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Serum muscular enzymes (CPK/AST) and plasma progesterone in Thoroughbred mares in training

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Summary

Blood samples were analysed in 111 Thoroughbred mares in training, to study the relationship between CPK, AST (GOT) and progesterone. The enzymes were measured spectophotometrically and the progesterone by RIA. The following effects were studied: 1.- Stud to which the animal belongs. 2.- Training stage. 3.-Period between the last hard exercise and the enzyme measurement. 4.- Animal's age. Two different groups were considered: group A: mares with progesterone levels less than 1 ng/ml; group B: mares with progesterone levels higher than or equal to 1 ng/ml. In group A, effects 1, 2 and 3 were significant ($p<0.05$) for CPK enzyme. Effects 1 and 2 were significant for AST(GOT) enzyme. Whereas in group B, for both enzymes (CPK and AST), none of the factors had a significant effect ($p<0.05$).

Keywords: rhabdomyolysis, progesterone, CPK, AST(GOT), mare, training, horse

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Muskelenzymaktivitäten im Serum (CK/AST) und Plasmaprogesterongehalt bei Vollblutstuten im Training

Die Autoren sammelten Blutproben von 111 Vollblutstuten im Training, um die Zusammenhänge zwischen den CK-, AST- und Progesteronkonzentrationen der Pferde zu ermitteln. Die Enzymaktivitäten wurde mit einer photometrischen Methode gemessen und der Progesteronspiegel mit einem Radioimmunoassay bestimmt.
Folgende Faktoren wurden studiert : 1) aus welchem Stall die Stute stammte, 2) das Trainingsstadium des Pferdes, 3) der Zeitraum zwischen der letzten harten Anstrengung und der Messung der Enzymkonzentrationen und 4) das Alter der Stute. Die Pferde wurden in zwei Guppen eingeteilt : die Gruppe A bestand aus Stuten mit einem Progesteronspiegel <1ng/ml, die Pferde der Gruppe B hatten Progesterongehalte von 1 ng/ml und mehr.
Folgende Enzymkonzentrationen wurden ermittelt : der Median der CK-Werte betrug im Durchschnitt 109 U/l, sie reichten allerdings von 24–1295 U/l, der Median AST-Aktivität lag bei 264 U/l (102–3740 U/l).
Es zeigte sich, daß in der Gruppe A die Faktoren 1, 2 und 3 die Höhe der Kreatinkinase-Aktivität beeinflußten. Weiterhin beeinflußten die Faktoren 1 und 2 die AST-Konzentration der Stuten in Gruppe A. Die Messungen der Gruppe B zeigten ganz andere Verhältnisse, da keiner der 4 Faktoren einen signifikanten Effekt auf die CK- und AST-Konzentration der Stuten besaß.
Bei der Gesamtbetrachtung der beiden Gruppen zeigten sich keine großen Unterschiede in den Medianen der CK- oder AST-Konzentration im Serum. Die Einflüsse der 4 Faktoren auf die Muskelenzymkonzentration unterschieden sich allerdings zwischen Stuten mit hohem und mit niedrigem Progesterongehalt im Plasma. Die Autoren vermuten, daß die Muskelzellmembran einer Stute im Diöstrus durch ihren höheren Progesterongehalt vermehrt geschützt wird. Im Einklang mit dieser These stand die Vermutung von *Snow* und *Valberg* (1994), daß eine Progesteronbehandlung bei manchen Stuten die Auswirkungen einer Myoglobinurie (d.h. einer Rhabdomyolyse) durch Überanstrengung mildern könnte.

Schlüsselwörter: Rhabdomyolyse, Progesteron, CK (CPK), AST (GOT), Stute, Training, Pferd

Introduction

Rhabdomyolysis has been one of the muscular diseases with a high incidence in horses in training, producing a decrease in their performance. Described for more than one hundred years, this disease has been, referred to as monday disease, myoglobinuria, tying-up, myositis, rhabdomyolysis, and exertional rhabdomyolysis. Strenuous exercise is the most common cause of rhabdomyolysis. Predisposing causes are still under discussion.
Carlstrom (1931) described this disease in horses that were exercised after a few days of rest with a high carbohydrate diet. Other factors which have been considered include: electrolyte imbalances *(Beech*, 1993), vitamin E and selenium deficiencies *(Owens* et al., 1987) and hypothyroidism *(Waldron-Mease,* 1979). *Brennan* (1959) and *Lindholm* (1987) suggested that rhabdomyolysis is more common in mares than in male horses, implying a hormonal predisposition. *Frauenfelder* et al. (1986) found no relationship between elevations in serum muscle enzyme levels and the stage of the oestrus cycle.
Diagnosis of rhabdomyolysis is based on clinical signs and elevated levels of creatinephosphokinase (CPK), aspartate

aminotransferase (AST/GOT), lactate dehydrogenase (LDH) and myoglobin in serum *(Cardinet* et al., 1963; *Snow* and *Valberg,* 1994). Clinical signs of acute rhabdomyolysis may include profuse sweating, elevated respiratory rate and myoglobinuria. Physical examination shows painful muscle cramps, especially in the gluteal area.
The purpose of this research was to study the influence of the following fixed effects: 1.– Stud to which the animal belonged, 2.– training stage, 3.– days elapsed between last hard exercise and the enzyme measurement and 4.– age, on CPK and AST (GOT) levels in two groups of Thoroughbred mares divided according to progesterone concentration, in training without changing their exercise schedule and general management.

Materials and methods

111 Thoroughbred mares between 2 and 4 years old, at San Isidro racetrack on the outskirts of Buenos Aires, were tested. They were located in 14 different studs each with a different trainer.
The samples were collected by venopunction in the morning (between 5.30 and 6.30 AM), and were placed into glass test tubes containing sodium EDTA anticoagulant for progesterone measurement, and without anticoagulant for enzyme measurement. Samples were centrifuged at room temperature within 2 hours of collection. Plasma was stored at -20°C until analysed. The enzymatic measurements were done within 12 hours of collection.
CPK and AST (GOT) measurements were made using a Shimadzu spectrophotometer UV, model 110–02, at 30°C[1]. Progesterone measurements were accomplished using a RIA *(Garcia Bienere* et al., 1975).
The mares were divided into two groups depending on their progesterone concentration: Group A.– mares with progesterone levels less than 1 ng/ml (46 observations). Group B.– mares with progesterone level higher than or equal to 1 ng/ml (65 observations). We considered 1 ng/ml as the limit, as mares with levels above 1 ng/ml are in diestrous *(Ginther,* 1992) with a functional corpus luteum.

Statistics

Linear methodology model was used *(Searle,* 1971) to study the action of 4 fixed effects on CPK and AST (GOT) serum levels. The fixed effects used were: 1.– Stud to which the animal belonged, 2.– training stage (stage 1: animals in training which had not started competing yet, stage 2: animals in training which had started competing) 3.– days elapsed between last hard exercise and the enzyme measurement 1, 2 and 3 days), 4.– age (2 ,3 and 4 years old). Progesterone levels were used as a covariable.
The data (CPK and AST) were transformed (-1/data) to achieve normal distribution.

Results

The concentration of enzymes observed in the 111 mares studied were expressed as median because of the wide range seen, they were CPK 109 U/l (range 24–1295 U/l) and AST (GOT) 264 U/l (range 102–3740 U/l).

[1]CPK UV (Wiener Lab.)/ GOT (AST) UV (Wiener Lab.), 2000- Rosario- Argentina

There was no significant difference ($p<0.05$) between the two groups. In group A, 46 mares with progesterone levels less than 1 ng/ml, the effect of age was not significant ($p<0.05$) either for CPK or AST (GOT). On the other hand the effects of stud and training stage were significant ($p<0.05$) for both enzymes (see tab. 1). Furthermore, the effect of the period between

Tab. 1: Serum levels of CPK and AST (GOT) in U/l in group A according to stage of training.

Stage	N	Median	Min.-Max.	
1	15	142[a]	65–546	CPK
		292[a]	155–3740	AST
2	31	105[b]	24–650	CPK
		255[b]	137–650	AST

N= Number of animals; Min.-Max.= Minimum and Maximum values; Stage 1= Animals in training which had not started competing yet. Stage 2= Animals in training which had started competing. a b values with different superscript are significantly different ($p<0.05$).

the last exercise and the enzyme measurements was only significant ($p<0.05$) for CPK (see tab. 2).

Tab. 2: Serum levels of CPK and AST (GOT) in U/l in group A according to days elapsed between the last hard exercise and the enzyme measurements.

Period	N	Median	Min.-Max.	
1	5	130[a]	84–154	CPK
		329[a]	182–400	AST
2	18	89[b]	24–650	CPK
		237[b]	137–650	AST
3	23	113[c]	57–546	CPK
		283[c]	155–3740	AST

N= Number of animals; Min.-Max.= Minimum and Maximum values. Period 1= 1 day elapsed between the last exercise and the enzyme measurements. Period 2= 2 days elapsed between the last exercise and the enzyme measurements. Period 3= 3 days elapsed between the last exercise and the enzyme measurements. [a b c] values with different superscript are significantly different ($p<0.05$).

In group B, 65 mares with progesterone levels higher or equal to 1 ng/ml, none of the effects were significant ($p<0.05$) for CPK or AST (GOT) (see tab. 3 and 4).

Discussion

The wide range observed in serum enzyme levels for CPK and AST (GOT), coincide with the references *(Harris* et al., 1990).
There was no significant difference ($p<0.05$) in enzyme levels between the group A and B but when it was studied the action of the fixed effects, there were differences in each group. The significant difference ($p<0.05$) found in group A, (mares with progesterone levels less than 1 ng/ml), for CPK and AST (GOT) when training stage and stud were considered, would highlight

Tab. 3: Serum levels of CPK and AST (GOT) in U/l in group B according to stage of training.

Stage	N	Median	Min.-Max.	
1	29	105[a]	65–1295	CPK
		274[a]	146–3290	AST
2	36	109[a]	65–445	CPK
		269[a]	101–1640	AST

N= Number of animals; Min.-Max.= Minimum and Maximum values; Stage 1= Animals in training which had not started competing yet. Stage 2= Animals in training which had started competing. [a] values with the same superscript are not significantly different ($p<0.05$).

Tab. 4: Serum levels of CPK and AST (GOT) in U/l in group B according to days elapsed between the last hard exercise and the enzyme measurements.

Period	N	Median	Min.-Max.	
1	5	105[a]	81–324	CPK
		219[a]	182–310	AST
2	32	125[a]	65–1295	CPK
		259[a]	102–2550	AST
3	28	91[a]	65–890	CPK
		275[a]	138–3290	AST

N= Number of animals; Min.-Max.= Minimum and Maximum values. Period 1= 1 day elapsed between the last exercise and the enzyme measurements. Period 2= 2 days elapsed between the last exercise and the enzyme measurements. Period 3= 3 days elapsed between the last exercise and the enzyme measurements. [a] values with the same superscript are not significantly different ($p<0.05$).

the importance of management on enzyme levels. Furthermore, the period between the last exercise with high intensity and the enzyme measurement was also significant for CPK. This can be explained by their different half life in plasma (24–48 hs. for CPK, and 7–14 days for AST).

In group B (mares with progesterone levels higher than or equal to 1 ng/ml) none of the effects studied were significant ($p<0.05$). This high progesterone level indicates the presence of a corpus luteum and therefore mares evidence they were cycling *(Ginther,* 1992).

We suspect that during the luteal phase the effects (stud to which the animal belonged, training stage and days elapsed between last hard exercise and the enzyme measurement), were hidden. It is possible that progesterone or another event present during diestrous has a protective effect on muscle cell membrane. *Snow* and *Valberg* (1994) suggested that progesterone treatment may have a beneficial effect in some mares with exertional rhabdomyolysis.

It would be of benefit to know which factors may protect cell membrane during dioestrus in order to help to reduce rhabdomyolysis incidence.

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