

PLASMA CORTISOL LEVEL ATTRIBUTABLE TO PHYSICAL EXERCISE IN ENDURANCE HORSES

CONCENTRAÇÃO PLASMÁTICA DE CORTISOL DECORRENTE DO EXERCÍCIO FÍSICO EM CAVALOS DE ENDURO

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SUMMARY

The objective of this study was to establish a relationship between exercise and plasma cortisol levels in endurance horses. A race in which only experienced animals may run longer distances. Thirty male and female, Arabians and crossbred Arabian horses that compete in endurance races were used. They were divided into three groups of 10 animals each: G1, ran more than 100km; G2, ran less than 100km; and G3, disqualified due to metabolic problems. Plasma cortisol was quantified at three different moments: t0, the day before the competition; t1, 30 to 60 minutes after the end of the circuit; and t2, 90 to 120 minutes after the end of the circuit. It was concluded that endurance exercise led to an increase of plasma cortisol levels; animals that run longer distances have lower cortisol increase; disqualified animals, who suffered great physical effort, tend to have high cortisol levels and less experienced animals have higher cortisol levels despite running shorter distances.

KEY-WORDS: Equine. Cortisol. Endurance. Stress. Effort.

RESUMO

O objetivo deste trabalho foi relacionar a intensidade do exercício físico e as concentrações de cortisol plasmático em cavalos de enduro, uma competição em que somente animais experientes podem competir nas provas mais longas. Foram utilizados 30 equinos Puro Sangue Árabe e mestiços Árabe, machos ou fêmeas participantes de provas de enduro. Foram divididos em três grupos de 10 animais: (G1): percorreram mais de 100km, (G2): percorreram menos de 100km, e (G3): desqualificados por causa metabólica. Foram realizadas dosagens de cortisol plasmático em três momentos diferentes: (t0): dia anterior à competição, (t1): 30 a 60 minutos após o término da prova e, (t2): 90 a 120 minutos após o término da prova. Concluiu-se que o enduro leva ao aumento do cortisol plasmático; animais que percorrem maiores distâncias apresentam menor aumento das concentrações de cortisol; animais desqualificados por causa metabólica, que passam por situações de extremo esforço físico, tendem a valores de cortisol mais elevados e animais menos experientes apresentam valores de cortisol mais elevados mesmo tendo percorrido menores distâncias.

PALAVRAS-CHAVE: Equino. Cortisol. Enduro. Estresse. Esforço.

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INTRODUCTION

A wide variety of physiological parameters like heart recovery rate, dehydration degree and hyperthermia is altered during training and competitions such as endurance races. The endurance riding is a sport in which the animal performs aerobic exercise of long duration and medium intensity, a course that ranges from 40 to 160 km, and horses with locomotor symptoms of instability or metabolic disease (claudication, pain, increased heart rate among others) are eliminated from the races. This modality is regulated by strict rules in order to preserve physical integrity of the horses, which is controlled systematically in veterinary stations, where these parameters are evaluated (FÉDÉRATION EQUESTRE INTERNATIONALE, 2009). In addition to these known parameters, it is necessary to study other physiological patterns of athletic horses so that accidents are avoided and their performances improved.

Plasma cortisol level is a physiological parameter that can assist in assessing the overall condition of the horse (FREESTONE et al., 1991; MARC et al., 2000; FERRAZ et al., 2010; MEDICA et al., 2011), since cortisol or hydrocortisone is the main glucocorticoid of the adrenal cortex in horses (FARREL et al., 1983; MCARDLE et al., 2002). The study of the hypothalamic-pituitary-adrenal axis is also important to evaluate the fitness of the animal, since this system is directly involved with the adaptation to exercise (MARC et al., 2000).

Cortisol, on one hand, increases the body's resistance to stress, but at high doses, it has an anti-inflammatory and suppressing cell-mediated immunity, namely acts to inhibit responses needed to combat various types of cellular lesions and infections. At normal levels, the hormone function would be to allow metabolic, circulatory and other responses, necessary to adapt to different types of stress. Conversely, higher levels of cortisol would act to limit the adaptive responses, preventing them from becoming excessive, deleterious to the body (AIRES, 1999). Repetitive exposure to stress factors often result in adaptation or habituation. This response is crucial for the welfare of the body by allowing it to better cope with stress. The speed of adaptation depends on the severity, duration and type of stress (VELLUCI, 1997).

Physical activity makes the response to cortisol change and depends on the sport type (DESMECHT et al., 1996), exercise duration and intensity (SNOW and ROSE, 1981; MARQUES, 2002), training (FREESTONE et al., 1991; SIGHIERI et al., 1996; NOGUEIRA; BARNABE, 1997; CHICHARRO; VAQUERO, 1998; MARC et al., 2000; FERRAZ et al., 2010), fitness level, nutritional status and place where the blood sample was collected (SNOW; MACKENZIE, 1977; COVALESKY et al., 1992).

Desmecht et al. (1996) compared plasma cortisol level of animals competing in different sports (equestrian classic, eventing, trotting, horse racing and endurance). The type of event affected both cortisol

levels, pre- and post-exercise. The increase of cortisol was directly related with the intensity and duration of physical effort. All types of competition studied increased plasma cortisol level, and the largest increase was observed in animals that participated in endurance race. Snow and Rose (1981) reported that endurance horses, after a 80-km course, had a marked increase of plasma cortisol levels (0.176 $\mu\text{mol/L}$ at rest, para 0.343 $\mu\text{mol/L}$, 30 minutes after the exercise).

Marques (2002) while studying race horses, subjected them to two types of short duration exercises, low and high intensity. The group of short duration and low intensity exercise had mean cortisol levels of 0.1515 and 0.1270 $\mu\text{mol/L}$ at rest and after exercise, respectively. In the second group, short duration and high intensity exercise, cortisol levels increased more, with means of 0.1397 and 0.1605 $\mu\text{mol/L}$ at rest and after exercise, respectively.

The study by Freestone et al. (1991) evaluated serum cortisol levels in 9 horses that underwent a 10-week conditioning program. In the first week, mean cortisol level was 0.144 $\mu\text{mol/L}$, and increased gradually until the fifth week when the mean was 0.187 $\mu\text{mol/L}$, when the mean value started to decrease. After the conditioning period, cortisol level of horses at rest was higher compared to pre-training.

In another study, where endurance horses in the early and later stage of training underwent a standard submaximal exercise on the treadmill, cortisol level increased less in trained horses compared to those who were in the initial phase of training (SIGHIERI et al., 1996). Another study in Brazil with race horses (NOGUEIRA e BARNABE, 1997) determined the difference in cortisol level of animals at different training stages. Mean cortisol level of highly trained horses was (0.101 $\mu\text{mol/L}$) lower than that of young horses at the beginning of training (0.149 $\mu\text{mol/L}$).

Marc et al. (2000) reported that horses submitted to 24 weeks of training had lower cortisol levels than the untrained animals, after exogenous application of ACTH.

On the other hand, Ferraz et al. (2010) showed that after the treadmill test, cortisol level is lower before the training compared to that after 90 days of constant exercise. According to the authors, this fact is justified by the non-adaptation of the hypothalamic-pituitary-adrenal axis to exercise.

In endurance races, horse and rider are required to hold an event that ranges from 65 to 80 km to qualify for the races between 80 and 120 km, and an event between 80 and 120 km to qualify for races longer than 120 km (FÉDÉRATION EQUESTRE INTERNATIONALE, 2009). Therefore, only experienced and properly conditioned animals can participate in long distance events.

The objective of this study was to relate the intensity of physical exercise to the plasma cortisol level in endurance horses. In addition, to establish a relationship between disqualified animals due to metabolic causes with the cortisol levels.

MATERIAL AND METHODS

We collected blood samples from 30 male and female, adult, purebred and crossbred Arabian horses that take part in official competitions of free speed endurance races (40 to 160km long) of the Confederação Brasileira de Hipismo. The animals were divided in three groups of 10 animals each: G1 - raced more than 100 km (130.56 ± 7.83 km); G2 - raced less than 100 km (71.1 ± 3.75 km); and G3 - animals disqualified due to metabolic causes that raced 57 ± 7.49 km.

Blood samples were collected at times: t0, before the race; t1, 30 to 60 minutes after the race; and t2, 90 to 120 minutes after the race. The blood samples were collected using Vacutainer[®] tubes containing the anticoagulant heparin. The samples at time t0 were collected the day before the races. Samples t1 and t2 were collected in the afternoon of the race day.

Blood samples were centrifuged during 15 minutes at 1150G to separate the plasma. Aliquots of the plasma were taken and stored at -20°C until further analysis.

Cortisol levels were determined in duplicate at the Laboratório de Dosagens Hormonais (LDH), of the Departamento de Reprodução Animal, FMVZ, USP, by solid phase radioimmunoassay using a commercially available system². Aliquots of 25 μl of samples were placed in tubes that had antibodies attached to the tube wall, in which the cortisol in the plasma binds itself. After cortisol marked with 125I, which is used as competitor, was added, the tubes were incubated in water bath at 37°C during 45 minutes; subsequently, the supernatant was removed by decantation. The tubes were taken to gamma³ radiation counter to measure the remaining radioactivity. The minimum detected dose was 0.5 $\mu\text{g/dL}$ and coefficients of variation within and between trials were 4% and 5.3%, respectively.

Statistical analysis was performed using a software⁴ to calculate the mean, median, standard error of the mean and coefficient of variation. Analysis of variance was performed for comparison between different groups and ages, with subsequent Tukey test. Pearson correlation test was used for the variables, cortisol levels and distance raced. Significance level was 5% for all tests.

The experiment is in accordance with ethical principles of animal experimentation of the Bioethics Committee of the Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo under protocol no. 650/2009.

RESULTS

Table 1 shows that horses of G1 had the lowest mean cortisol levels at rest (3.46 ± 0.68 $\mu\text{g/dL}$)

compared to G2 (7.84 ± 0.81 $\mu\text{g/dL}$) and G3 (8.02 ± 2.41 $\mu\text{g/dL}$).

At times t1 and t2, cortisol levels of G1 (t1: 12.38 ± 2.07 $\mu\text{g/dl}$ and t2: 9.04 ± 1.89 $\mu\text{g/dL}$) were similar to G2 (t1: 18.69 ± 2.42 $\mu\text{g/dL}$ and t2: 12.56 ± 1.47 $\mu\text{g/dL}$) and lower than G3 (t1: 24.40 ± 4.43 $\mu\text{g/dL}$ and t2: 17.23 ± 3.07 $\mu\text{g/dL}$). Horses of G3 had high levels of cortisol at all times, from resting to post-exercise (Table 1).

Pearson linear correlation test related the distance raced and cortisol levels after the race in all groups ($p = -0.269$), but value was not significant ($p = 0.158$). When G3 was excluded, the correlation was significant ($p = 0.045$), with a negative linear correlation ($p = -0.465$). The disqualified animals had high cortisol levels, which were not correlated with the distance traveled.

Table 2 shows that G1 raced the longest distance (130.56 ± 7.83 km) compared to G2 (71.10 ± 3.75 km) and G3 (57.00 ± 7.49 km).

DISCUSSION

The evaluation of the mean cortisol levels of groups 1 and 2 at different times shows that exercise increased significantly the hormone level in the blood, thus confirming results that indicate that physical activity leads to an increased release of cortisol. According to the study by Snow and Rose (1981) there was a marked increase of cortisol levels in the plasma of all horses that participated of an 80-km endurance race.

Before the exercise (t0), cortisol level of G3 was higher than G1. This difference can be explained by the inexperience of horses of G3 in competitions, travels, shorter training period or the causes of disqualification. The mere fact that the blood samples were drawn in the place where the competition was taking place increases plasma levels as observed by Covalsky et al. (1992) in a study, in which experienced animals had lower cortisol levels than beginners. The training time affects this hormone behavior, Nogueira and Barnabe (1997) conducted a study in which animals with longer training periods displayed mean cortisol concentration lower than the horses in early training. In another study by Sighieri (1996), endurance horses in the early and later stages of training underwent a standard submaximal treadmill exercise, which induced smaller increases in the trained horses compared to the ones who were in the initial phase of training.

Once the animals must go through qualifying stages to participate in longer races (FÉDÉRATION EQUESTRE INTERNATIONALE, 2009), as well as a period of adaptation to the competition environment and travels. The horses of G2 had higher cortisol levels compared to G1, which can also be explained by the horse inexperience. It would be expected that animals of G1 had higher cortisol levels since they raced longer distances; however, as stated by Sighieri et al. (1996), in addition to intensity and duration of exercise, it should be taken into account the training stage of the

² DPC-Med Lab Coat a Count[®] Cortisol

³ Cobra Auto-Gama Packard[®]

⁴ MINITAB v. 13.1.

Table 1 – Mean and standard deviation of (EP) plasma cortisol ($\mu\text{g/dL}$) and significance level of the comparison test between groups and moments.

Groups	Times						P(t)
	t0		t1		t2		
	Mean	EP	Mean	EP	Mean	EP	
G1	3,46 ^{Aa}	0,68	12,38 ^{Bc}	2,07	9,04 ^{Be}	1,89	0,006
G2	7,84 ^{Cb}	0,81	18,69 ^{Dcd}	2,42	12,56 ^{Cef}	1,47	0,00
G3	8,02 ^{Eb}	2,41	24,40 ^{Ed}	4,43	17,23 ^{Ef}	3,07	0,064
P(G)	0,009		0,04		0,056		

P(G): significance level between groups. P(t): significance level between times. Uppercase letters indicate comparison between times and lowercase letters between groups. Different letters mean significant statistical difference ($P < 0.05$).

Table 2 – Sample number, mean, median, standard deviation, minimum and maximum raced distances for different groups.

Group	N	Mean	Median	EP	Minimum	Maximum
		(km)	(km)		(km)	(km)
1	9	130,56 ^a	123,0	7,83	101	160
2	10	71,10 ^b	65,5	3,75	60	88
3	10	57,00 ^b	50,50	7,49	28	100

Different letters mean significant statistical difference ($P < 0.05$).

animal as well. Thus, although G2 horses raced shorter distances (Table 2), they displayed a tendency to higher cortisol levels compared to G1.

Pearson linear correlation test showed that as the traveled distance increased, cortisol levels decreased. This fact is justified by the Fédération Equestre Internationale rule, since the horses should go through classificatory stages in order to take part in longer endurance races, this works as a preparatory period to fulfill the race requirements. The disqualified animals had high cortisol levels, independent of the traveled distance. A horse can be eliminated from the competition having traveled long or short distances, and still present increased cortisol levels in the blood.

CONCLUSIONS

The study confirms that exercise with long duration and medium intensity leads to increased plasma cortisol level and that more fit animals display smaller cortisol level increase. Horses that were disqualified due to metabolic causes, which include situations of

extreme physical exertion, tend to have higher cortisol levels, while less experienced animals have higher cortisol levels despite having traveled shorter distances.

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