EFFECTS OF TWO DIFFERENT EXERCISES ON PHYSIOLOGICAL STRESS RESPONSES OF TRAINING TROTTERS

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INTRODUCTION

The multidisciplinary strategy has been already used in the medicine field of sporting horse. Researchers have developed a series of analysis to monitor the psycho-physical condition of horses exposed to agonistic activity. In order to assess true horse adaptation response to physical strain endocrine-metabolic parameters have been used (Siciliano et al., 1995; Fazio et al., 1998; Nagata et al., 1999; Diverio et al., 2003a; 2003b; Barone et al., 2003a; 2003b). Nevertheless further studies are required in order to provide information useful for the protection of athlete horse welfare.

AIM OF THE STUDY

To assess the influence of intensity and duration of physical exercise on the magnitude of the physiological stress response in trotters.

MATERIALS AND METHODS

Animals

The experiment was carried out on March-July 2004, on a group of 12 trotters, aged 2-3 years, after the beginning of their training period. They were twice a day fed with a ration composed by hay and pellet feed.

Treatments

The 12 trotters were randomly subjected, with at least one-month interval, to two different treatments: a Standard Training (ST) and a High Standard Training (HST). The ST consisted in: after a warm-up of 3 laps horses went at steady trot for 2 laps (1600 m) (15 minutes long). The HST consisted in: after a warm-up of 3 laps horses went at their maximum trotting speed for 2 laps (1600 m) (15 minutes long).

Heart rate monitoring

All 12 experimental subjects were equipped with a Polar Accurex Plus HR monitor to record heart rate (HR) at T0 and T1.

Laboratory Analysis

From each subject, at every treatment (ST and HST), blood samples were collected from the jugular vein:

- just before beginning exercise (T0)
- immediately after the end of exercise (T1)
- after 60 minutes the end of exercise (T2)
- after 120 minutes the end of exercise (T3)

At each time, 15 ml blood samples were collected and divided into two evacuated tubes with anticoagulant (K-ETDA), 5 ml also added with 0.05 ml of aprotinine for avoiding protein

denaturation (for the β-endorphin assay). Samples were centrifuged at 2500 rpm and stored at –20°C until analysis.
Plasma were assayed for β-endorphin (Immunoassay, kit EIA Peninsula Laboratories), cortisol (Kit RIA Cortisol Orthoclinical Diagnostics), packed cell volume (PCV) by micromethod and total proteins, blood urea nitrogen (BUN), glucose, Ca, P, Fe, Mg, Cl and lactate deidrogenase (LDH) by colorimetric method (Analyser Express Plus 560, Bayer Diagnostics).

Statistical Analysis
Repeated-measures GLM was used to analyse the data for sampling time and treatments (ST vs HST). Least square means were evaluated by t-test. Adrenal cortex and opioid responses were further evaluated by Correlation Analysis (MINITAB® Release 14).

RESULTS AND DISCUSSION
A significant effect of sampling time ($P<0.001$) on plasma β-endorphin, cortisol, PCV, glucose, total proteins and HR was recorded, independently from intensity of physical exercise. In particular, plasma β-endorphin and total proteins concentration significantly increased at T1 ($P<0.001$) in both treatments (ST and HST), but returned to pre-exercise baseline values at T2 (Graph. 1 and 4; Table 1). Large increases of plasma β-endorphin have been reported in horses after a fast gallop (Li and Chen, 1987), walking and trotting (Mehl et al., 1999) and a treadmill exercise test (Alberghina et al., 2001).
Plasma cortisol and PCV concentrations showed a similar trend: they significantly increased at T1 but plasma cortisol values remained elevated up to T2, whereas PCV up to T3 (Graph. 2 and 3; Table 1).
Plasma Glucose concentrations were affected by sampling time after HST (from 4,36 mmol/l at T0 to 6.16 mmol/l at T1; $P<0.001$) (Graph. 5, Table 1).
Increasing intensity of exercise significantly affected ($P<0.001$) plasma cortisol, glucose and LDH concentrations (Table 1). However, all these parameters were higher after HST compared with those recorded after ST, with the exception of LDH activities (Table 1). Such an unexpected result, may due to differences in the pre-exercise muscle metabolic status. These findings seem to confirm that HST induced a greater endocrine-metabolic response to stress compared with ST.
A positive correlation between adrenal cortex and opioid responses was found, suggesting the presence of a mechanism of precursor co-release from the pituitary gland.
A large inter-individual variation ($P<0.001$) in electrolytes concentrations was observed.

CONCLUSIONS
Independently from intensity of exercise, training always elicited a transient endocrino-metabolic response, which was significantly related to sampling time.
Plasma cortisol and glucose concentrations seem to better represent the degree of physical exercise, as they further significantly increased after high-speed training.
Plasma β-endorphin release showed a significant relathionship with plasma cortisol release, confirming that physical stress can exert their precursor co-release from the pituitary gland.
The large interindividual variation in plasma electrolytes concentrations seem to suggest these parameters cannot easily be used as indicator of physical stress in trotters.

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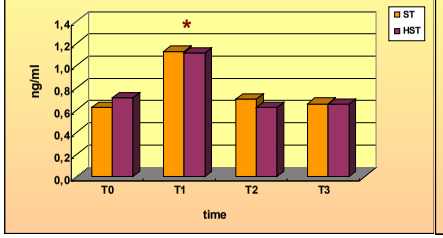
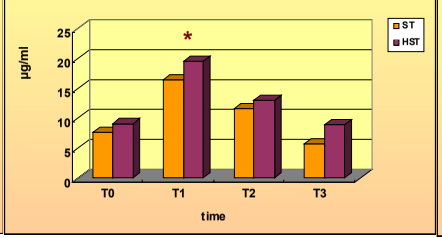
ACKNOWLEDGEMENTS

Research supported by the Perugia University (MURST-COFIN). The authors wish to thank Ermanno Mori and the personnel of the "Mori Trotter Stable" (Civitanova Marche, AN) for their precious technical assistance. The enthusiastic and patient help of the intern students of the Physiology Section, Dpt. Biopathological Science and Food and Animal Production Hygiene of Perugia University was mostly appreciated.

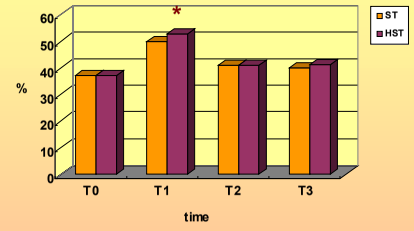
Graph. 1: Mean plasma b-endorphin concentrations for treatments and sampling time



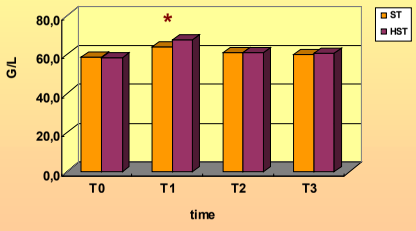
Graph. 2: Mean plasma cortisol concentrations for treatments and sampling time



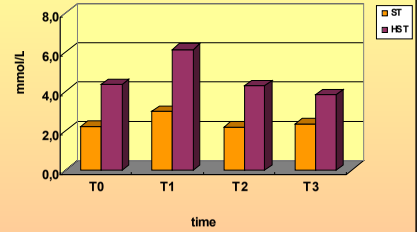
Graph. 3: Mean PCV values for treatments and sampling time



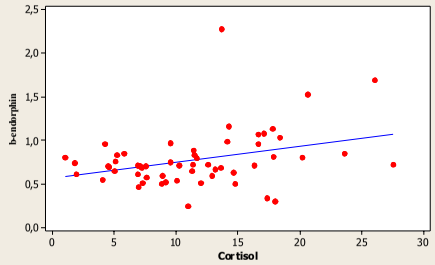
Graph. 4: Mean plasma total protein concentrations for treatments and sampling time



Graph. 5: Mean plasma glucose concentrations for treatments and sampling time



Regression between plasma cortisol and b-endorphin concentrations.



The regression equation is
ENDO = 0,565 + 0,0183 Cort.SIE
P<0.01

Table 1: Estimated means ± S.E. of endocrine-metabolic parameters for sampling time and treatments

	T0		T1		T2		T3			
Parameters	**ST**	**HST**	**ST**	**HST**	**ST**	**HST**	**ST**	**HST**	**Time**	**ST vs HST**
	means±S.E.	means±S.E.	means±S.E.	means±S.E.	means±S.E.	means±S.E.	means±S.E.	means±S.E.	**P <**	**P <**
β-endorphin (ng/ml)	0.62±0.06**B**	0.71±0.06**B**	1.13±0.17**A**	1.11±0.17**A**	0.70±0.05**B**	0.62±0.05**B**	0.66±0.07**B**	0.65±0.07**B**	**0.001**	**NS**
Cortisol (µg/dl)	7.59±1.61**B**	9.14±1.61**B**	16.54±2.35**A**	19.52±2.35**A**	11.64±1.24**C**	13.12±1.24**C**	5.78±1.04**B**	8.86±1.04**B**	**0.001**	**0.01**
PCV (%)	37.00±1.39**B**	37.00±1.39**B**	50.00±1.66**A**	52.67±1.66**A**	41.00±1.00**C**	41.00±1.00**C**	40.00±1.30**C**	41.33±1.30**C**	**0.001**	**NS**
Tot. proteins (g/l)	58.94±1.38**B**	58.67±1.38**B**	64.29±1.28**A**	67.56±1.28**A**	61.20±1.30**b**	61.23±1.30**B**	60.34±1.38**B**	60.66±1.38**B**	**0.001**	**NS**
BUN (mmol/l)	5.28±0.50	5.57±0.50	5.43±0.47	5.82±0.47	5.44±0.49	5.65±0.49	5.48±0.44	5.66±0.44	**NS**	**NS**
Glucose (mmol/l)	2.23±0.34	4.36±0.34**B**	2.98±0.46**A**	6.16±0.46**AB**	2.19±0.29**A**	4.31±0.29**BB**	2.37±0.20**A**	3.82±0.20**BB**	**0.001**	**0.001**
Ca (mmol/l)	2.69 ±0.11	2.68±0.11	2.53±0.05	2.81±0.05	2.70±0.19	2.56±0.19	2.67±0.14	2.33±0.14	**NS**	**NS**
P (mmol/l)	1.46±0.35	1.01±0.35	1.52±0.12	1.63±0.12	1.22±0.11	1.84±0.11	1.21±0.12	1.21±0.12	**NS**	**NS**
Fe (Umol/l)	39.40±8.34	33.14±8.34	42.71±10.05	44.29±10.05	43.13±8.64	34.80±8.64	42.83±13.17	34.03±13.17	**NS**	**NS**
Mg (mmol/l)	1.97±0.08	1.84±0.08**b**	1.94±0.07	2.03±0.07	1.93±0.08	1.83±0.08	1.95±0.10	1.90±0.10	**NS**	**NS**
Cl (mmol/l)	106.72±1.28	107.10±1.28	104.97±1.54	106.51±1.54	103.77±1.37	106.01±1.37	104.61±2.08	105.98±2.08	**NS**	**0.001**
LDH (IU/l)	874.57±50.51**A**	614.00±50.51**B**	927.86±93.68	838.71±93.68	926.57±54.35**A**	699.71±54.35**B**	973.00±58.97**A**	708.28±58.97**B**	**NS**	**NS**