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Effect of familiar and unfamiliar riders on Cortisol, Oxytocin and Beta–endorphin levels in horses

Efecto de los jinetes familiares y no familiares sobre los niveles de Cortisol, Oxitocina y beta–endorfina en caballos

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ABSTRACT

Stress response of the horse may be related to the behavioral and physiologic factors e.g., stress hormones. The aim of this study was to evaluate the different rider contact on the stress hormones of the horses that are used for javelin sport in a local riding club. Seven Arabian horses were ridden by familiar riders with the horses in the first and second weeks and by additional unfamiliar riders with the horses in the third and fourth weeks. Cortisol, oxytocin and beta–endorphin levels in sera samples and cortisol levels in saliva were measured before and after the riding. There was no statistical difference in serum cortisol and β–endorphin and salivary cortisol values between the groups with regard to the familiarity ($P>0.05$). Behavioral scoring did not differ between the groups. However, there was an increase in the oxytocin level of the horses ridden by the familiar riders in the second week compared with the first week ($P<0.05$). The horses did not respond stressfully associated with the hormone levels and behavioral changes; besides, they responded well to the familiar riders by elevating the oxytocin level. In conclusion, the horses used herein responded well to the familiar and unfamiliar riders with regard to potential stress factors. It has been therefore suggested that the familiarity towards the interaction between the human and the horse may not alter the physiological stress of the horses that are regularly ridden by various people in a riding club.

Key words: Horse; β–endorphin; cortisol; oxytocin; stress

RESUMEN

La respuesta al estrés del caballo puede estar relacionada con factores fisiológicos y de comportamiento, por ejemplo, las hormonas del estrés. El objetivo de este estudio fue evaluar los diferentes contactos de los jinetes sobre las hormonas del estrés de los caballos que se utilizan para el deporte de jabalina en un club de equitación local. Siete caballos árabes fueron montados por jinetes familiarizados con los caballos en la primera y segunda semanas y por jinetes desconocidos adicionales con los caballos en la tercera y cuarta semanas. Se midieron los niveles de cortisol, oxitocina y betaendorfina en muestras de suero y los niveles de cortisol en saliva antes y después de montar. No hubo diferencias estadísticas en los valores de cortisol sérico y β–endorfina y cortisol salival entre los grupos con respecto a la familiaridad ($P>0,05$). La puntuación conductual no difirió entre los grupos. Sin embargo, hubo un aumento en el nivel de oxitocina de los caballos montados por jinetes familiares en la segunda semana en comparación con la primera semana ($P<0,05$). Los caballos no respondieron al estrés asociado con los niveles hormonales y los cambios de comportamiento; Además, respondieron bien a los jinetes familiares elevando el nivel de oxitocina. En conclusión, los caballos utilizados aquí respondieron bien a los jinetes familiares y desconocidos con respecto a los posibles factores de estrés. Por lo tanto, se ha sugerido que la familiaridad con la interacción entre el ser humano y el caballo no puede alterar el estrés fisiológico de los caballos que regularmente son montados por varias personas en un club de equitación.

Palabras clave: Caballo; β–endorfina; cortisol; oxitocina; estrés

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INTRODUCTION

Since ancient times, the horse (*Equus caballus*) has been the closest animal to humans with many activities such as transport, chevalier and sports. Equestrian sport has been adopted as an ancestral sport in modern culture including physiotherapy and psychotherapy associated with the horse and human interaction. Currently, horses are used in the fields of racing, riding, and therapy (hippotherapy) as well as equestrian sports (javelin, etc.). Emotional or physical interaction depends on the relationship between the horse and the human. Although there are previous reports about the value of the horse for human health [1], there is limited information on the stress response and well-being of the horse. With the increased number of horses Equine-Assisted Activities and Therapies (EAAT) programs and the growing concern for animal welfare, it has become important to understand the effects of these programs on the stress levels, stress-related disorders, and quality of horse life. Also, stress in the horse can negatively affect the horse-rider couple and increase the risk of accidents. The measurement of pain and stress level as a result of stress exposure depends directly on observations with regard to behavioral and physiologic changes including circulating stress hormones. Behavioral and physiological indicators for assessing the emotional state and well-being of the horse should include both positive and negative outcomes [2]. Behavioral scoring is an objective, non-invasive and easy to assess for welfare in animals [3, 4].

Stress can be defined as a condition in which an animal "must make abnormal or extreme adjustments to its physiology or behavior to cope with the negative aspects of its environment and management" [5]. Responses to stressful stimuli involve behavioral changes, decreased immunity, and activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis and Autonomic Nervous System [6, 7]. Adrenocorticotropic hormone (ACTH) is released into the systemic circulation from the pituitary gland following the activation of HPA axis through hypothalamic integration induced by signals from the peripheral and central nervous system (CNS). Thus, ACTH further stimulates the synthesis and secretion of stress hormone cortisol from the adrenal gland [8]. Stress may cause daily changes in heart rates and endocrinological changes such as plasma cortisol, beta-endorphins, and catecholamines in racing or training horses [5] as well as in other animals under stress with metabolic adaptation [9] and cardiotoxicity [10]. Corticotropic hormones, serotonin and catecholamines are of the stress hormones in order to assess the level of response in the horse [11, 12]. Adrenal gland secretes catecholamines and glucocorticoid cortisol hormone after exposure of physical and/or psychological stressor. When stress is evaluated, it is more useful and appropriate to measure free cortisol rather than total cortisol in serum [13]. Since cortisol diffuses rapidly into saliva, salivary cortisol concentrations reflect changes in cortisol concentrations in blood plasma. Thus, salivary cortisol concentrations are used as an index of serum-free cortisol [14]. Blood cortisol secretion follows a circadian rhythm with the highest concentrations in the morning and lowest in the afternoon and evening [15, 16] in horses, as in humans, and other species [17]. Therefore, samples were taken at the same time of the day.

The activation of oxytocin neurons causes an increase in oxytocin secretion not only after reproductive stimuli but also after stressful stimuli [18]. Oxytocin can further modulate response of the body against stressful fear, panic, and extreme exercise. Besides, oxytocin is released during pleasant social activities and has antistress or antidepressant effects by reducing cortisol due to an increased parasympathetic function [19]. Previous studies in human-beings indicate that animal interaction stimulates the production of circulating oxytocin resulting in a calming effect and relaxation [20, 21].

Beta-endorphins, opiate-like peptides of pituitary origin, play roles in learning and memory, feeding behavior, thermoregulation, blood pressure regulation, and reproductive behavior [11, 21]. Beta endorphins can also modulate excitability of CNS by activating motor function and pain perception during exercise-induced catecholamine secretion and lactic acid accumulation [5]. The release of beta-endorphins into the blood is particularly evident in horses during stress reactions [22]. In the present study, behavioral scoring and the levels of cortisol, serum oxytocin and beta-endorphin levels in saliva and serum were investigated in the horses before and after riding to compare the weekly stress response in between the riders who were familiar with the horses and the additional riders who were not familiar with the horses.

MATERIALS AND METHODS

Animals

This study was approved by the Local Ethics Committee for Animal Experiments of Ataturk University (Protocol No.: 2020/170). The horses used in this study, with permission from their owners, were from a local private equestrian sports club where they were kept for sporting activities (e.g., javelin riding). The horse and human interaction during the sportive activity has been illustrated in FIG. 1. The study was conducted in 7 clinically healthy intact male 4-16-year-old Arabian horses. The weight of the horses ranged from 400 to 500 kg. All horses were fed with a standard horse ration in the same housing conditions. Clinical health status of the horses was evaluated by anamnesis and full clinical examinations of respiratory system, digestive system, circulatory system, lymph nodes, mucous membranes, secretion, and excretion findings and musculo-skeletal motor functions. Complete blood count was also performed to evaluate the hematological status of the horses. The horses that represent normal clinical and hematological findings were included in the study.

Behavioral assessment

The behavioral scoring system was selected based on the previous reports [3, 23, 24]. Parameters were determined as 1: no stress, 2: low stress, 3: medium stress and 4: severe stress, depending on the horse's general attitude, neck position, tail movements, ear and mouth position. On the days of the study, each horse was observed for 15 min during the preparation for riding, contact with the rider and going for a ride. After the ride, the horses were also observed for 15 min when they entered the barn.

Study design

All tests were carried out in the equestrian area where the horses are routinely housed at all times. The method of this study was a measurement design repeated at weekly intervals over four weeks on horses ridden at a slow walk by licensed riders on sampling days. The horse's gait is shown in FIG 2. On the remaining d of the week, the horses were allowed to continue their daily routine activities. On the sampling days in the first and second weeks, the horses were ridden for one-hour by two riders who were familiar with the horses, i.e., the people who had often ridden these horses. On the sampling days in the third and fourth weeks, the same horses were ridden for one-hour by two additional riders who were not familiar with the horses, i.e., the people who had never ridden these horses.

FIGURE 1. Illustrations for the horse and human interaction during javelin sport (A) and sportive activity (B). The illustrations have been provided here courtesy of the rider, the owner and the riding club

FIGURE 2. The horse's gait with the familiar rider

Sample Collection

Samples were taken gently by an experienced veterinarian without stressing the horse. To evaluate hematological status of the horses before the study initiation, blood was drawn using 18G needle from jugular vein into spray–coated vacutainers with anti–coagulant K2EDTA (Becton Dickinson Co., USA). Complete blood count was performed in a veterinary hematology analyzer (Abacus Junior Vet5, Diatron, Hungary) at the beginning of the study to check the health status of the animals. Sampling procedure of the repeated measurement was performed before and after the riding of all horses on the days of sampling weekly for four weeks. Weekly blood and saliva samples were obtained from the horses before contacting the riders (pre–riding) and just after the riding (post–riding) at the time period of the d (15:00 to 16:00) to account for the same daily rhythm of hormone levels [25]. The saliva samples were taken before blood sampling to refrain from possible effects of blood drawing. Saliva was sampled by using Salivettes (Sarstedt, Nümbrecht, Germany) without restraining the horse. A swab was held inside the mouth of the horse above and below the tongue for one minute with a metal clamp and inserted into the Salivates. The samples were immediately stored at +4°C during the activity and taken to the laboratory at the end of each study. The saliva samples were centrifuged at 1,500 G for 10 min and saliva was aspirated then placed in godets and stored at -20°C (Vestel, SC47011, Türkiye) until analysis [25, 26]. Blood was also drawn from jugular vein on the days of sampling (pre–riding and post–riding) into spray–coated silica vacutainers containing polymer gel without anti–coagulant (Becton Dickinson Co., USA) for hormone analysis to obtain serum samples weekly for four weeks after the collection of the saliva. Following coagulation, the blood samples was centrifuged at 4,000 G for 10 min (Beckman Coulter, Allegra X–30R, USA) and sera samples were allocated into sterile tubes and stored at -80°C until analysis.

Cortisol, β–endorphin and oxytocin analysis

Saliva and serum cortisol (Horse cortisol, BTLaB, China), serum β–endorphin (Horse Beta–Endorphin, BTLaB, China), and serum oxytocin (Horse Oxytocine, Sunred, China) hormone levels were measured by using horse–specific ELISA test kits according to the manufacturers' instructions.

Statistical analysis

Data comparison was made in a model to evaluate both effects of riding and period on hormone levels. The data showed normal distribution in the Shapiro–Wilk test. The Paired–samples T test and repeated measures design were used to compare the hormone levels in the SPSS 18 package software program for statistical analysis. Chi Square analysis was used to compare behavioral scoring between groups.

RESULTS AND DISCUSSION

The owners of the horses no complaint for the anamnesis of the horses. There were no abnormalities in physical examination findings with regard to full clinical examination of respiratory system, digestive system, circulatory system, lymph nodes, mucous membranes, secretion, and excretion findings and musculo-skeletal motor functions. Erythrocyte, leukocyte and platelet parameters were within normal ranges related with complete blood count analysis of the blood samples. FIG. 3 shows the blood serum level of oxytocin (A), cortisol (B) and β–endorphin (C) and saliva level of cortisol (D) at the pre–riding and post–riding time points. The levels of serum oxytocin

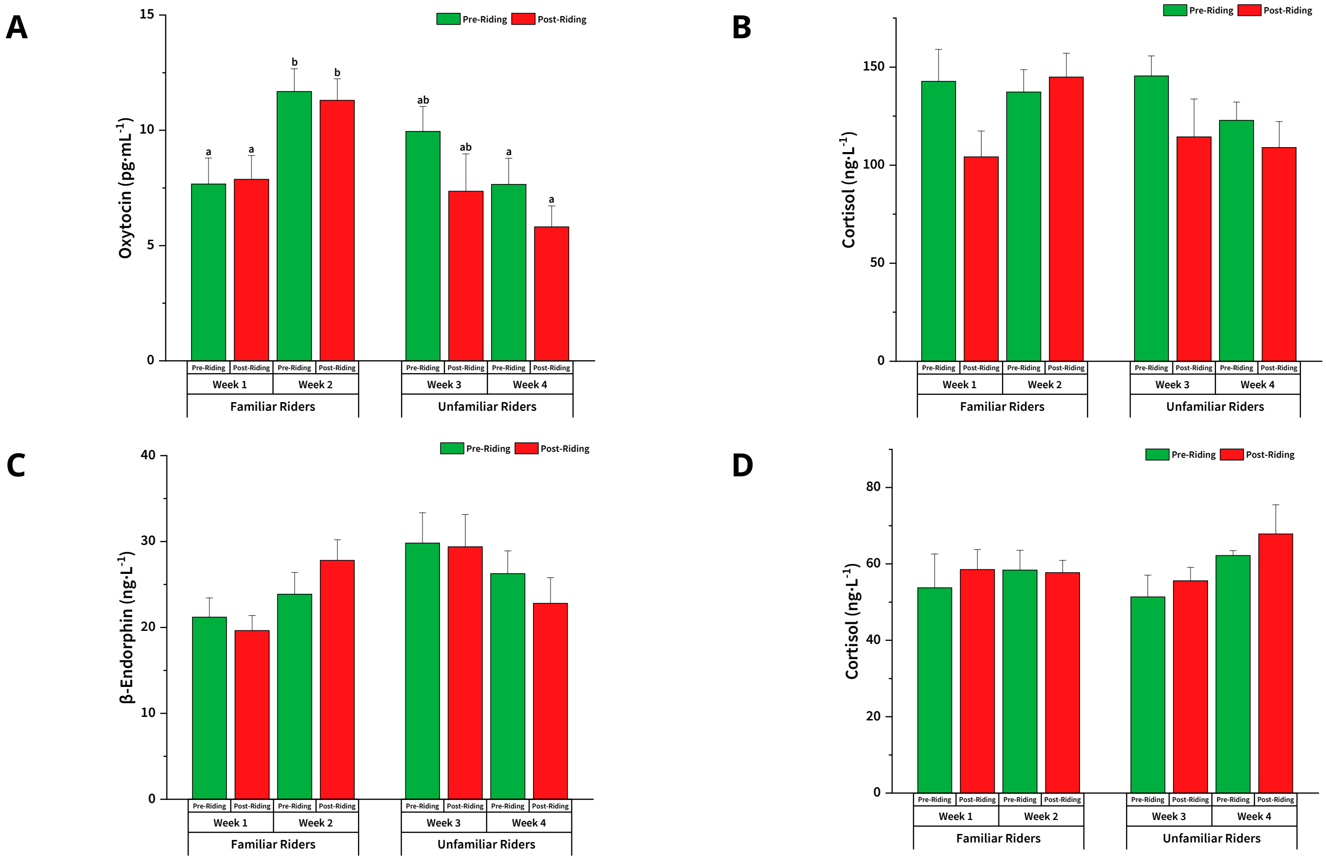

FIGURE 3. The pre–riding and post–riding levels of oxytocin (A), cortisol (B) and β–endorphin (C) in blood serum and levels of cortisol in saliva (D) of the horses (n=7) for the days with a one–week interval. Note that oxytocin levels are higher on the day of the second week than on the day of the first week in the horses ridden by familiar riders (*P*<0.05). Different letters in the same graph indicate a statistical difference (*P*<0.05) and no letters in the same graph indicate no statistical difference (*P*>0.05) between mean levels. The data are presented as mean ± standard deviation

hormone levels increased on the day in the second week at pre–riding and post–riding time points compared with the levels on the day in the first week (*P*<0.05) in the horses ridden by familiar riders. The increased oxytocin levels were gradually decreased on the fourth week (*P*<0.05) in the horses that were ridden by unfamiliar riders. There were no statistical changes for serum β–endorphin, serum cortisol, and salivary cortisol levels during the study (*P*>0.05) in the horses that were ridden by familiar riders for two weeks and by unfamiliar riders for additional two weeks. It was also noted that there was a tendency to decrease in cortisol level in saliva samples between pre–riding and post riding periods on the day in the first week (*P*=0.096).

The mean values of behavioral scoring were 1.6 ± 0.12 in the familiar rider group and 1.7 ± 0.18 in the unfamiliar rider group. There was no statistical difference in between behavioral stress scoring of the riding groups (*P*>0.05).

The stress response is defined by physiological changes to short – or long–term unpredictable changes in environmental conditions that cause a redirection of resources to vital processes and disrupt (or threaten to disrupt) homeostasis [27]. Stress response varies according to the stimulus causing special consequences. Responses to stimuli (e.g., stress) include behavioral and hormonal changes [28]. Beta–endorphin [29], ACTH, cortisol, total and free iodothyronines [11], or reproductive hormones and oxytocin are used to evaluate the response to the stress [19]. The horse should be refrained from the stress to perform better in the equestrian sport or not to harm the riders in EAAT studies. Stress may also affect the performance of the horse in javelin sport or any other competitions depending on the physiological and hormonal mechanisms. Thus, the results of this study evaluated the effect of the different rider factor on stress hormone levels and behavioral changes in the horses that are regularly ridden by various people in an equestrian club.

The measurement of pain and stress level as a result of stress exposure depends directly on observations with regard to behavioral and physiologic changes including circulating stress hormones in animals [2]. The previous assessments are most likely to have some misinterpretation due to an over – or under–measurement of the pain and stress [8]. Behavioral scoring is an objective, non–invasive and easy to assess for welfare in animals [3, 30]. In this study, there was no statistical difference in between behavioral stress scoring of the riding groups. Potier and Louzier [24] investigated the stress levels of eight healthy horses during hippotherapy in two riding sessions on separate days, one with disabled riders and one with beginners. They found no significant difference between stress scores as seen in this study. Hovey *et al.* [23] studied differences in behavior and serum cortisol concentrations in horses used in a therapeutic riding program. They reported that overall behaviour scores were relatively low in the horses in the two groups included in the study similarly.

In animal welfare studies, glucocorticoids are used widely as an animal–based welfare evaluation [19]. Cortisol measurement is an indicator of Hypothalamus–Pituitary–Adrenocortical Axis (HPA) activity, which reflects the physiological response to acute or prolonged stress [31, 32]. The release of cortisol is stimulated by the sympathetic nervous system, and cortisol maintains the homeostasis of biochemical processes by mobilizing energy during physical and psychological stress [33]. In horses, as in humans and other species, blood cortisol secretion is in a circadian rhythm, with the highest concentrations in the morning and lowest in the afternoon and evening [15]. The samples were therefore taken at the same time period in the afternoon to ensure uniformity and to prevent hormonal fluctuations in this study.

Serum cortisol concentration is used to evaluate the type of stress level in horses [14] associated with transport [34, 35, 36], rival [33], training and exercises [37, 38]. The serum cortisol level mainly increases during acute stress associated with the free cortisol level. Free cortisol measurement is more useful to evaluate the stress rather than total cortisol in serum [13, 32]. Salivary cortisol values have been shown to correspond to serum cortisol levels, and many studies to assess the acute stress response in horses measure salivary cortisol concentration [25]. Salivary cortisol, by the passive diffusion to the salivary glands, provides information about the free cortisol concentration [14]. It is widely used as a biomarker in mental, physical diseases or psychological stress [13].

Previous studies reported the association of blood and saliva cortisol concentrations suggesting the saliva samples can be used as a non–invasive technique for cortisol level in the horse [14]. Salivary cortisol concentrations have been previously measured in horses during therapeutic riding, conventional riding, and resting conditions, and no significant difference has been detected in delta cortisol values between riding conditions [39]. Smith *et al.* [40] measured blood cortisol values before and after transport in horses and reported that cortisol values increase after transport. Horses used in therapeutic riding have presented lower cortisol concentrations 5 and 30 min after the session, and the therapeutic riding session had minor effects on HPA response [41].

Arfuso *et al.* [42], investigated the effects of environmental temperature and work on cortisol in tourism carriage horses in Italy. They emphasized that there was no change in cortisol levels because the horses were used to the work they were doing. Cortisol has been evaluated as acute stress in this study. Similar to the study by Arfuso *et al.* [42] blood and saliva cortisol values did not change in between different rider groups before and after one–hour riding period suggesting that the horses regularly used for riding purposes with various people adopted themselves well to the stress factors arise from the rider contact in an equestrian sport facility.

Emotional contagion is also thought to occur between animals and humans, and horses are also thought to be sensitive to human emotions [43]. The activation of oxytocin neurons causes increased oxytocin secretion not only after reproductive stimuli but also after various stressful stimuli [18]. Recent studies have shown that oxytocin levels are associated with positive human–animal interactions [44]. Oxytocin released by positive social interaction causes anti–stress effects by reducing glucocorticoid stress hormones in humans and animals and is associated with increased parasympathetic function [19]. In a previous study, oxytocin level was found to be unchanged in the horses after the exposure to audio–visual and unfamiliar field stimuli [45]. The change in oxytocin value is difficult to determine due to the short half–life of oxytocin [19]. Indeed, plasma oxytocin level increases in humans and dogs and plasma cortisol decreases in humans with relational interaction [21].

In this study, oxytocin values have not been differed significantly except for the second week. Oxytocin values may not change with constant contact with humans because riding horses may clear it from the bloodstream within an hour of riding [19]. The increase on the second week is considered to be a neurophysiological consequence of relational behavior in the horse–human contact with the familiar riders.

The release of beta–endorphins from the pituitary gland in horses is synchronized with the first stage of the stress reaction, reducing the negative effects of cortisol on the organism [11, 21]. The release of beta–endorphins into the blood is a particular evident during stress reactions in the horse [22]. Potential physical and emotional stress factors might occur commonly in horses with the elevations of plasma beta–endorphin concentrations with severe abdominal pain [46]. However, horses with painful but chronic lameness have a plasma beta–endorphin concentration similar to that of normal horses. The similarity of beta–endorphin values of the horses before and after riding in the present study indicates the horses are not physically or emotionally under stress. The similarities during riding with familiar and unfamiliar riders may be related to the adapted horses accustoming to the riders. The similar stress parameters in present the horses might be the adaptation of the horses to the potential stressful factors caused by different rider contact.

CONCLUSIONS

Stress hormone levels of serum and saliva cortisol and serum beta–endorphin values in familiar and unfamiliar rider groups were found similar in the horses that are regularly ridden by different people in a javelin sport club conforming with the steady behavioral scoring. The increase in the oxytocin values on the second week in the horses ridden by the familiar riders could be related with the well response to the riders indicating positive social interaction and a familiarity of the people and the horses. Therefore, the riding the horses by familiar or unfamiliar certified horse riders may not cause physiological stress on the horses that are regularly ridden by different people for hobby and sportive activities.

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Authors' contributions

NU and OA contributed to the design and process of the study. NU, OA and SB were including sample collection and processing materials, and data analysis and presentation. All the authors made substantial inputs in drafting and editing the final manuscript. MI contributed to the biochemical analysis of the samples and OE contributed to the statistical analysis of the data. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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