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Original article

Relationship between maximum eye temperature and plasma cortisol concentration in racehorses during intensive training

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Abstract

The aim of the study was to determine the utility of maximum eye temperature measured by infrared thermography (IRT) as a stress indicator compared with plasma cortisol concentration in Thoroughbred and Arabian racehorses. The study included thirty racehorses undergoing standard training for racing. Measurements of maximum eye temperature and blood collection for plasma cortisol concentration were carried out before training (BT), and within 5 (5AT) and 120 minutes (120AT) after the end of the each training session in three repetitions, with a monthly interval. Both parameters were elevated at 5AT compared to BT ($p<0.001$). Compared to BT, at 120AT the maximum eye temperature remained elevated ($p<0.001$) and plasma cortisol concentration decreased ($p<0.001$). The study indicated significant weak correlations ($r=0.220$; $p<0.001$) between both measurements at all time points. The results support the use of IRT technique to monitor the response of horses to stress, potentially improving animal management and welfare.

Key words: racehorses, stress, eye temperature, cortisol, training

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Introduction

In the recent years, a lot of research has been done on the factors that contribute to stress in performance horses. Studies have indicated that competition (Valera et al. 2012, Becker-Birck et al. 2013), training practice (Christensen et al. 2014) and intensive training (Schmidt et al. 2010) are potential stressors for horses. In order to determine or partially reduce the level of stress to which animals are exposed, reliable methods of measuring the strength of stress stimuli are needed.

The assessment of the stress hormone cortisol is currently a well-accepted indicator for the analysis of adrenocortical responses to environmental conditions (Mormède et al. 2007). Plasma cortisol concentration has been found to be increased in response to training in different western riding events (Fazio et al. 2006), and after race training of Arabian horses (Kędzierski et al. 2014). However, blood sampling for cortisol level analysis often requires the horse to be restrained and may itself potentially confound results (Stewart et al. 2005). Therefore, there is a need to develop non-invasive stress assessment methods.

Infrared thermography (IRT) has been employed to non-invasively measure maximum surface temperature around the posterior border of the eyelid and the caruncula lacrimalis in horses (Valera et al. 2012). This measurement is termed commonly maximum eye temperature (Tmax), and reflects changes in peripheral blood flow as a result of vasoconstriction or dilation, caused by activation of the sympathetic nervous system in response to stress (Stewart et al. 2007, Valera et al. 2012, Soroko et al. 2016). A recent study correlated Tmax with salivary cortisol concentration (Valera et al. 2012, Soroko et al. 2016). However, bearing in mind the published data on plasma cortisol concentration as the preferred indicator of stress levels, it is necessary to investigate whether there is such correlation with cortisol levels in the blood of the racehorses. Therefore, the aim of our study was to determine the effect of physical training of racehorses on Tmax, and to correlate these results with plasma cortisol concentration.

Materials and Methods

All experimental procedures were approved by the Local Ethics Committee (Krakow, Poland, resolution no. 62/2020). Thirty racing horses of two breeds (19 Arabian Horses – [aged 3-5 years, 7 mares, 11 stallions, 1 gelding] and 11 Thoroughbred [all 3 years old, 5 mares, 6 stallions]) were assigned to the study. Horses were clinically healthy with no signs of illness or lameness, and without stereotypical behaviour. Animals were housed in individual stalls with *ad libitum* access to water and salt blocks. The study took place at Partynice Racetrack (Wroclaw, Poland) from April to July 2020, when horses were in regular training for the racing season. Everyday training (Monday to Saturday) of horses consisted of initial warm-up in walker (walk and trot for 20 min) followed by the training with riders: approximately 5 min walk, then trot, canter and gallop for a 10 to 20 min depending on training program for each breed. Throughout the whole research period horses were ridden by their usual riders, and trained by the same trainers.

Horses were fed three times a day at 05.30 a.m., 12.30 p.m. and 6.30 p.m. Diets prepared individually for each horse met Nutrient Requirements of Horses (2007) and consisted of meadow hay, concentrate mixture (oats grain with muesli “Livery Mix”, Saracen, United Kingdom) and vegetable oil (0.5 ml/kg of body weight).

Experiment lasted for 74 days, with 14 days of adaptation period. Horses underwent physical efficiency test (PET) together with thermographic examination and blood sampling at day 0, 43 and 73 of study. On these days horses were trained by the same riders on their regular track. The PET included a warm-up (walk and trot in walker for 20 min), canter (with riders) at distance 2.200 m and cooling down in an automatic horse walker for 20 min.

Thermographic examination and blood sampling was performed thrice on each PET day: before training (BT, between 6.00 a.m. and 7.00 a.m. when horses were at rest), within 5 minutes after the end of training (5AT) and 120 min after training (120AT), when the horses were resting in the stable.

Images of the left eye were captured as described previously by Soroko et al. [2016], using a VarioCam hr Resolution infrared camera (uncooled microbolometer focal plane array, resolution 640 x 480 pixels, spectral range 7.5 - 14 μm, InfraTec, Dresden, Germany). To minimize the effect of environmental factors, thermography was always performed in the horse’s stall where ambient humidity and temperature was also measured (Soroko et al. 2019). The distance of the animal from the camera was fixed for all imaging at 1m, at an angle of 90° to the head, and the emissivity (ε) was set to 1 for all readings. The ambient temperature in the stable was measured with a TES 1314 thermometer (TES, Taipei, Taiwan). As previously described (Valera et al. 2012, Soroko et al. 2016), Tmax was measured by identifying the peak pixel temperature from a region of interest at the lacrimal caruncle. Data analysis was performed with IRBIS 3 Professional software (InfraTec, Dresden, Germany).

Blood for plasma cortisol concentration (PCC) measurements was taken from the jugular vein, imme-

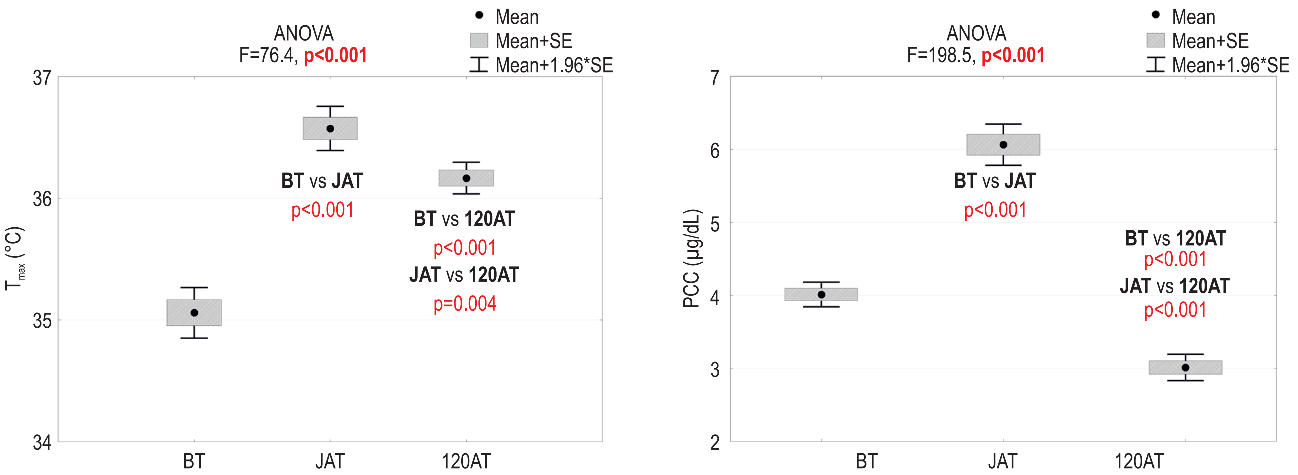

Fig 1. Maximum eye temperature (T_{max}) and plasma cortisol concentration (PCC) before training (BT), immediately after training (5AT) and 2 hours after training (120AT) (mean of three measurement sessions) and results of analysis of variance (one-way ANOVA and Friedman ANOVA) and multiple comparisons with tests post-hoc (Tukey HSD and Dunn's test).

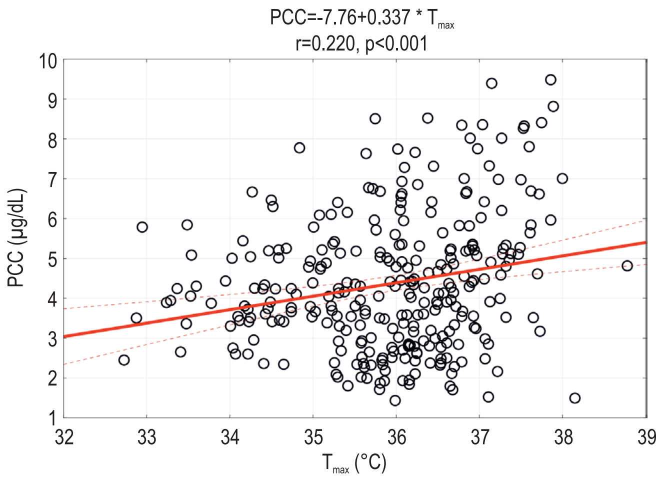

Fig. 2. Correlation diagram between maximum eye temperature (T_{max}) and plasma cortisol concentration (PCC), mean of three measurement sessions (BT, 5AT and 120AT).

diately after each thermographic examination. The horses tolerated these procedures without resistance. Blood for serum samples (4.5 ml) was collected into tubes containing coagulation activator (Sarstedt, Nümbrecht, Germany) and was immediately sent to the commercial laboratory (Vetlab, Wroclaw, Poland). Cortisol analysis was performed using the chemiluminescence method (IMMULITE 2000 XPi Immunoassay System) with IMMULITE® 2000 Cortisol kit (Ref. no. L2KCO2) and Immulite 2000 XPi automat (Siemens Healthcare, Warsaw, Poland).

Data were analyzed using Statistica v.13.3 (TIBCO Software Inc.). The normality of data distribution was tested using the Shapiro-Wilk test. Data on T_{max} with a normal distribution were analyzed using analysis of variance (one-way ANOVA), and for multiple comparisons (post hoc tests) using the Tukey test (Tukey honest significant difference). Data on PCC, that was not normally distributed was analyzed using non-parametric Friedman test (Friedman ANOVA), and for multiple comparisons (post hoc tests) by Dunn's test. Differences between means were considered significant at $p<0.05$. Data are presented as means and standard error of the mean (SEM).

Results

Considering the mean of all three measurement sessions, T_{max} at BT was significantly lower than at 5AT ($p<0.001$) and at 120AT ($p=0.04$). T_{max} was also significantly lower at 120AT compared to the value measured at 5AT (Fig. 1). Considering the median of all three measurement sessions, PCC before training was significantly lower than at 5AT ($p<0.001$). There was a significant correlation between T_{max} and PCC at BT, at 5AT and at 120AT in all sessions (Fig. 2).

Discussion

Our study indicated statistically significant differences for T_{max} and PCC before and after training. Both measured parameters were significantly elevated at 5AT. At 120AT, the PCC level decreased below pretraining levels, whereas maximum eye temperature remained elevated. These results are in agreement with previous findings from racing and sport horses (Valera et al. 2012, Soroko et al. 2016, Redaelli et al. 2019).

Interestingly, throughout the three-month period of the study, there was a significant increase in T_{max}, whereas PCC had consistent values throughout the whole study period. This temperature trend was probably influenced by environmental factors, especially ambient temperature, which increased progressively about 5 ºC across the three research periods (from April to June). A previous study confirmed that surface temperature is strongly influenced by ambient temperature (Soroko et al. 2017).

Our finding that cortisol concentration level was at its highest just after training has also been observed in other studies of eventing horses (Fazio et al. 2008) and jumping and dressage horses (Cayado et al. 2006). Changes of cortisol level could also be influenced by circadian rhythm, with peak levels in the morning falling throughout the day (Irvine and Alexander 1994).

Our data showed a significant correlation between T_{max} and PCC, suggesting that both techniques measure a physiological response to external stressors. Of note, a previous study of ours on racing horses subjected to intensive training did not find any correlation between T_{max} and saliva cortisol concentration (Soroko et al. 2016), and the same findings have been reported in competition horses (Valera et al. 2012). A study based on endurance horses did not indicate any correlation between blood cortisol concentration and eye temperature (Redaelli et al. 2019). Cook et al. (2001) found a correlation between plasma cortisol and eye temperature, but this was in response to ACTH injection as the stressor. Therefore, the relationship between eye temperature and cortisol level seems to be complex, and the results can be influenced by different factors such as changes of PCC due to circadian rhythm (Irvine and Alexander 1994) and basal values of PCC differing greatly between individual horses.

The main limitation of our study is the variety of age and level of performance of the horses. Considering previous results, age and performance level had significant influence on the stress responses in sport horses (Valera et al. 2012, Becker-Birck et al. 2013).

The significant correlation found between eye temperature and plasma cortisol concentration supports the potential use of IRT to monitor the response of horses to stress situations. IRT can provide real-time information that could be used to improve animal management and welfare. Further investigations on eye temperature assessment using IRT in racehorses are required, with careful monitoring of environmental conditions. These studies should be conducted on a larger number of horses, and during racing, to confirm our preliminary findings.

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