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Original Research

Salivary Cortisol Concentration in Exercised Thoroughbred Horses

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ABSTRACT

Both physical activity and stress result in an increase in plasma cortisol level. The measurement of cortisol in plasma requires taking blood samples, which is stressful itself. Therefore, the aim of this study was to evaluate the use of saliva sampling for the determination of cortisol concentrations, indicating the intensity of exercise in horses during race training. Twelve Thoroughbred horses aged 2-3 years were examined during their speed training sessions. The horses galloped on the 1,200-m sand track at a speed of 14.4-15.3 m/s. Three saliva samples and three blood samples were collected from each horse. Both types of samples were taken when the horse was at rest, immediately after returning from the track and 30 minutes after the end of exercise. Blood lactic acid (LA) concentration was determined using the enzymatic cuvette test. The concentrations of cortisol in saliva and plasma samples were measured by enzyme immunoassay methods. Statistically significant correlations were found between salivary cortisol level determined 30 minutes after the end of exercise and blood LA concentration obtained immediately after exercise ($P = .003$) and between salivary and plasma cortisol levels measured 30 minutes after the end of training session ($P = .015$). The measurement of cortisol concentration in saliva samples taken from race horses 30 minutes after the end of exercise can be recommended for use in practice under field conditions to estimate the level of relative intensity of exercise in race horses.

1. Introduction

Horses that are subjected to training are exposed to various potentially stressful situations like transport [1-3], change of residence [4,5], work under human direction [6], physical exercise [7], participation in competitions [8], and veterinary procedures [9]. All these stimuli can result in emotional excitation that increases heart rate and cortisol release [2,3,6,7,9]. Blood cortisol concentration can reflect not only mental stress level [8,10] but also the physiological reaction to various types of exertion. It has been reported that the cortisol release rate depends on the duration rather than the intensity of exercise [7,11,12].

Blood plasma cortisol concentrations partially correlate with those in saliva, and across the diurnal rhythm [13]. Cortisol, like all steroids, is poorly soluble in water. In blood, steroids are bound mainly with plasma protein carriers, and only 2%-15% of cortisol is free and biologically active [14]. This non–protein-bound fraction of cortisol can reach other body fluids like saliva. Therefore, salivary cortisol concentration represents this free and biologically active fraction. The high correlation coefficient found between blood and salivary cortisol concentrations in horses supports saliva sampling as a useful technique for the measurement of cortisol release [15,16]. Salivary cortisol level was successfully used as the indicator of stress levels in horses during road transport [2,3,17], initial training [6], and competition

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in various equestrian disciplines [18-20]. To the best of our knowledge, race horses have never been examined in this context. During race training, horses are subjected to anaerobic, short, but very intensive exertion. This type of effort has been reported to produce an increase in cortisol level, measured in blood plasma [21]. On the other hand, cortisol release is a time-dependent process; it takes almost 20 minutes to reach peak values in plasma after the stress stimulation [22]. Moreover, the maximum cortisol concentration in saliva appears later than in blood [16].

Therefore, the aim of this study was to compare a salivary cortisol concentration with blood lactic acid (LA; routinely used for the evaluation of the reaction for anaerobic effort) and plasma cortisol levels in Thoroughbred horses during race training session.

2. Materials and Methods

2.1. Horses

This study was performed in Poland at the Sluzeviec (Warsaw) racetrack. The group of 12 Thoroughbred horses, aged 2-3 years old, consisted of an equal number of stallions and mares and was examined in August. The horses were maintained in one stable and trained by one trainer. They were fed with typical fodder used as a standard diet for race horses. Prior to the study, the horses were trained 5 days a week. All the horses competed in official races held from May to July.

The study was accepted by the Local Ethics Review Committee for Animal Experimentation and conducted according to the European Community regulations concerning the protection rules of experimental animals.

2.2. Exercise Protocol

The horses were examined during their training session, which was a part of the routine race training. They were in the last phase of preparation for a start in races. On the day of the study, the training session was performed in the morning, from 7 AM-9 AM. Mares were exercised first, then stallions. All horses had a 10-minute warm-up trot with a rider. Then they galloped on the 1,200-m sand track at a speed of 14.4-15.3 m/s. Finally, they returned, trotting to the stable. After being unsaddled, the horses were cooled on an automatic horse walker for approximately 20 minutes.

Three saliva samples and three blood samples were collected from each horse. All horses were sampled by the same operators: one person sampled saliva and the other one collected blood samples. Both types of samples were taken according to the following protocol: 1) at rest, 2) immediately after return from the track, and 3) 30 minutes after the end of exercise. In each case, saliva was sampled first and then blood was taken. The saliva samples were collected with a small piece of sponge which was inserted into the horse's mouth, and then, after soaking in saliva, it was placed in a plastic tube as described previously [19]. Blood samples were collected by jugular venipuncture into EDTA K3 tubes. Blood LA concentrations were determined immediately using enzymatic test kit (Dr. Lange, Berlin, Germany) and expressed in mmol/L. The remaining blood was immediately centrifuged at 2,000 $\times$ g for 10 minutes, and plasma was stored at -20°C until assayed.

2.3. Laboratory Analysis

Before laboratory analysis, the saliva samples were warmed to room temperature and centrifuged at 500 $\times$ g for 15 minutes at room temperature. Next, the sponge with the straw was removed, and the saliva was transferred to test tubes. The concentrations of cortisol in saliva samples were measured by the enzyme-immunoassay (EIA) method using the cortisol EIA kit (Diagnostic System Laboratories Inc., Webster, USA). For plasma cortisol determination, cortisol enzyme-linked immunosorbent assay kit (DRG Instruments GmbH, Marburg, Germany) was used. All samples were analyzed in duplicate. Absorbance was measured by using a Multiscan reader (Labsystem, Helsinki, Finland) using Genesis V version 3.00 software. The intra- and interassay coefficients of variation (CV) for salivary cortisol determined in the laboratory amounted to 9% and 11%, respectively, and 5% and 8%, respectively, for plasma cortisol concentration. The results were expressed in nmol/L.

2.4. Statistical Analysis

The results are presented as means $\pm$ SD. Statistical analyses were performed using Statistica version 6.0 software. Comparisons between the results obtained before the training session, immediately after training, and 30 minutes after the end of exercise in the blood and saliva samples were made by the Tukey test (analysis of variance). The coefficient of correlation was assessed by Pearson test. The statistical significance was accepted at a P value of $<.05$.

3. Results

The results of LA and cortisol measurements are presented in Table 1. Blood LA concentration increased significantly after exercise ($P < .001$). The mean value of this parameter determined after 30 minutes of restoration was lower than the results achieved immediately after exercise ($P < .001$) but did not reach the levels at rest ($P = .011$). Plasma cortisol level increased significantly after exercise ($P = .008$). The results obtained 30 minutes after exercise did not differ significantly from the values measured at rest and immediately after exercise. Salivary cortisol concentrations determined at rest, immediately after exercise, and after 30

Table 1
Mean $\pm$ SD blood lactic acid and plasma and salivary cortisol levels determined in Thoroughbred horses (n = 12) during training sessions

Sample	At Rest (R)	Immediately After Exercise (S)	30 min After Exercise (T)
Blood lactic acid (mmol/L)	0.86 ± 0.14^{a}	13.4 ± 3.36^{b}	2.89 ± 1.71^{c}
Plasma cortisol (nmol/L)	249 ± 83.4^{a}	335 ± 88.5^{b}	281 ± 78.7^{ab}
Salivary cortisol (nmol/L)	1.63 ± 2.09^{a}	2.57 ± 2.59^{a}	3.82 ± 2.55^{a}

R, samples taken at rest; S, immediately after the exercise; T, 30 min after the exercise.

[a, b, c]Mean values in rows with different superscripts differ significantly at $P < .05$.