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Critical Evaluation of Whole-Body Cryostimulation Protocol in Race Horses

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ABSTRACT

Cold therapy is commonly used to relieve pain and inflammation and to aid in muscle recovery after exercise in human medicine. A number of applications have also been observed in veterinary practice. In this article, a critical evaluation of equine protocol applied with a new commercial concept of equine whole-body cryostimulation (WBC) was made. With this new concept of WBC, the protocol usually utilized for relieving pain and discomfort in humans has been extended to horses. The investigations described herein focus on the reduction of horse skin temperature when applying human WBC protocols. Based on infrared thermography measurements, results show that exposing a horse for 3 minutes to a temperature of −140°C, which are conventional parameters used for humans, does not induce sufficient skin thermal gradients in horses. Consequently, beneficial cold reflexes such as vasomotor, neuro-conduction, and biochemical reactions cannot be triggered. Further investigations should therefore be carried out to design an adequate protocol specifically aimed at horses.

1. Introduction

Localized cold, whether by means of ice, snow or cold water, has been used in humans for over 2000 years mostly because of its analgesic effects. It also helps reduce muscular spasms and provides muscle relaxation by blocking nerve conduction [1,2]. This applies to both nociceptive and sensory fibers; thus the decrease in sensory influx would lead to a decrease in muscle spasms contributing to analgesia in the affected area [3]. It is highly prized by athletes in their muscle recovery process [4,5] and explains why the practice of WBC as a complementary therapy or a natural recovery strategy in humans has become widespread in recent years [6,7]. Indeed, numerous studies [8–10] have accumulated scientific evidence supporting the beneficial effects of WBC in the human medical domain as an alternate treatment or rehabilitation technique. Similar findings have been made in the sporting realm, especially in the context of postexercise recovery [1,11]. The principle commonly implemented is to cause a thermal stress in a confined space at a temperature generally comprised between −160°C and −110°C, for a limited time of about 3 minutes.

Applications of cold to mammals are few [12] and only horses actually undergo cryotherapy sessions in local treatment [13–15]. Several articles in the literature recommend the use of cold and provide advice related to methods and local duration of action on horses [16–18]. Used by veterinarians since the early 2000s, cryotherapy techniques for horses consist in ice applied through a continuous cold boot, sprayed liquid nitrogen, or the use of a cryotherapy probe and gas cryotherapy [1,17,18]. It has been shown that these localized treatment techniques can bring real benefits in post-traumatic treatment [19–21]. However, reaching the same

Animal welfare/ethical statement: Based on European directive 2010/63 transposed into French law (decree 2013–118), no ethical issue arises from this study as experimental data are only gathered from observational field studies. Observations were made in the frame of normal cryosessions scheduled by the trainer, under his full responsibility. The horses were familiar with these sessions: every effort was made to ensure their welfare by minimizing disturbance caused by researchers and the horses showed no sign of stress.

Conflict of interest statement: The authors have no conflict of interest to declare.

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temperature in the subcutaneous tissue as on the skin involves the onset of a thermal shock. If not, the temperature of deeper tissues will only decrease a few minutes after the application of cold; the cool-down being gradual and therefore the magnitude of cooling will be less than at the skin level [22]. Analgesia is believed to result from slowed nerve conduction, inhibiting the transmission of pain signals to the dorsal horn of the spinal cord [23–25]. This phenomenon is observed in humans when muscle temperature drops down to a threshold of 27°C [18].

Today, in the absence of any scientific guidelines for horses, experimental commercial WBC systems for horses are based on the same concept as for humans. More precisely, the whole horse body undergoes a thermal shock at −140°C, caused by exposure to gaseous nitrogen in a semiclosed space over a limited time frame (typically 3 minutes). In humans, this duration is required to trigger cold reflexes, such as vasoconstriction, slowdown, or even blocking of nerve conduction, ultimately leading to pain relief [26].

The purpose of this study was to test the validity of this human WBC technique transposed to horses, by measuring the level of the skin thermal variations thus obtained. To this end, the change in cutaneous temperature was measured over the whole body of the horse after exposure to an extremely cold atmosphere generated by WBC embedded in a new commercial device. Measurements were performed using infrared thermography (IRT) to acquire skin temperature, its response being sensitive to extreme cold. The potential role of IRT in the diagnosis and treatment monitoring is mentioned in veterinary applications [22], especially in equine medicine [27–33]. This powerful, noninvasive, and easy-to-handle technique has a broad range of application. It is used in horses to diagnose symptoms in the fields of equine orthopedics and lameness [34], palmar or plantar digital neurectomy [35], detection of jugular venipuncture [36], and design of training protocols [37,38]. Then, the critical evaluation of the WBC protocol used in humans at −140°C and extended to race horses, as it is used today, will be carried out by IRT.

2. Materials and Methods

2.1. Thermal Behavior and Dissipation in Horses and Humans

Horses and humans share thermal characteristics. First, they are both homeothermic mammals [25], with a core temperature independent of the surrounding environment and tending to constancy at rest due to neurophysiologic mechanisms balancing heat production and heat losses. This balance is maintained at rest in an environmental temperature range called thermoneutral zone, estimated in horses in the range 5°C–25°C [39,40] and in the range 25°C–30°C for a naked man. Core temperatures are very similar, namely 38°C [41] for horses and 37°C for humans. Same goes for the average skin temperature, namely 33°C for horses and humans. The thermal balance is therefore governed by the relationship:

$$\text{Heat}_{\text{production}} = \text{Heat}_{\text{dissipation}} \pm \text{Heat}_{\text{storage}} \tag{1}$$

In humans, it has been shown that the internal temperature does not vary during WBC sessions [42]. The reason is that the exposure to cold is too short to induce a decrease in internal temperature. If a net internal heat loss had initiated, heat storage would have been negative and the body temperature would have fallen (S < 0) [43]. If the internal temperature is constant, it can be assumed that the rate of heat storage ($\text{Heat}_{\text{storage}}$) is zero (S = 0). This assumption in humans can reasonably be transposed to horses.

Major differences between horses and humans may lie in the way heat dissipates even if for both species, convective (including respiration) and radiative thermal exchanges are the main heat transfer modes:

$$\phi_{\text{heat losses (W/m}^2)} = (\phi_{\text{conv}} + \phi_{\text{resp}}) + \phi_{\text{rad}} \tag{2}$$

Convective and radiative exchanges ϕ_{conv} and ϕ_{rad} are expressed using Newton and Boltzmann laws, respectively:

$$\phi_{\text{heat losses (W/m}^2)} = h_c(T_S - T_\alpha) + \phi_{\text{resp}} + \sigma\varepsilon\left(T_S^4 - T_\propto^4\right) \tag{3}$$

where h_c is the free convective heat transfer coefficient, σ is the Stefan–Boltzmann constant ($\sigma = 5.67 \times 10^{-8} Wm^{-2}K^{-4}$) and ε is the skin radiative emissivity. Parameters T_S and $T_\propto$ stand for the skin and ambient temperatures respectively, expressed in K. In horses, the heat loss related to the convective respiratory mechanism ϕ_{resp} is estimated between 15% and 25% of total loss [42], while it lies around only 10.5% in humans [44].

2.2. Horses Studied

Five healthy thoroughbred mares (3 years ± 3 months of age, 448 ± 18 kg) from a racing stable in Chantilly (France) were recruited for the study. All were followed by a veterinarian who declared them sound, with no musculoskeletal disease history and no clinical symptoms of illness. In the opinion of the horse trainer, no external sign of estrus was observed at the time of the trials, which took place at the end of October, outside the breeding season [45]. Before the experiments, early in the morning, the horses performed a horse's standard exercise protocol composed of 800 m at promenade allure ($\approx$7 km h^{-1}), 1,200 m trotting ($\approx$14 km h^{-1}), 1,400 m hunting canter ($\approx$20 km h^{-1}), and 1,500 m quick canter ($\approx$50 km h^{-1}). They stayed in the stable for 3 hours to cool down afterward. The owners of the horses as well as the horse trainer who owns the race stable gave their consent for this study.

2.3. Whole Body Cryostimulation Chamber for Horses

The development of this cryotherapy device for horses was inspired by the mobile units for athletes that enable cryotherapy devices to be brought directly onto sporting events or race sites. In the present case, the truck is replaced by a two-horses van (Figs. 1A and 1B). The technology is based on the decompression of liquid nitrogen to reach extreme temperatures very quickly inside the cryostimulation chamber. For safety considerations, the head of the horses remained outside the cooled zone to avoid any nitrogen respiration (Fig. 1B). This principle is found in cryocabins for humans [1], where the top face of the cylinder constituting the cryotherapy chamber is open such that the free surface of nitrogen lies at the neck level. Moreover, horses were equipped with earplugs to avoid possible stress due to noises emitted by the device. It should be noted that all the mares were familiar with this kind of cryostimulation and none of them showed any resistance to entering the chamber during the experiments, nor appeared to be under any stress according to the horse trainer feedback. However, it is worth reminding that naïve horses must be gradually acquainted with entering the mobile chamber to minimize stress.

For all horses, the selected protocol was identical to the one conventionally used for humans and commonly adopted in cryocabins, that is, an exposure of 3 minutes at −140°C. Each horse was thermally photographed (instantaneous capture) on its left side before the trainer led it into the van (Fig. 1A) for the cryostimulation session. This picture is the reference thermal level. After the cryostimulation session, immediately after leaving the van, the horse is thermally photographed again on the same side. This last picture makes it possible to evaluate the influence of thermal shock on the horse's skin temperature in comparison with the reference image. Although all five horses were evaluated in the

Fig. 1. Mobile cryostimulation chamber (A); horse's head out of the cryotherapy device (B).

same way, the experiments were not repeated over several days, which may be a limitation of the study.

2.4. Infrared Thermography and Environmental Conditions

Skin temperatures were recorded using a ThermoVision SC620 thermal imaging camera (FLIR Systems, Wilsonville, OR) in accordance with the standard protocol of infrared imaging in medicine [46]. For each horse, two IRT pictures were acquired, the first one just before entering the cryotherapy chamber, and the second just after leaving it.

It is worth noting that healthy horses display thermal symmetry similarly to humans. Indeed, healthy humans have a high degree of thermal symmetry in terms of both magnitude and pattern [47]. The results can easily be extended to mammals and more specifically to horses. This is the reason why only the left laterality of the horses was thermally treated.

Because the experiments took place outdoors, knowledge of the atmospheric conditions is necessary to qualify dissipative heat transfers. Measurements of outdoor temperature, atmospheric pressure, humidity, and wind speed were performed throughout the experiment and are summarized in Table 1. Experiments were conducted in a shaded area away from the sun.

For accurate measurement by IRT, the knowledge of skin emissivity is key because it reflects the effectiveness of the emission of infrared radiation by the skin. In published works on equine thermography, the emissivity has not been accurately defined, ranging from 0.95 to 1. An estimated emissivity value of 0.98, already adopted in the literature [47], was selected.

It should be noted that in race horses, the use of IRT to access skin temperatures comes without generating measurement bias because these horses are characterized by a coat that is often short [48].

Quantitatively, thermal imaging was analyzed according to regions of interest that we identified as being some of the areas where muscle tensions appear in the horse's trunk [49]. In detail, nine areas have been identified, corresponding, respectively, to the subcutaneous positions of the trapezius muscles over the shoulder, latissimus dorsi muscle of the back, medium, and superficial buttock muscles, semitendinosus and gluteofemoral muscles of the hind limb, latissimus dorsi muscle, and brachiocephalic muscle of the neck. These areas are depicted in Fig. 2. We hypothesize that in horses, as in humans [50,51], there is a relationship between surface temperature and intramuscular temperature. Thus, measuring the surface temperatures over the 9 identified areas must make it possible to obtain information on the corresponding deep tissues, which are ordinarily the target tissues of cryotherapy applications.

3. Results

Fig. 3 presents the IRT mappings of the same mare just before and immediately after the cryostimulation session, at −140°C for a 3 minutes' duration, with identical color-coded scales. It clearly appears in Figure 3A, at rest and before the cryostimulation session, that the skin temperature in not uniform over the body surface, the maximum amplitude measured being 10.5°C (maximum at the throat latch: 32.4°C and minimum at the fetlock of the hind leg: 21.9°C). Higher skin temperatures are generally obtained in the vicinity of vital organs that must be thermally preserved. Indeed, in these areas, greater heat production (to preserve vital organs) induces greater heat dissipation to maintain the thermal balance, resulting in an increase in skin temperature.

It should be noted that for each mare and each area investigated, the measurement obtained is not a one-point measurement but an average measurement over an area of approximately 100 cm^2. Fig. 4 represents the statistically averaged measured temperature over all mares before and immediately post cryostimulation treatment, for the different body regions of interest. The * indicates a significant skin temperature difference between before and after treatment ($P \leq .05$), β indicates a significant skin temperature difference between before and after treatment ($P \leq .001$). Muscular area numbers are presented in Fig. 2.

Standard deviation is also reported. The observation that can be drawn from these results seems to be that temperature decreases too slightly between before and after cryostimulation. For a −140°C cryostimulation during 3 minutes, corresponding to human protocol, the induced skin cooling varies from −1.12°C for the large dorsal to −2.80°C for medium buttock in horses.

4. Discussion

It is reminded that as a consequence of the dissimilar morphologies between humans and horses, all the comparisons herein

Table 1
Atmospheric working conditions during IRT measurements.

Outdoor temperature	Relative humidity	Outdoor pressure	Wind speed	Weather
12.7°C	69.35%	1,025 hPa	0.7 m s^{-1}	Sunny

Abbreviation: IRT, infrared thermography.

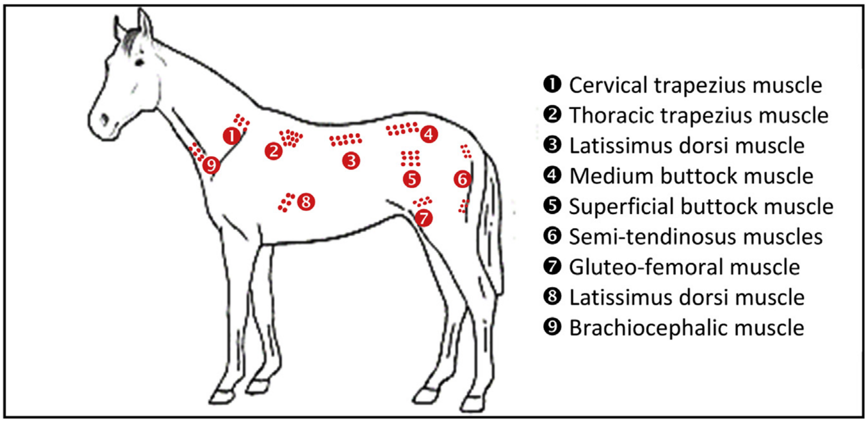

Fig. 2. Areas of muscle tension in the horse's trunk [49] selected for the IRT imaging.

are questionable. Nevertheless, it can be estimated from the results that skin cooling level observed in horses is 5–6 times lower than that of humans, when the same cryostimulation protocol at −140°C for a duration of 3 minutes is applied.

For comparison purposes about Fig. 3, we can refer to a recent study in which 10 human subjects (50% female; means ± S.D.: age 45.8 ± 5.5 years, height 168.7 ± 9.3 cm, weight 75.3 ± 13.1 kg, body fat percentage 19.3 ± 9.8) participated in cryostimulation sessions with the same protocol [52]. Thermal imaging was quantitatively analyzed according to given regions of interest, namely chest, upper back, arms, stomach, lower back, and legs. In humans, the induced skin cooling was found to vary from −6.30°C for the upper back to −14.35°C for the legs.

This difference could be attributed to several factors. First, contrary to radiative dissipative heat transfer which is independent of the body shape, convective heat transfer is quite different between horses and humans. Their body shapes are actually quite dissimilar; the human body can roughly be assimilated in shape to a volumetric heat source embedded in a thin vertical cylinder while horses can be considered as a large horizontal one. Convective heat transfer stemming from these simplified geometries is therefore different [53,54]. Subsequently, at an ambient temperature between −3°C and 37°C, horses undergo a total heat loss [40] corresponding to a dissipative heat flux density of about 142 W/m^2 (without evaporating losses), whereas humans at rest present a value close to 50 W/m^2. This order of magnitude may be calculated by considering a key physiological parameter in cryotherapy, which is the ratio between the mass (kg) and body surface area (m^2) of humans and horses alike. It can reasonably be estimated at 80 kg/1.8 m^2 for an average male human, and 500 kg/5 m^2 for horses [55], which shows that horses need to dissipate 2.5 to 3 times as much heat through each square meter of body surface area than men. Therefore, thermal balance is ensured by a higher heat production in horses than in humans in the same proportion. When facing extreme cold conditions, the lower the body mass relative to body surface, the higher the heat dissipation compared to basal metabolic rate; this explains in part why humans are more subject to hypothermia than horses, and why horses may tolerate temperatures as low as −40°C [56]. Another key parameter when it comes to facing cold is the tissue thermal insulance. This value, which is rarely studied in animals, is nevertheless fundamental in the way the body reacts to cold. The reference value of tissue thermal insulance in horses (0.10 m^2K/W [40]) is twice that of humans (0.05 m^2K/W—derived from the study by Polidori et al [42]), which also explains why the latter cannot withstand temperatures as low as horses do. One of the major reasons why the discrepancies between tissue thermal insulance in horses and humans lies in their skin structures, which provides a physiological barrier between the external and internal environments as well as higher thermal inertia [57] than humans. Horses and humans strongly differ in this matter, even though skin is the largest organ in both these species and constitutes a temperature regulator as well as the key factor when it comes to heat loss issued from the body. Its thickness is estimated on average to be about 3.8 mm in horses [58], whereas it is commonly accepted that the average value in humans lies around 0.6 mm. For obvious reasons, the thickness and structure (presence

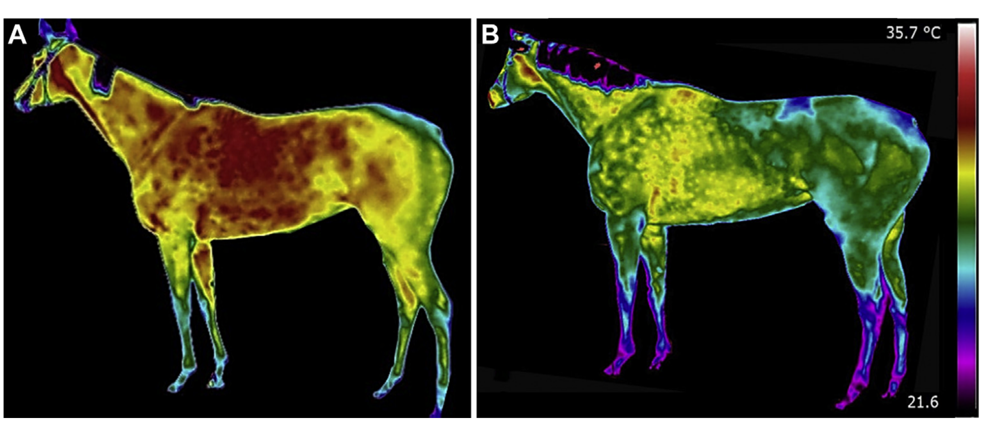

Fig. 3. « Red-Whisper » (3 years old, 466 kg) just before the cryosession (A), immediately after session (B).

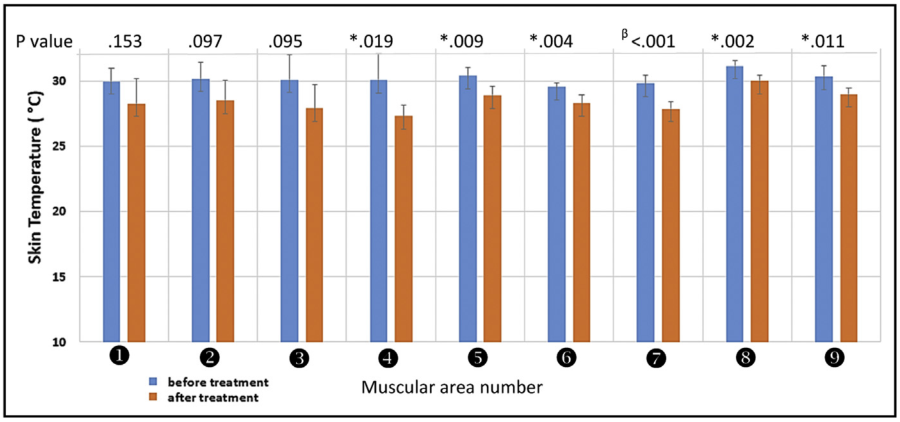

Fig. 4. Average skin temperature measurements and standard deviation obtained through IRT.

of leather) of the skin may affect the thermal insulance of tissues. These different thermal characteristics account for the discrepancies in the reactions of horses and humans to extreme cold. In the end, these differences prevented the cold from penetrating rapidly into the horse's tissues during the reduced time frame of the cryostimulation session.

A number of potential limitations need to be considered. First, the thermal mapping depicted in Fig. 3 and obtained before cryostimulation indicates values lower than the average skin temperature in horses mentioned in the literature, that is, around 33°C. One of the reasons is that experiments were conducted in a real outdoor situation in October in northern France at a temperature of 12.7°C and a light breeze of 0.7 m s^{-1}. Under these climatic conditions, the characteristics of dissipative heat transfers are obviously different from those that could be obtained under laboratory conditions. This would support in part an assumption found in the literature, which states that wind speed has an influence on the amplitude of skin temperature variation [59]. Another limitation may lie in the reduced number of horses involved in the study.

In spite of the discrepancies in thermal characteristics mentioned previously, the principle of equine thermoregulation in the face of extreme cold remains similar to that of humans. Thus, one may reasonably think that WBC can also produce beneficial effects in horses. Given the current state of knowledge, transferring human protocols to horses produces insufficient benefits in terms of skin temperature decrease.

One possible solution would be to select colder treatment temperatures, but nitrogen-based WBC technologies do not allow it [60]. Therefore, only the extension of the exposure time may be considered. To define the optimal duration of exposure to cold, a test campaign related to the skin cooling kinetics of the horse should be performed by measuring thermal fields at increasing time intervals of exposure and on a larger number of animals. This would make it possible to estimate the thermoregulatory behavior of the horse in WBC, which remains unknown to date, unlike that of humans [61]. Knowledge of this behavior will provide essential information in the definition of a WBC protocol adapted to the horse's particularities.

5. Conclusion

This experimental study focused on a critical evaluation of whole body cryostimulation protocol in race horses. For this purpose, the reaction to extreme cold of five race horses was analyzed by means of IRT. The benefits of WBC on muscle recovery in equestrian sport seem illusory at the current state when protocols are transposed from humans (3 minutes at 140°C) to horses. Indeed, the recorded skin variations seem far too small to be able to induce interesting therapeutic or muscle recovery effects. Future studies appear necessary and should therefore focus on analyzing cold exposure duration to define an appropriate protocol for race horses.

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