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Original article

The effect of training on infrared thermographic images of the forelimb and hindlimb joints of healthy racehorses

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Abstract

The aim of this study was to evaluate the influence of training on body surface temperature over the joints in racehorses, measured by infrared thermography. The study involved monitoring of 14 Thoroughbred racehorses in 6 imaging sessions over a period of 3 months. Temperature measurements of the forelimb and hindlimb joints were made before and just after training. Joint temperature of limbs increased significantly after training. Environmental temperature had a statistically significant influence on surface temperature over the joints. The lowest surface temperatures were recorded over the metacarpophalangeal and metatarsophalangeal joint and the highest temperatures in the shoulder, elbow, hip and stifle joint. The metacarpophalangeal and metatarsophalangeal joints warmed the least during training, but were influenced the most by differences in environmental temperature. The surface temperature difference before and after training is an important indicator of the thermoregulatory response to exercise in racing horses. Understanding surface temperature changes in response to regular training is necessary for future studies on diagnosing injuries of joints.

Key words: thermography, horses, surface temperature, exercises, limbs

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Introduction

Homeothermic animals produce heat that is lost from the body surface in several ways including radiation, conduction, evaporation, and convection (Piccione et al. 2005). The evaluation of body surface temperature provides valuable information to monitor the physiological status, welfare and stress responses in performance horses (Simon et al. 2006, McGreevy et al. 2012). Body surface temperature is influenced by the local metabolism, blood flow (vascular tone), perfusion in the subcutaneous tissue, and coat thermal insulation properties (Palmer 1983, Langman et al. 2015). Environmental conditions have a big impact on body surface temperature, especially ambient temperature, direct radiant energy, airflow and humidity (Palmer 1983, Soroko et al. 2016, 2017a). Any physical exercise causes surface temperature homeostasis disruption due to changes in peripheral blood flow and evaporative heat loss (Hodgson et al. 1994). During exercise bones, ligaments, tendons and muscles are subject to overloads. This causes an elevation of metabolic activity taking place in individual tissues (Arfuso et al. 2016). Muscles increase their oxygen uptake from blood flow through blood vessels (Shephard 1982). This contributes to increased skin circulation and thus to improved dissipation of excess heat to the environment (Lindinger and Waller 2008).

Infrared thermography (IRT) has been suggested as a useful non-invasive modality for measuring local changes in body surface temperature distribution in animals (Luzi et al. 2013, Howell et al. 2020). IRT has been widely used as a tool in environmental physiology, and as a diagnostic tool in veterinary medicine (Ciutacu et al. 2006, Purohit et al. 2006, Soroko and Howell 2018). Clinical conditions in equine veterinary medicine that can be recognized in initial phase of diagnosis by thermography include inflammation of the stifle (Purohit et al. 2006) and experimentally induced inflammation of the carpal and tarsal joints (Bowman et al. 1983, Turner 2001).

In the field of exercise physiology, recent studies have suggested the use of IRT in monitoring the influence of training on body surface temperature in performance horses (Soroko et al. 2014, 2015, 2017a). The surface temperature distribution of the forelimbs and hindlimbs has been described in detail in horses at rest, with proximal areas warmer compared to the distal parts of the limbs (Palmer 1983). IRT has been used to assess forelimb and hindlimb surface temperature change after treadmill exercise as a measure of muscle activity (Simon et al. 2006, Yarnell et al. 2014). Another study found a significant increase in surface temperature over the neck, shoulder, elbow and gluteus in routinely-ridden horses just after exercise on a treadmill (Soroko et al. 2018). To the best of our knowledge, however, no previous studies have measured the changes of surface temperature overlying the joints in racehorses subjected to regular racing training. The present study reports infrared thermographic imaging measurements of forelimb and hindlimb joints, which are the most common sites of injury or sub-clinical inflammation in performance racehorses (O'Sullivan and Lumsden 2003, Muir et al. 2008, Reed et al. 2012).

The aim of this study was to evaluate the influence of training on body surface temperature over the joints in racehorses, measured by IRT. The hypothesis of the study was that regular training increases surface temperature over the joints, and that the increase in temperature of the skin overlying the proximal joints is greater than the increase at the distal forelimbs and hindlimbs.

Materials and Methods

All horses recruited to the study were subjected to standard procedures without any harm or discomfort and therefore the study did not require the consent of the Local Ethics Commission for Animal Experiments (European directive EU/2010/63).

Study population

The study was conducted on 14 clinically healthy Thoroughbreds, all aged 2 years and housed in individual stalls at the Partynice Race Course in Wroclaw (Poland). The study took place in the middle of the racing season, when horses were in regular training for their first flat racing season. Included horses had been in regular training for 6 months prior to the study. Before inclusion, all research horses were evaluated for soundness by standard clinical examination, including manual palpation of the limbs and observation in movement. All the examined horses were trained at the same intensity levels, consisting of trotting for a distance of 2 km, cantering for a distance of 1.000 m, and then introducing elements of speed training from 200 m up to 500 m. Measurement of body temperature was performed on each horse in 6 separate sessions, scheduled every two weeks over a period of 3 months (July-September 2019).

On each examination day, horses underwent training consisting initially of warm-up in walk and trot on an automatic horse walker for approximately 40 minutes. Immediately after warming up, had walk for 5 minutes, then trot over 1.000 m for 5 minutes and canter over 500 m for 10 minutes in not constant directions on the racetrack with dirt surface, with the length

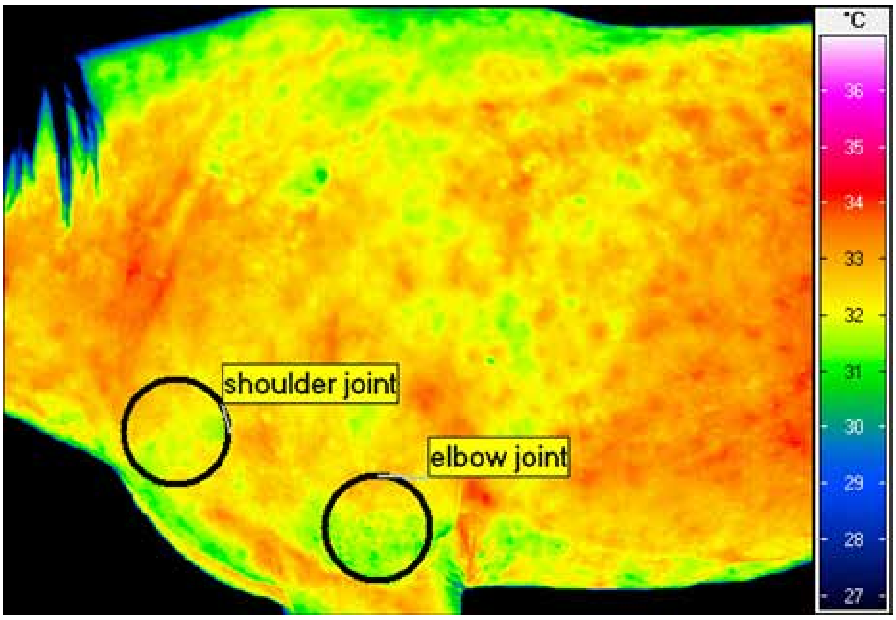


Fig. 1. Thermographic image of the horse's left side of the proximal forelimb area before training with the shoulder joint and elbow joint region of interests indicated.

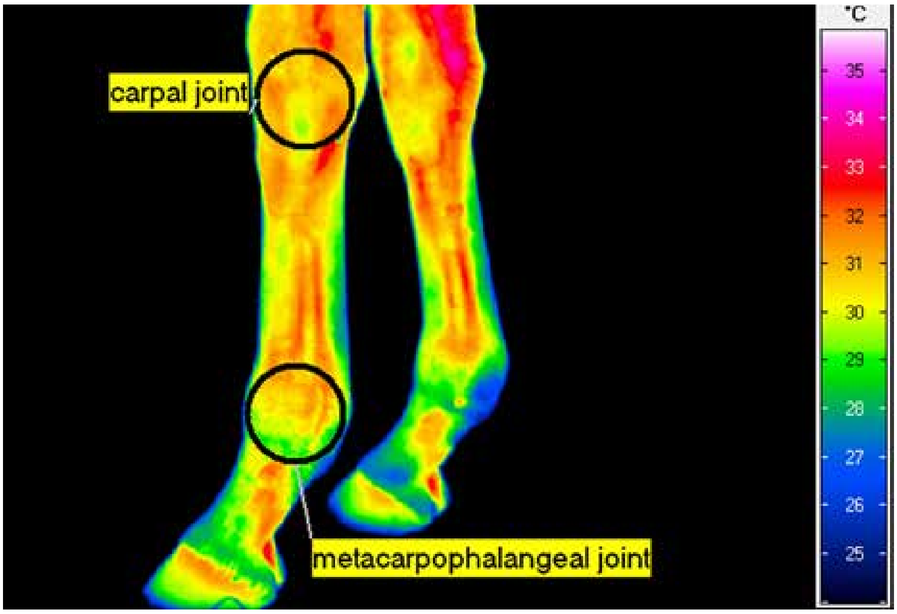


Fig. 2. Thermographic image of the horse's left side of the distal forelimbs before training with carpus and metacarpophalangeal joint region of interests indicated.

of 700 m. The racetrack was maintained twice a day (at 9.00 am and at 12.00 after finishing training). Horses were trained between 7.00 and 10.30 am at each session by a different rider from a team of four, each of whom were of similar riding experience and weight.

Infrared thermography

At each of the six sessions, thermographic images were acquired always in the morning before training (after night rest at the stable, between 6.00 and 7.00 am) and immediately (2-3 minutes) after training of each horse. Thermograms before and after training included 4 areas: the shoulder, distal part of the forelimb, croup, and distal part of the hindlimb were imaged from both sides of the body. All measurements were made indoors in an enclosed stable, in the area adjacent to the stall of each horse, to eliminate errors due to reflected solar radiation and air flow. Images acquired before training followed the protocol for thermography examination described by Soroko and Howell (2018). Dirt and mud present in the imaging field of view was brushed away 15 minutes before taking the images. Thermographic images were recorded from a distance of approximately

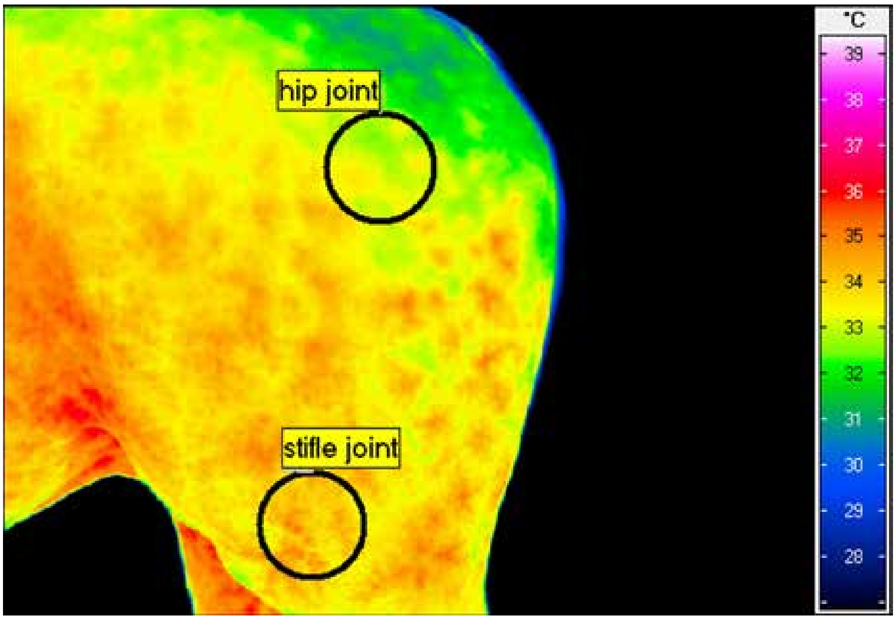


Fig. 3. Thermographic image of the horse's left side of the proximal hindlimb area before training with hip joint and stifle joint region of interests indicated.

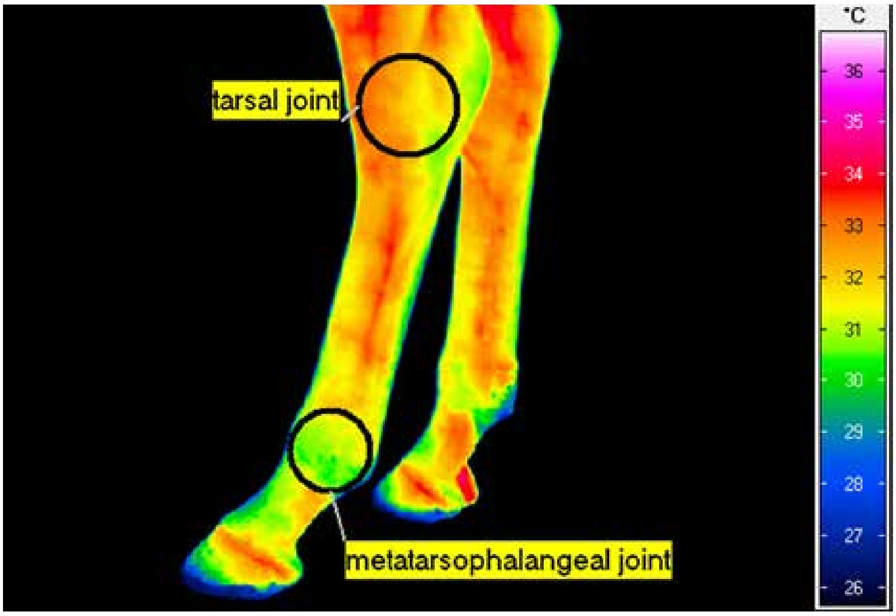


Fig. 4. Thermographic image of the horse's left side of the distal hindlimbs before training with tarsus and metatarsophalangeal joint region of interests indicated.

1.5 m from the examined horse in the stable corridor. Images were captured using a VarioCam hr Resolution infrared camera (resolution 640 x 480 pixels, spectral range 7.5–14 mm, InfraTec, Dresden, Germany). The emissivity (ε) was set to 1 for all readings. On each thermographic image the mean surface temperature within circular regions of interest was calculated for: shoulder and elbow joint (Fig. 1), carpal and metacarpophalangeal (Fig. 2), hip and stifle joint (Fig. 3), tarsal and metatarsophalangeal joint (Fig. 4).

IRBIS 3 Professional software (InfraTec, Dresden, Germany) was used to calculate mean temperature in all ROIs (regions of interest). The ambient temperature and humidity inside and outside the stable were measured using a handheld HM 34 Humidity and Temperature Meter (Vaisala HUMICAP®, Finland).

Statistical analysis

Simple two-group t-tests were used to confirm that there were no significant differences between the measurement sides for each measurement obtained as was suggested in previous papers (Palmer 1983, Soroko et al. 2017b). The measurements from the left and right sides of each horse therefore were averaged.

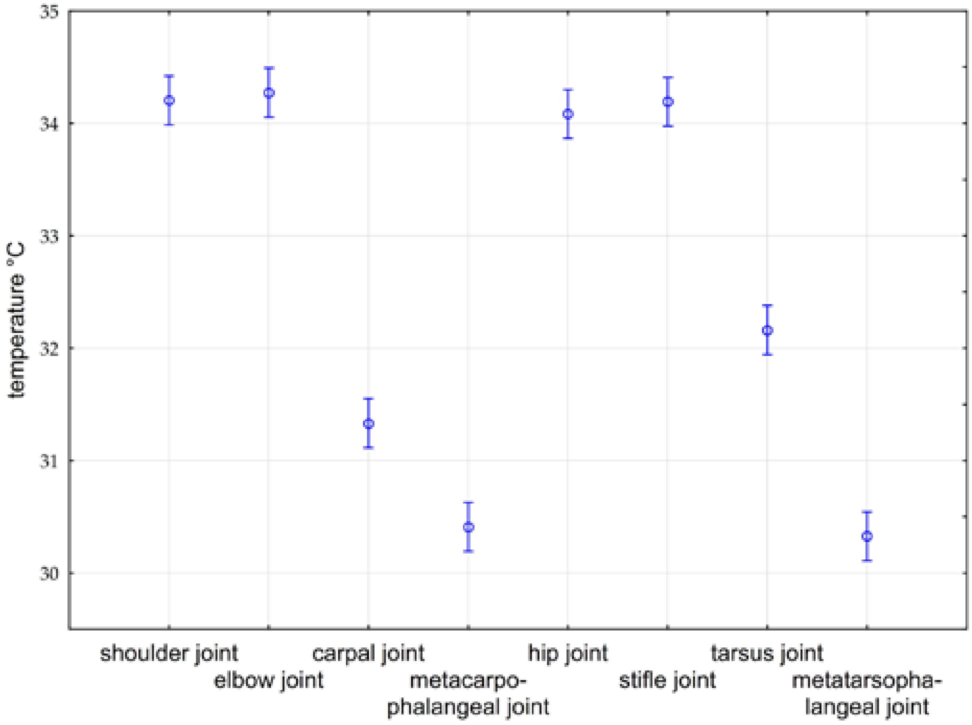


Fig. 5. Surface temperature over each joint. Data shown are mean ± SD from all horses, including both before and after training measurements ($p<0.001$).

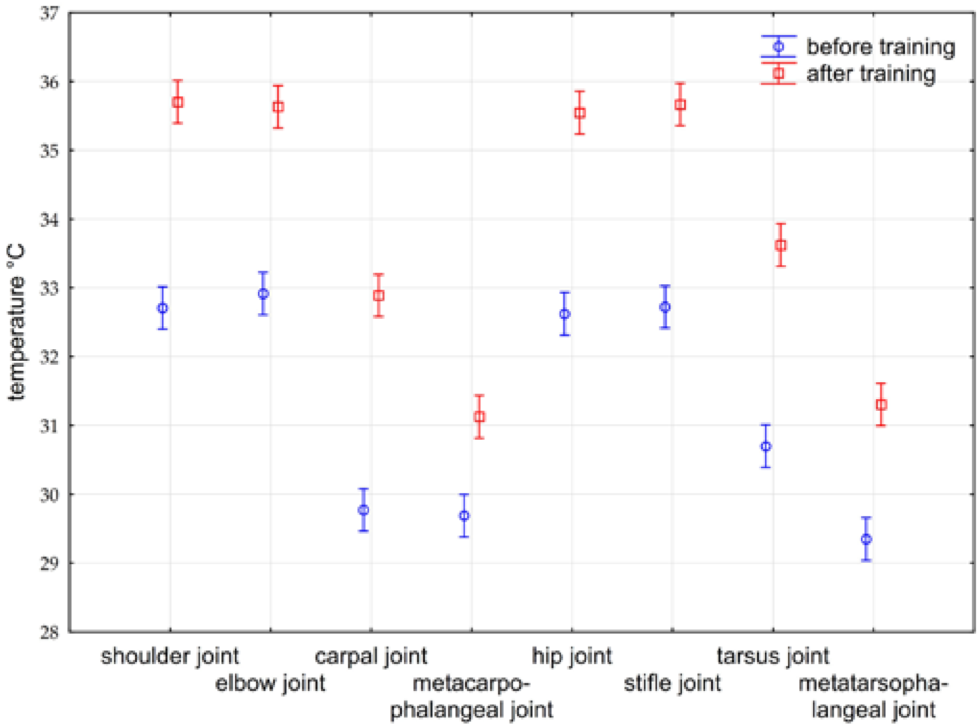
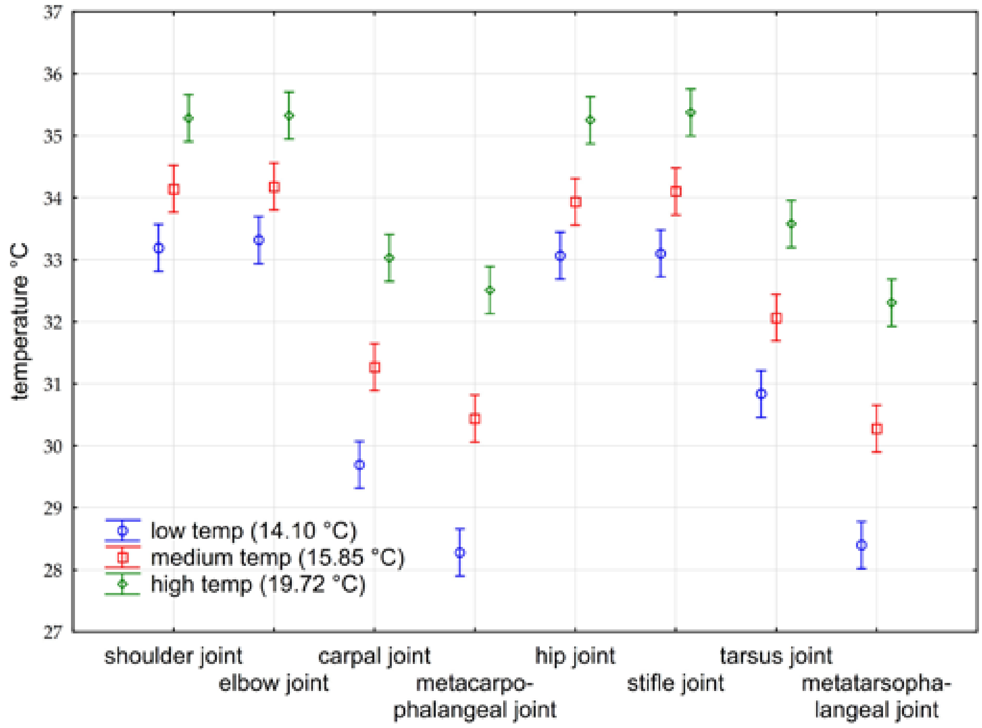


Fig. 6. Surface temperature overlying individual joints before and after training, showing a statistically significant interaction effect. Data shown are mean ± SD from all horses ($p<0.001$).

As ambient temperatures were measured for entire sessions, they were treated as an between-subject factor. In order to simplify the research plan, a few trial ANOVA models were made in which were checked whether it was possible to reduce six sessions due to the lack of differences in the results between some sessions. This preliminary analysis of ambient temperature differences indicated that sessions: first and second, and sessions three, four and five did not significantly differentiate results obtained, therefore were merged (sixth



Fig, 7. Surface temperature overlying the joints at different environmental temperatures, showing a statistically significant interaction effect. Data shown are mean ± SD from all horses, including both before and after training measurements (p<0.001).

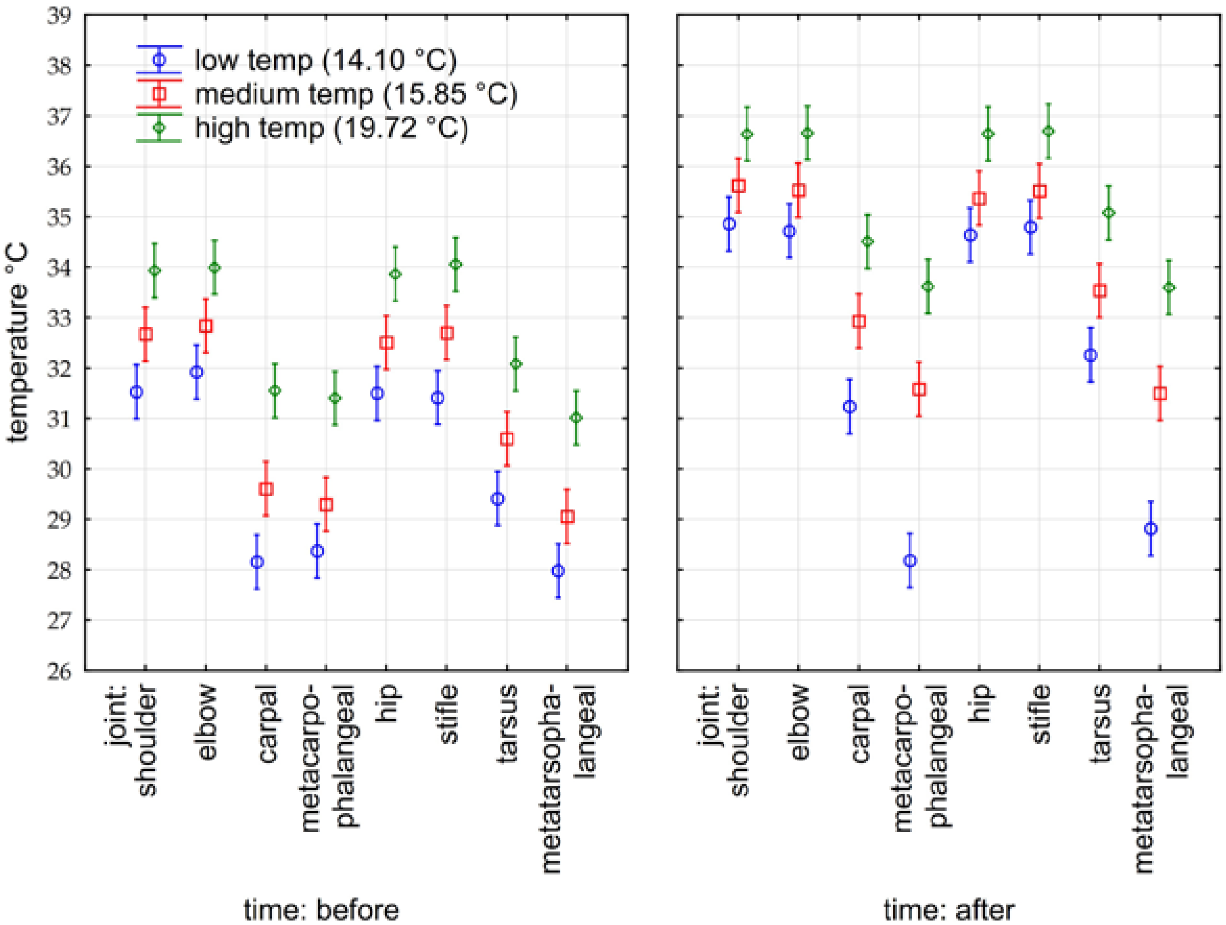


Fig. 8. The influence of joint, training and environmental temperature on surface temperature at each region of interest (ROI). Data shown are mean ± SD from all horses (p<0.001).

Table 1. Average ambient temperature (inside and outside) during measurements in sessions 1-6.

Ambient temperature	Session1	Session2	Session3	Sesson4	Sesson5	Session6
inside	20.9°C	17.5°C	20.7°C	21.9°C	22.9°C	17.1°C
outside	16.0°C	15.7°C	17.6°C	19.6°C	22.0°C	14.1°C

session was significantly different from any other) (Table 1). Sessions were divided into three groups of mean outside ambient temperature: low (sixth session, 14.1°C) vs medium (first and second sessions, mean 15.8°C) vs high temperature (third, fourth and fifth sessions, mean 19.7°C). For each session group (outside ambient temperature threshold level), the results were averaged. The model used was 2 (time) x 8 (joints) x 3 (outside temperature), in mixed ANOVA design. For the joint variable ($\chi2(27) = 290.8$, $p<0.001$), and time-joint interaction ($\chi2(27) = 255.3$, $p<0.001$) the tests were statistically significant. The statistics were calculated with corrections for degrees of freedom. Due to the epsilon values $\varepsilon = 0.28$ and $\varepsilon = 0.3$, the Greenhouse-Geisser correction was chosen (Field 2009).

Results

The main effect of time (before and after training), was statistically significant, $F(1.00, 39.0) = 404.6$, $p<0.001$, $\eta^2 = 0.91$. Horses before training (31.3°C ± 1.9°C) were characterized by a significantly lower surface temperature over the joints than after training (33.9 ± 2.6°C). Outside temperature also had a statistically significant effect, $F(2, 39) = 65.0$, $p<0.001$, $\eta^2 = 0.7$. At the lowest outside temperature (14.1°C) (31.2°C ± 2.9°C) horses demonstrated significantly lower surface temperature over the joints than at moderate (15.8°C) (32.5 ± 2.2) and high outside temperatures (19.7°C) (34 ± 1.9); these two last also differed from each other (all differences significant at $p<0.001$ level).

The main effect of the horse joint was also statistically significant, $F(2.00, 77.9) = 428.9$, $p<0.001$, $\eta^2 = 0.8$. The trends presented in Fig. 5 can be described as follows: the lowest (around 30°C) and almost the same temperature were observed for the metacarpophalangeal joint and metatarsophalangeal joint (there were no statistical differences between the two joints); then higher temperature for carpal and tarsus joints, which were statistically different from each other $p<0.001$; the highest, but comparable temperature (above 34°C) was observed for the four joints: shoulder, elbow, hip and stifle, between which there were no significant differences.

The interaction effect of time and outside ambient temperature was not statistically significant, $F(2.00, 39.00) = 0.79$, $p = 0.462$, $\eta^2 = 0.00$. The interaction effect of time and joint factors was statistically significant: $F(2.12, 82.81) = 21.76$, $p<0.001$, $\eta^2 = 0.28$. The increase in joint ROI temperature after training was similar for all joints, with the exception of the metacarpophalangeal and metatarsophalangeal joint, which was a smaller increase (Fig. 6).

The interaction effect of joint and outside ambient temperature factors was also statistically significant, $F(4.00, 77.92) = 8.79$, $p<0.001$, $\eta^2 = 0.04$ (Fig. 7). The temperature over each joint increased with rising environmental temperature. This effect was strongest at the metatarsophalangeal joint, but much weaker at the shoulder, elbow, hip and stifle joints.

The interaction effect of all factors also proved to be statistically significant, $F(4.25, 82.81) = 8.43$, $p < 0.001$, $\eta^2 = 0.22$ (Fig. 8). It can be seen that, at "low" environmental temperatures, the metacarpophalangeal joint did not warm during training. A similar relationship can also be seen in the case of the metatarsophalangeal joint, but at a "medium" environmental temperature the increase in temperature in response to exercise was almost the same as in other joints.

Discussion

Our study investigated the influence of physical exercise on body surface temperature over the joints in healthy racehorses. After training, the surface temperatures of joints were significantly higher than before training. Increases of body surface temperature in response to exercise are indirect markers of the underlying heat produced by muscle contraction, metabolic activity and alterations in blood flow. These occur in order to meet the oxygen demand of the working tissues (Yarnell et al. 2014). Temperature distribution is strongly correlated with muscle and vein distribution. Joint surface temperature in the proximal part of the body is lower compared with surrounding structures, because of synovial fluid and cover by subcutaneous fat (Turner 1996). The stifle joint, however, can be warmer than surrounding tissue due to its proximity to major blood vessels. In contrast, body areas with muscle tissues which have a rich blood supply (e.g. around the neck, upper forelimbs and hindlimbs) have a higher surface temperature compared to less muscular areas (e.g.

around the forearm and gaskin) or areas with no muscle (e.g. the distal forelimbs, hindlimbs) (Palmer 1983). The establishment of a uniform, reliable and repeatable temperature pattern across the back, neck, shoulders, chest and croup is difficult due to the presence of the muscle tissue, which is regulated by physiological mechanisms and influenced by type of exercise or performance (Soroko et al. 2018). The consequence of increased perfusion in the muscle tissue was noted by Simon et al. (2006), who evaluated the activity of limb muscles during training on a treadmill. It was found that body surface temperature of the proximal and distal forelimbs and hindlimbs was significantly higher immediately after exercise, and 5 and 15 minutes after exercise, while no statistically significant differences were noted 45 minutes after the end of the training. Areas with high muscle mass reduced temperature after training faster than less muscular areas, because of blood flow differences.

The utility of IRT for detecting changes in body surface temperature has been previously examined by Turner et al. (2001) where Thoroughbred horses were evaluated in training for 10 weeks. Similar results were found in the study of Purohit and McCoy (1980), where the surface temperature of the distal limbs increased significantly after training, with a high degree of symmetry in temperature distribution between contralateral parts of the body.

A series of studies in Poland further examined the effect of exercise on the body surface temperature in horses (Jodkowska and Dudek 2000, Jodkowska 2005). The surface temperature distribution of the forelimbs and hindlimbs was evaluated. The temperature of the limb surfaces increased significantly with physical effort, and it was shown that surface temperature depended on the type of exercise, and was considerably higher in forelimbs in comparison to hindlimbs (Jodkowska et al. 2001).

Our present study indicated that surface temperature over the joints in the proximal part of the body (shoulder, elbow, hip and stifle) were warmer (above 34°C), compared to the surface temperature at the distal joints (carpal and tarsus joint, and metacarpophalangeal and metatarsophalangeal joint), where the lowest temperature (around 30°C) were recorded. There were no differences between body surface temperature over the metacarpophalangeal and metatarsophalangeal joint of the forelimb and hindlimb. Similarity in temperature between the metacarpophalangeal and metatarsophalangeal joints was also found in our previous study (Soroko et al. 2017b).

Increased or decreased blood flow in the distal limbs can be most reliably detected by IRT when viewing the major blood vessels from the lateral and medial aspects. The distribution of the body surface temperature depends on thermoregulatory processes, is marked by a considerable inter-individual variability and is affected by environmental conditions (Mogg and Pollitt 1992, Soroko et al. 2017a, b).

Our data confirmed that ambient temperature had a statistically significant influence on body surface temperature. Horses trained at lower ambient temperature (14.1°C), had significantly lower joint temperature than at medium (mean 15.8°C) and high temperature (mean 19.7°C). This is in broad agreement with data from previous studies (Jodkowska 2005, Soroko et al. 2014, 2017a, b). In future studies it will be interesting to consider a greater environmental temperature variation and obtain a more precise determination of the relationship between environmental and joint surface temperature.

Our data also clearly indicated that the metacarpophalangeal and metatarsophalangeal joint surface temperature warmed up the least during training, but at the same time their temperature was the most sensitive to ambient temperature. The issue of increased temperature of the metacarpophalangeal and metatarsophalangeal joint was discussed in the study by Prochno et al. (2020), where both joints showed temperature differences even in the early stages of training, but this was not accompanied by clinical signs of inflammation. According to some authors, this may be related to the fact that the metacarpophalangeal/metatarsophalangeal joint undergoes the greatest adaptive changes during training, in the palmar/plantar region of the third metacarpal/metatarsal bone (Gaschen and Burba 2012), especially predisposing these structures to injuries or fractures (Whitton et al. 2010).

IRT is a useful diagnostic tool in monitoring blood perfusion after exercise (Dyson et al, 2001). In our study, surface temperature of all measured joints significantly increased after training. The surface temperature increments occurred in a similar trend over all joints, suggesting a physiological adaptation to regular exercise. The latest research by Prochno et al. (2020) used thermography to evaluate musculoskeletal adaption to initial training of two year old Thoroughbreds. Images taken at rest clearly indicated an increase in body surface temperature at the last thermographic evaluation, compared with those at the beginning of the study. The increase in temperature was not linear throughout the training period, however changes were more gradual in the trot and canter phase than in the previous stages of training. In contrast, our study indicated temperature changes to constant exercises, where the major effect on body surface changes had ambient temperature.

Nonetheless, it is important to remember that IRT is strongly influenced by external environmental factors, which can create misleading results. Also, horses demonstrate considerable variability in their individual body surface temperature, which is also changing during the day as a result of physical variations like vascular tone, and metabolism (Soroko et al. 2017b).

Regular exercise caused an increment of surface temperature over the joints in healthy horses. We showed that surface temperature over the joints in the proximal part of the body (shoulder, elbow, hip, stifle) was higher than the surface temperature over the joints in the distal part of the limbs (carpal and tarsus joint, metacarpophalangeal/metatarsophalangeal joint of the forelimb and hindlimb). Ambient temperature had a significant influence on surface temperature over the joints. The metacarpophalangeal and metatarsophalangeal joints warmed the least during training, but were influenced the most by differences in ambient temperature. The surface temperature differences before and after training may be an important indicator of the of the thermoregulatory response to exercise in racing horses. Understanding surface temperature changes in response to regular training is necessary for future studies on diagnosing injuries of joints in young racehorses.

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