Equine Exercise Physiology 3: 391–396, 1991

Effect of 5 Different Types of Exercise, Transportation and ACTH Administration on Plasma Cortisol Concentration in Sport Horses

A. LINDEN, T. ART, H. AMORY, D. DESMECHT and P. LEKEUX

Equine Sports Medicine Unit, Faculty of Veterinary Medicine, University of Liege, Bât B42-Sart Tilman, B-4000 Liege, Belgium

ABSTRACT. This study was carried out to compare the changes in plasma cortisol concentration produced by 5 different types of exercise, transportation and ACTH administration. Venous blood was analysed for cortisol concentration before and after each stimulus. The types of exercise were show jumping ($n=28$), cross country phase of a three-day event ($n=32$), trot race ($n=9$), gallop race ($n=10$) and endurance ride ($n=8$). The response following the intravenous injection of synthetic ACTH (200 µg) in 6 of the event horses was determined. The ACTH test was carried out one week after competition. In 5 horses, the response to road transport of one hour was studied. All stimuli, except transportation produced a significant increase in plasma cortisol concentration. The endurance ride produced the largest increase and the show jumping the smallest. ACTH administration resulted in a 164% rise in plasma cortisol. It was concluded that 1) the degree of increase in plasma cortisol concentration appeared to reflect both intensity and duration of workload; endurance ride appeared to be the most exhaustive and show jumping the least; 2) there was a correlation ($r^2=0.82$) between the individual cortisol response to cross country exercise and to exogenous ACTH in the same horses and 3) there was an important individual effect on transport-induced cortisol changes.

INTRODUCTION

During the last few decades, there has been an ever increasing popularity of equestrian competitions. Numerous studies have been carried out to investigate the "competition stress" produced by such events.[13,16,20,21] Indeed, sport horses are submitted to intensive training, frequent transportation and exhaustive competition. All these situations cause important physiological and biochemical fluctuations.[6,15,22] In many species, the increase in adrenocortical secretion associated with stress has been studied.[1,8] In sport horses, the exercise-induced changes in plasma corticosteroids have been shown to be closely related to physiological stress.[14,18]

The aim of this work was 1) to compare the effect of 5 types of equine competition on plasma cortisol concentration. The events consisted of different kinds of exertion, i.e., very high intensity and short duration such as gallop and trot races; high intensity and long duration such as cross country; low intensity and long duration such as endurance ride; mild intensity and short duration such as show jumping, 2) to compare the cortisol response to cross country exercise with the response to exogenous ACTH in the same horses and 3) to study the effect of transportation on plasma cortisol concentration.

MATERIALS AND METHODS

Animals

The research was carried out on 92 clinically healthy horses, aged from 3 to 16 years. For the exercise investigation, 5 groups of sport

Table 1. *Characteristics of the 5 equine sports disciplines*

n = number of horses

Discipline	*n*	Type	Age (years)	Speed (m/min)	Duration (sec)	Length (m)
Jumping	28	Saddlebred	8–13[a]	331.20[b] ±31.43	96.6[b] ±10.8	500
Cross-country	32	Saddlebred	5–16	448.46 ±14.88	471.8 ±28.5	3 526
Trot	9	Standardbred	5–7	740.82 ±5.28	178.2 ±1.3	2 200
Gallop	10	Thoroughbred	3–5	926.50 ±6.19	129.5 ±0.9	2 000
Endurance	8	Saddlebred	6–11	183.33 ±39.30	14 000 ±720	44 000

[a] Range.
[b] Mean ± standard error

horses were studied during their respective competitive events: show jumping, cross country of a three-day event, trot race, gallop race and endurance ride. The characteristics of each discipline are given in Table 1. All the horses of a given group were investigated on the same day, during an official national competition. All had been transported to the event on the day of the competition. For the pharmacological investigation, 6 of the 32 event horses were randomly chosen and 200 μg of a synthetic ACTH 1–24 preparation (Synacthen R: tetracosactrin, 250 $\mu g\,ml^{-1}$, Ciba Geigy N.V.) was injected intravenously. Synacthen was diluted with 10 ml of acidified saline (0.9% NaCl). The test was performed one week after the cross country and with animals in a resting state.

The transport consisted of a 1 hour period of transportation by road in a conventional horse trailer.

Methods of collection and analysis

Venous blood was collected from the jugular vein into tubes containing potassium EDTA, at rest (8.00–10.00 a.m.) and after each experiment: i.e. 2 min after the completion of competitive events (2.00–4.00 p.m.), 1 hour after intravenous ACTH injection (8.00–9.00 a.m.) and 2 min after transportation (8.00–9.00 a.m.). Blood samples were immediately centrifuged at 3000 rpm for 20 min. After separation, plasma was frozen at −20°C until assayed for cortisol. Cortisol was determined by a radioimmunoassay method (Gamma coat ^{125}I Cortisol Radioimmunoassay Kit; Baxter Travenol Diagn. Inc.). The coefficient of intra-assay and inter-assay variation were 5.1 and 6.6% respectively. All assays were performed in the same laboratory, using the same procedure. The assay detection limit was 6.62 $nmol\,l^{-1}$ and the cross reactivity of the antiserum to other steroids has been previously described.[17] All samples were assayed in duplicate.

Statistical analysis

Values were expressed as mean ± standard error of the mean (SEM). The post-plasma cortisol values were compared to the resting ones by a Student's *t*-test for paired data. Square root transformations were used to obtain a normal distribution. A fixed linear model including the discipline effect (ANOVA 1) was adjusted to the resting (absolute values) and post-exercise (relative values) plasma cortisol data.

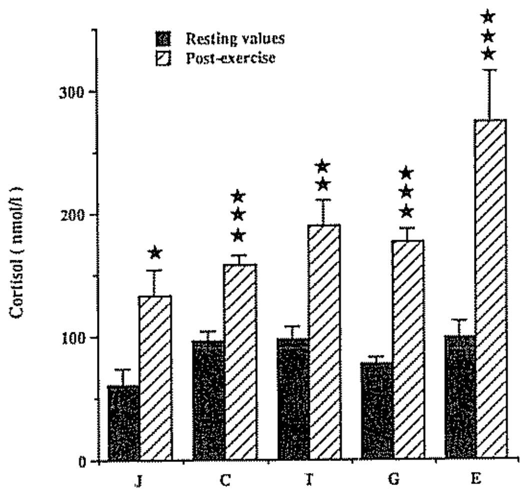

Fig. 1. Plasma cortisol concentration (mean ± SEM) in 87 horses, at rest and 2 min after exercise. J = show jumping, C = cross country, T = trot, G = gallop and E = endurance. $*p \leqslant 0.05$, $**p \leqslant 0.01$, $***p \leqslant 0.001$: significantly different from resting values with paired *t*-test.

RESULTS

Fig. 1 displays the mean values ± SEM of the resting and post-exercise plasma cortisol levels of the 87 horses in the 5 disciplines. All competitions produced a significant increase in plasma cortisol. The exercise to rest ratio was 1.72±0.19, 1.78±0.14, 2.09±0.28, 2.47±0.18 and 2.76±0.41 for show jumping, cross country, trot, gallop and endurance, respectively. The fixed linear model gives r^2 of 0.282 ($p \leqslant 0.05$) and 0.377 ($p \leqslant 0.01$) for the resting and post-exercise plasma cortisol values, respectively. In this model, the resting and post-exercise plasma cortisol values of jumping horses were significantly lower ($p \leqslant 0.01$) than the mean resting and post-exercise values of the other disciplines. The post-endurance plasma cortisol values were significantly higher ($p \leqslant 0.001$) than the mean post-exercise values of the other disciplines.

The mean values ± SEM of the resting and post-transport plasma cortisol levels are given in Fig. 2. The coefficient of variation (CV) of post-transport values was 91.43%.

As shown in Fig. 3, for each horse, ACTH test and exercise produced a high plasma cortisol increase. The ACTH-induced cortisol changes were strongly correlated ($r^2 = 0.82$) with the exercise-induced cortisol changes in these 6 horses.

DISCUSSION

This is the first study reporting the comparison of plasma cortisol changes in 5 groups of sport horses studied in their respective disciplines during official competitions, and thus including exercises of various intensity and duration. The fact that the protocol was the same during the whole investigation and that all analyses were performed in the same laboratory using the same methods, allowed us to make a reliable comparison between disciplines.

Large fluctuations throughout the day in the resting plasma cortisol values have been previously reported in man[11] and horses.[9,12] Therefore, in order to limit variations due to this circadian rhythm and to allow an accurate comparison between groups, the resting samples have been taken between 8.00–10.00 a.m. at the time of the highest plasma values of the glucocorticoids in the horse.[10,26] The mean resting plasma cortisol concentration reported in this study is in

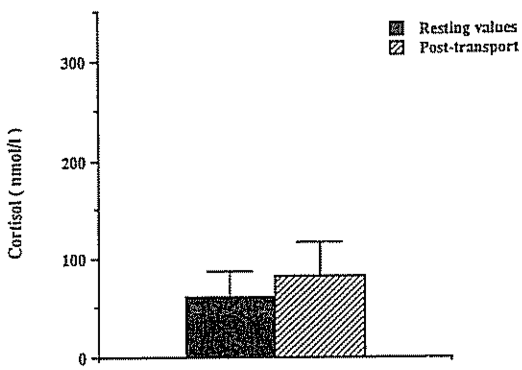

Fig. 2. Plasma cortisol concentration (mean ± SEM) in 5 horses, at rest and 2 min after transportation.

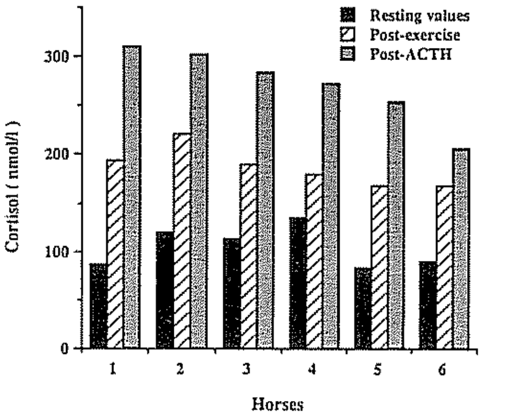

Fig 3 Plasma cortisol concentration in 6 horses, at rest, 2 min after exercise (Test 1) and 60 min after 200 µg ACTH administration (Test 2 performed one week after Test 1).

agreement with the results reported earlier and obtained with a similar technique.[3,13,17] With all samples investigated in the same way, no significant difference has been demonstrated between Thoroughbreds and Standardbreds.

The exercise-induced plasma cortisol changes have been reported previously in man,[2,5] dogs[7] and horses,[6,13,18,25,27] but conflicting results exist, reporting either an increase or a decrease in the exercise-induced changes. Basically, moderate exercise causes little alteration in plasma cortisol concentration while strenuous and exhaustive exercise produces a marked increase.[24,25] In man, a workload that could be maintained for 1 hour, at approximately 60% of the maximal oxygen uptake, appears to be the critical level above which a rise in plasma cortisol occurs.[5] In the present study, all the disciplines produced a significant increase in plasma cortisol concentration. The relative changes after exercise are in agreement with the 3-fold rise following endurance,[19] and the 2-fold rise after gallop race.[17] The cortisol values reported by Rose[16] after the cross country are higher than those reported in this study but were measured at an international event, which was longer and faster. Cortisol changes after show jumping have never been reported previously. In the present study, show jumping produced the smallest post-exercise plasma cortisol changes. This could be explained by the short duration and mild intensity of this exercise compared to the other disciplines.

The post-endurance plasma cortisol values were significantly ($p \leqslant 0.001$) higher than the mean of the post-exercise values of the other disciplines, indicating that an exercise of long duration and low intensity produces a higher plasma cortisol change than one of high intensity and short duration, like a gallop race. It must be emphasized that the post-exercise samples were taken immediately after the trial, so, for exercises of short duration, cortisol concentration may have continued to rise after collection of the post-exercise samples as has been demonstrated in man[2] and in other equine studies.[4,16] The fixed linear model shows that the discipline effect is significant on the resting cortisol values ($p \leqslant 0.05$). This could be explained by the fact that the resting cortisol is partly influenced by the type of training. A previous study suggested that the training stress stimulates the adrenocortical function.[14] So, the significantly lower resting values of jumping horses could be explained by the fact that these horses are subjected to a less intensive training than the others. For the post-exercise cortisol values, the discipline effect is more significant ($p \leqslant 0.01$). The data in Fig. 1 support this supposition. It should be emphasized that this discipline effect, although significant did not explain all the variations. Factors other than the exercise workload may also contribute to the variability observed after exercise.[25]

The effect of transport on the glucocorticoid changes have been reported.[23] In this study, the transport did not produce a significant plasma cortisol increase. The CV of the post-transport values was high, suggesting that while the 5 investigated horses were loaded and sampled in the same conditions, individual responses are important. This could be explained by transport-induced stress being highly influenced by individual psychological factors.

In the pharmacological study, the ACTH-induced plasma cortisol increase reported was of the same magnitude as the cortisol response described in ponies after intravenous ACTH injection at dosages of 100 or 200 µg.[10] When the post-ACTH cortisol values were compared to the corresponding post-exercise values, the relative increase in plasma cortisol was higher after the pharmacological experiment than after the physiological one ($p \leq 0.001$). This observation suggests that the effort performed by horses did not produce a maximal cortisol response. The strong correlation between the post-exercise and post-ACTH relative increase suggests that each animal has a specific stress-induced adrenocortical response independent of the type of stress experienced. Indeed, the horses which showed the lowest and the highest post-exercise cortisol increase were those which showed the lowest and the highest post-ACTH cortisol increase, respectively.

The results of this study suggest that 1) the discipline effect is significant on the plasma cortisol concentration both at rest and immediately after exercise, 2) the plasma cortisol increase produced by a standardized pharmacological test could be used to predict the exercise-induced cortisol response and 3) there is an important individual effect on transport-induced cortisol changes.

ACKNOWLEDGEMENTS

The authors are grateful to the organisers and riders participating in the competitive sports for permission to sample their horses. We wish to acknowledge Dr N. Kafidi, Prof. P. Leroy and Dr M. Buchet for valuable assistance and M. Leblond for typing the manuscript. Financial support was provided by Equine Research Funds.

REFERENCES

1 Beckert, B A, Nienaber, J A, Christenson, R K, Manak, R C DeShzer, J A and Hahn, G L (1985) Peripheral concentrations of cortisol as an indicator of stress in the pig Am J Vet Res 46, 1034–1038

2 Buono, M J, Yeager, J E and Hodgdon, J A (1986) Plasma adrenocorticotropin and cortisol responses to brief high-intensity exercise in humans J Appl Physiol 61, 1337–1339.

3 Burguez, P N., Ousey, J, Cash, R S G and Rossdale, P D (1983) Changes in blood neutrophil and lymphocyte counts following administration of cortisol to horses and foals Equine Vet J 15, 58–60

4 Church, D B, Evans, D L., Lewis, D R and Rose, R J (1987) The effect of exercise on plasma adrenocorticotrophin, cortisol and insulin in the horse and adaptations with training *In* Gillespie, J. R and Robinson, N E (eds): *Equine Exercise Physiology 2.* ICEEP Publications, Davis, CA, pp 506–515.

5 Davies, C T M and Few, J D (1973) Effects of exercise on adrenocortical function J Appl Physiol 35, 887–891.

6 Dybdal, N O, Gribble, D, Madigan, J E and Stabenfeldt, G H (1980) Alterations in plasma corticosteroids, insulin and selected metabolites in horses used in endurance rides Equine Vet. J 12, 137–140

7 Foss, M L, Barnard, R. J and Tipton, C M (1971) Free 11-hydroxycorticosteroid levels in working dogs as affected by exercise training Endocrinology 89, 96–104

8 Fulkerson, W J and Jamieson, P A (1982) Pattern of cortisol release in sheep following administration of synthetic ACTH or imposition of various stressor agents Austr J. Biol Sci 35, 215–222.

9 Hoffsis, G F, Murdick, P W, Tharp, V L and Ault, K (1970) Plasma concentrations of cortisol and corticosterone in the normal horse Am J Vet. Res 31, 1379–1387.

10 James, V H T, Horner, M W, Moss, M S and Rippon, A E (1970) Adrenocortical function in the horse J Endocr 48, 319–335

11 Krieger, D T, Allen, W, Rizzo, F and Krieger, H P (1971) Characterization of the temporal pattern of corticosteroid levels J Clin Endocr Metab 32, 267

12 Larsson, M., Edquist, L -E, Ekman, L and Persson, S (1979) Plasma cortisol in the horse, diurnal rhythm and effects of exogenous ACTH Acta Vet Scand 20, 16–24

13 Martinez, R., Godoy, A, Naretto, E and White, A (1988) Neuroendocrine changes produced by competition stress on the Thoroughbred race horse Comp Biochem Physiol 91A, 599–602.

14 Persson, S G B, Larsson, M and Lindholm, A (1980) Effects of training on adreno-cortical function and red-cell volume in trotters Zbl Vet Med A 27, 261–268.

15 Revington, M (1983) Haematology of the racing Thoroughbred in Australia 1: reference values and the effect of excitement Equine Vet J 15, 141–144.

16 Rose, R J, Ilkiw, J E, Sampson, D and Backhouse, J W (1980) Changes in blood gas, acid–base and

metabolic parameters in horses during three-day event competition Res Vet. Sci , 28, 393–395
17 Rossdale, P D , Burguez, P. N and Cash, R S G (1982) Changes in blood neutrophil/lymphocyte ratio related to adrenocortical function in the horse Equine Vet J 14, 293–298.
18 Snow, D H and MacKenzie, G (1977) Some metabolic effects of maximal exercise in the horse and adaptations with training Equine Vet J. 9, 134–140
19 Snow, D H and MacKenzie, G. (1977). Effect of training on some metabolic changes associated with submaximal endurance exercise in the horse Equine Vet J 9, 226–230.
20 Snow, D H and Munro, C. D (1982) Changes in blood levels of several hormones following ACTH administration and during exercise *In* Kitchen, H. and Krehbiel, J. D (eds): *Proc First Int Symp Equine Hematology*. Am. Ass Equine Practnrs , pp 481–489
21 Snow, D H , Kerr, M G., Nimmo, M A. and Abbott, E M (1982). Alterations in blood, sweat, urine and muscle composition during prolonged exercise in the horse Vet Rec 110, 377–384
22 Snow, D H , Mason, D K., Ricketts, S W and Douglas, T. A (1983). Post-race blood biochemistry in Thoroughbreds *In* Snow, D. H , Persson, S. G. B and Rose, R J. (eds): *Equine Exercise Physiology I*. Granta Editions, Cambridge, pp 389–399
23 Sonnichsen, H V. and Jorgenssen, K (1988) Untersuchungen zum Transportstress beim Pferd. Pferdeheilkunde 4, 35–36.
24 Tharp, G. D (1975) The role of glucocorticoids in exercise Med. Sci Sports 7, 6–11
25 Thornton, J. R (1985). Hormonal responses to exercise and training Vet Clin Nth Am , Equine Pract 1, 477–496
26 Toutain, P L , Oukessou, M., Autefage, A and Alvineric, M (1988) Diurnal and episodic variations of plasma hydrocortisone concentrations in horses. Domestic Anim Endocrinol 5, 55–59.
27 Valberg, S., Essén-Gustavsson, B , Lindholm, A and Persson, S G B (1989). Blood chemistry and skeletal muscle metabolic responses during and after different speeds and durations of trotting. Equine Vet J. 21, 91–95