randomized trial. *Am. J. Sports Med.* **36**, 2128-2138 (2008).

3. Knight, K. L. & Londeree, B. R. Comparison of blood flow in the ankle of uninjured subjects during therapeutic applications of heat, cold, and exercise. *Med. Sci. Sports Exerc.* **12**, 76-80 (1980).

4. Knight, KL. Cryotherapy in sports injury management. *Int Perspec Physiother.***4**, 163-185 (1989).

5. Heidi L. Reesink, et al. Measurements of digital laminar and venous temperatures as a means of comparing three methods of topically applied cold treatment for digits of horses. *Am. J. of Vet. Res.* **73**(6), p 860-866 (2012).

6. Pollitt, C. C. & van Eps, A. W. Prolonged, continuous distal limb cryotherapy in the horse. *Equine Vet. J.* **36**, 216-220 (2004).

7. Swenson, C., Sward, L. & Karlsson, J. Cryotherapy in sports medicine. *Scand. J. Med. Sci. Sports* **6**, 193-200 (1996).

8. Manning, A. Practical approach to the rehabilitation of soft tissue injuries. In: Jumping Amsterdam 2014 55th edition, 10th Veterinary sport horse congress, 24-25 January 2014.

9.Smith, R. Rational treatment options for tendon and ligament injuries. In: Jumping Amsterdam 2014 55th edition, 10th Veterinary sport horse congress, 24-25 January 2014.

10. McMaster, W. C., Liddle, S. & Waugh, T. R. Laboratory evaluation of various cold therapy modalities. *Am. J. Sports Med.* **6**, 291-294 (1978).

11. http://intl.gamereadyequine.com/index.htm.

Annex

Four incisions were made in the forelimb:

1. An incision (1cm) was made lateral of the carpal joint, from cranial to caudal.
2. An incision (1cm) was made lateral midcarpal, from proximal to distal, for the thermocouple. The thermocouple was used for temperature measurement of the palmar tissue midmetacarpal against the 3rd metacarpal bone.
3. An incision (3cm) was made under the superficial and deep digital flexor tendon proximal of the metacarpo-phalangeal joint.
4. An incision (1cm) was made medial of the forelimb from cranial to caudal on the metacarpo-phalangeal joint.

A copper tube (diameter: 1 cm) was placed through the four incisions. The copper tube was meant to function as a heating element (figure 2). Subsequently, the skin was sutured with a continuous suture using Vicryl 3-0.

Figure 2

Four incisions were made in the hind limb:

1. An incision (1cm) was made lateral of the hock joint, from cranial to caudal.
2. An incision (1cm) was made lateral midmetatarsal, from proximal to distal, for the thermocouple. The thermocouple was used for temperature measurement of the plantar tissue midmetatarsal against the 3rd metatarsal bone.
3. An incision (3cm) was made under the superficial and deep tendon flexor tendon proximal of the metatarso-phalangeal joint.
4. An incision (1cm) was made medial of the hind limb from cranial to caudal on the metatarso-phalangeal joint.

A copper tube (diameter: 1 cm) was placed through the four incisions. The copper tube was meant to function as a heating element. Subsequently the skin was sutured with a continuous suture using Vicryl 3-0 (figure 3).

Figure 3

The limb was placed in a container of water (35-40°C) for one half hour. Then the limb was placed on a table.
The thermocouple and a thermometer were inserted in the incision as described above. The temperature will be measured palmar midmetacarpal against the 3rd metacarpal bone (fore limb) or plantar midmetatarsal against the 3rd metatarsal bone (hind limb).
The thermometer (0.1°C accurate), was more accurate than the thermocouple (1°C accurate). In addition, the thermometer and the Game Ready Equine System could not be used simultaneously. Therefore, the thermometer was only used while heating the limb.

Instructions for initiating the Game Ready Equine System:

1. After connecting the water tank to the copper tube, the limb will start heating to 37°C (figure 3).
2. When the limb temperature is steady at 37°C for 30 minutes, remove the thermometer.
3. Secure the Game Ready utility wrap around the limb.

4. Connect the wrap to the control unit.
5. Select the pressure and timer settings (30 minutes).
6. Press the start button.
7. Measure the temperature for 30 minutes.