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Serum cortisol, lactate and creatinine concentrations in Thoroughbred fillies of different ages and states of training

Cortisol sérico, concentração de lactato e creatinina em cavalos de corrida Puro Sangue Inglês com diferentes idades e estágios de treinamento

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SUMMARY

Exercise can be defined as “normal stress” stimulating body functions. Some reports suggest lactate as a stimulator of cortisol levels, while creatinine varies according to the amount of muscle tissue. In the present study we investigated the relationship between creatinine, serum lactate concentration and cortisol levels in training horses. Twenty-three Thoroughbred fillies were used, divided into 3 groups according to age and training protocol: G1, 1-2 years of age (N=7) on pasture, G2, 2-3 years (N=9) starting to be mounted, and G3, 3-4 years (N=7) racing at the Jockey Club. Blood samples were collected weekly during a six-month period at about 1:00 p.m. while the animals were resting. Cortisol was quantified with a commercial kit (Coat-a Count®) and serum creatinine and lactate were evaluated with an autoanalyzer with commercial reagents. Data were evaluated using non-parametric statistical tests, with the level of significance set at $P < 0.05$. Cortisol concentrations were $149^a \pm 7$, $126^b \pm 6$, and $101^c \pm 5$ nmol/l, lactate concentrations were $2.1^a \pm 0.1$, $2.0^a \pm 0.1$, and $1.75^b \pm 0.1$ mmol/l, and creatinine concentrations were $125^a \pm 2$, $132^a \pm 2$, $145^b \pm 3$ mmol/l in G1, G2 and G3, respectively. Only G2 showed a low but significant positive correlation of cortisol with lactate and a negative correlation of cortisol with creatinine levels. It was possible to conclude that cortisol, lactate and creatinine varied during horse aging and physical conditioning. The decrease of cortisol concentration (G2) suggests that the better physical condition acquired during training led to the increase of creatinine concentration, possibly related to muscle mass. The lower cortisol and lactate concentrations observed in G3 animals may have been due to greater muscle mass inducing an increase in creatinine concentrations or changes in muscle fiber type during training.

KEY-WORDS: Hydrocortisone. Horses. Lactates. Creatinine. Exercise.

INTRODUCTION

Exercise is probably the main physiological stimulus to the body and the best example of a “normal stress” to which an animal can be submitted¹. Cortisol is an important regulatory glucocorticoid secreted by the horse’s adrenal cortex that increases blood glucose concentration when released². Cortisol secretion was found to be related to anaerobic activity, suggesting that lactate may be a stimulator of cortisol concentration^{3,4}. According to Blomqvist and Saltin⁵, physical training increases cardiocirculatory function as well as maximal oxygen uptake, thereby decreasing muscle anaerobiosis. Almost half of the animal’s body is composed of skeletal muscle fibers, which make up a huge cellular mass with the same kind of activity⁶.

Muscle activity can be evaluated by measuring serum enzymes and metabolites that are released from cells during exercise. Lactate is released from muscle cells during

anaerobic glycolysis^{7,8}. Since lactate concentration can be used as an indicator of physical condition⁹, it can also be related to performance¹⁰. Creatinine is produced from the decomposition of creatine, a nitrogen compound used by muscle cells to store energy. The serum concentration of creatinine varies according to creatine synthesis and the amount of muscle tissue of the animal¹¹.

The performance of a horse during competition is the result of a combination of many complex interactions including age, breed, genetic potential, strength and fitness. Because competition is an essential part of the evolutionary process and it is present throughout the animal kingdom¹² a physiological study conducted in competing horses, may be more similar to reality than indoor studies.

The objective of the present study was to investigate the relationship between serum muscle biochemistry and cortisol secretion, in resting Thoroughbred horses that are training at the study farm and racing at the Jockey Club.

MATERIAL AND METHOD

The study was conducted on 23 Thoroughbred fillies from the Equilia Stud Farm located in the town of Avaré, São Paulo State, Brazil. The animals were divided into three groups according to age and training protocol. Group 1 consisted of 7 fillies aged 1-2 years which remained on pasture (coast-cross) receiving a supplementary diet. Group 2 consisted of 9 fillies aged 2-3 years which were starting to be mounted, acquiring physical condition. The amount of daily exercise was gradually increased for these fillies but most individuals did not canter until late August and only began full galloping in November or December. Group 3 consisted of 7 fillies aged 3-4 years that were training and already racing at the Jockey Club. The fillies from G2 and G3, remained in stables and were exercised early in the morning, from 5:30 to 8:00. All horses were subjected to a similar general training program but individual adjustments were unavoidable. Blood samples were collected weekly, from the jugular vein during a period of six months corresponding to the breeding season of horses, always around 1:00 p.m. while the animals were resting. Blood sample tubes were centrifuged at the stud laboratory and serum was immediately stored in a freezer until the time for assay.

Cortisol was quantified with a commercial kit (Coat-a-Count[®], DPC) according to the method of Freestone et al.¹³. The assay had a sensitivity of 1.9 nmol/l and an inter- and intra-assay coefficient of variation of 15.06% and 12.05%, respectively. Serum biochemistry was evaluated with an

autoanalyzer (Cobas Mira Roche Diagnostic System - Suisse) using commercial kits. Serum lactate was quantified using an MPR1 kit (n° 149993 - Boehringer Mannheim, Germany), and serum creatinine concentration was evaluated with a kit (n° 035, Lab Test Diagnostic S/A - MG Brazil). Data were analyzed statistically by comparing month and semester average within groups, using the non-parametric Kruskal-Wallis and Spearman tests due to the small number of animals studied, with the level of significance set at $P < 0.05$.

RESULTS

Statistical analysis of the data showed that there were differences between groups during the semester both when the month average and the semester average were considered. Due to the wide variation of the results during the semester, only the semester average was considered for each group (Tab. 1).

The cortisol concentration of G1 showed a positive correlation coefficient during the semester, $r = 0.220$ ($P < 0.05$), differing from G2 and G3, which showed a negative correlation during the semester, $r = -0.111$ ($P < 0.05$) and $r = -0.115$ ($P < 0.05$), respectively. Comparing the cortisol average for the semester, the results were significantly different between G1 and G3 and also between G2 and G3, in a decreasing order (Table 1). Only G2 showed significant positive correlation ($r = 0.16$) between cortisol and lactate concentrations and a negative correlation ($r = -0.14$) between cortisol and creatinine concentrations.

Table 1

Resting serum cortisol, lactate and creatinine concentrations during a six-month period (from July to December) in 3 groups of Thoroughbred fillies submitted to different training programs. Group 1 (G1) was 1 to 2 years old, G 2, 2 to 3 years old and G 3, 3 to 4 years old. The fillies were respectively on pasture, acquiring physical conditioning and racing at the Jockey Club. Data are reported as means \pm SEM. Groups that do not share the same superscript letter differed significantly ($P < 0.05$, Kruskal-Wallis). São Paulo, 2001.

	Cortisol (nmol/l)			Lactate (mmol/l)			Creatinine (mmol/l)		
	G1	G2	G3	G1	G2	G3	G1	G2	G3
Jul	137 \pm 11	131 \pm 15	121 \pm 11	2.1 ^a \pm 0.3	1.8 ^a \pm 0.1	1.7 ^b \pm 0.1	122 ^a \pm 5	116 ^a \pm 4	141 ^b \pm 6
Aug	127 ^{ab} \pm 15	156 ^a \pm 9	98 ^b \pm 11	2.2 ^a \pm 0.2	2.3 ^a \pm 0.2	1.8 ^b \pm 0.2	126 ^a \pm 4	119 ^b \pm 3	144 ^a \pm 4
Sept	158 ^{ab} \pm 30	152 ^a \pm 11	98 ^b \pm 17	1.9 \pm 0.1	2.0 \pm 0.1	1.7 \pm 0.1	132 ^a \pm 5	122 ^b \pm 3	140 ^a \pm 7
Oct	137 \pm 15	138 \pm 7	103 \pm 12	2.2 ^a \pm 0.2	2.0 ^a \pm 0.1	1.8 ^b \pm 0.2	122 \pm 6	139 \pm 9	148 \pm 8
Nov	165 ^a \pm 12	123 ^b \pm 7	79 ^c \pm 13	2.0 ^a \pm 0.1	1.9 ^b \pm 0.0	1.7 ^b \pm 0.2	130 \pm 5	146 \pm 5	153 \pm 8
Dec	163 ^a \pm 13	97 ^b \pm 17	104 ^b \pm 13	2.1 ^a \pm 0.1	1.8 ^b \pm 0.1	1.8 ^b \pm 0.2	114 ^a \pm 5	145 ^b \pm 7	148 ^b \pm 6
Mean	149 ^a \pm 7	126 ^b \pm 6	101 ^c \pm 5	2.1 ^a \pm 0.1	1.95 ^a \pm 0.1	1.75 ^b \pm 0.1	125 ^a \pm 2	132 ^a \pm 2	145 ^b \pm 3

DISCUSSION

Cortisol, lactate and creatinine varied during physical conditioning and aging in Thoroughbred fillies. There was a decrease on cortisol concentration in 2-3 years old fillies and an increase of creatinine concentration. Cortisol and

lactate concentrations were lower in 3-4 years old fillies.

The lower serum cortisol concentration in the older fillies (G3) was probably the consequence of the different training programs to which the animals were submitted. Luna¹⁴ reported that exercises of low intensity lasting for a longer period of time was more efficient in triggering adrenocortical

secretion than a shorter high-intensity work load. G3 fillies may also be under chronic stress since they had been exercising for a longer period of time¹⁵. The decrease in cortisol concentration observed with increasing age in G2 fillies may also have been related to the increase in cardiocirculatory capacity due to habituation to exercise⁵. The higher cortisol concentration on G1 may be explained by the low suppression of cortisol secretion, as opioids inhibit the equine hypothalamo-pituitary-adrenal axis under basal conditions¹⁶.

The higher serum lactate concentration in G1 fillies, which were on pasture, suggests that horses walking on pasture may have higher lactate concentrations at rest than horses maintained in stables. The lower lactate concentration observed in G2 and G3 fillies may have been a consequence of training^{10,17}. This decrease in serum lactate concentration may represent a better athletic capacity as described by Sciliano⁹. The increase in serum creatinine concentration in G2 and G3, considering that exercising horses maintains a normal renal function¹⁸, indicates that creatinine concentration may increase together with muscle mass and exercise¹⁷.

Since only G2 fillies showed a low positive correlation between serum concentration of lactate and cortisol, we may propose that lactate is not the major stimulus of cortisol secretion during exercise. There are several factors involved in control of cortisol secretion, explaining the individual pattern of cortisol secretion observed during this experiment

and also reported by Meirleir³ and Port⁴. Sheeherman and Morris¹⁹ reported a more rapid decrease in venous lactate and a more rapid return to initial rest values in the adult horses relative to younger untrained horses, indicating that aerobic power and exercise capacity increased with age and training. The lower venous lactate in the adult indicates a more rapid rate of lactate use when compared to young horses.

CONCLUSIONS

We conclude that through age and training protocol there were changes in the resting biochemical pattern in Thoroughbred race horses, probably related to a better physical condition, an increase of muscle mass acquired during exercise or lower response to the stressor, but the changes (creatinine and lactate) were not correlated to cortisol concentration.

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RESUMO

Exercício pode ser definido como um “estressor normal” estimulando as funções corpóreas. Alguns trabalhos sugerem o lactato como estimulador da secreção de cortisol, enquanto que a creatinina varia em função da quantidade de tecido muscular. No presente estudo é investigada a relação entre creatinina, lactato e cortisol séricos em cavalos em treinamento. Vinte e três potras Puro Sangue Inglês foram utilizadas, divididas em 3 grupos de acordo com a idade e protocolo de treinamento: G1, 1-2 anos de idade (n=7) mantidas a pasto, G2, 2-3 anos (n=9) começando a ser montadas e G3, 3-4 anos (n=7) competindo no Jockey Club. Amostras de sangue foram colhidas semanalmente durante 6 meses próximo às 13 h, enquanto os animais descansavam. O cortisol foi quantificado através de kits comerciais (Coat-a Count[®]) e a creatinina sérica e o lactato foram avaliados através de um auto-analyzer, usando reagentes comerciais. Os resultados foram avaliados utilizando testes estatísticos não-paramétricos com nível de significância $P < 0,05$. As concentrações de cortisol foram $149^a \pm 7$, $126^b \pm 6$, e $101^c \pm 5$ nmol/l, as concentrações de lactato foram $2,1^a \pm 0,1$, $2,0^a \pm 0,1$, e $1,75^b \pm 0,1$ mmol/l, e as concentrações de creatinina foram $125^a \pm 2$, $132^a \pm 2$, $145^b \pm 3$ mmol/l nos grupos G1, G2 e G3, respectivamente. Somente o G2 apresentou uma pequena, mas significativa correlação positiva do cortisol com o lactato e correlação negativa do cortisol com a concentração de creatinina. Foi possível concluir que o cortisol, lactato e a creatinina variaram em função da idade e do condicionamento físico. A diminuição do cortisol observada nos animais do G2, reflete o melhor condicionamento físico adquirido durante o treinamento, que pode ser inferido através do aumento da concentração de creatinina, relacionada a quantidade de massa muscular. A diminuição do cortisol observada nos animais do G3 pode também ser consequência do aumento da massa muscular em função do condicionamento, que repercutiu no aumento da creatinina, ou mudanças nos tipos de fibras musculares durante o treinamento.

PALAVRAS-CHAVE: Hidrocortisona. Equinos. Lactatos. Creatinina. Exercício.

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