GLUTATHIONE PEROXIDASE ACTIVITY AND ITS RELATIONSHIPS WITH CORTISOL AND OPPIOID RESPONSES TO TRAINING IN TROTTERS

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SUMMARY

Aim of the study was to assess how physical exercise influenced Glutathione Peroxidase (GPx) activity in trotter athletic horses. The experiment was carried out on October-November 2004, on a group of 7 trotters, aged 2-3 years, after the beginning of their training period. Each trotter was subject to a standard training (ST) (20 minutes): after a warm up of 3 laps horses went at a steady trot for two laps. Blood samples were taken just before beginning the ST (T1), soon after (T2), and after 60 (T3), 120 (T4), 180 (T5) and 240 minutes (T6) the end of the ST. Blood β-endorphin, cortisol, PCV and GPx were determined. Data were statistically analysed using GLM and correlation analysis. Training induced significant increases ($P<0,001$) of cortisol, PCV and HR at T2 ($P<0,05$), with values returning to baseline within 1 hour after ST (T3). Plasma β-endorphin concentrations showed a similar trend but differences were not significant. GPx activity also significantly ($P<0,001$) increased after the ST, but peak values were observed at T4 and values remained still elevated to T6. Non significant variations of GPx activities were recorded for age, sex, and individuals.

INTRODUCTION

In Equine Sport Medicine becomes very important to study the physiological adaptations induced by physical exercise. It is well known that the severity of exercise challenge can influence the physiological response to stress in horses (Nagata et al., 1999). A range of endocrine variables have been used to measure horse adaptation to physical stress but also to evaluate its degree of fitness (McCarthy et al., 1991**; Marc et al., 2000)**. Good training programmes for athletic competitive horses should aim to obtain good performances but, at the same time, to safeguard animal welfare.

However, the effect of physical stress by the analysis of such physiological and clinical responses could be better highlighted integrating also the evaluation of the concomitant oxidative stress (Hargreaves et al., 2002). Physical exercise has been shown to increase Reactive Oxygen Species (ROS) (Avellini et al., 1999; Clarkson and Thompson, 2000). However, training seems also induce a reduction of the oxidative stress through an increase of GPx activity in active skeletal muscles (for review; Powers et al., 1999). Oxidative stress may compromise horse welfare as it has also been associated to the development of some equine diseases, i.e. inducing membrane damage in recurrent exertional rhabdomyolysis (Valberg et al., 1993) or vascular defects in exercise-induced pulmonary haemorrhage (Williams et al., 2004).

In previous studies, evidences of oxidative stress in horses dealing with intense (Chiaradia et al., 1998; White et al., 2001) and endurance exercises (Marlin et al., 2002; Williams et al., 2004) have been already described. As far as we are aware, there are only few reports in the literature of antioxidant status and markers of oxidative damage in trotters (Trombetta and Falaschini, 2003). Aim of this study was to assess the effect of a standard physical exercise on Glutathione Peroxidase (GPx) activity and its relationship with stress induced endocrine-metabolic changes in training trotters.

MATERIALS AND METHODS

Animals

The experiment was carried out on October-November 2004, on a group of 7 trotters, aged 2-3 years, after the beginning of their training period. Horses were twice a day fed with a ration composed by hay and pellet feed. Every subject, three times a week, was subjected to a training section, and occasionally was left grazing in an external paddock (about 1000 m^2).

Treatment

For the experiment, each trotter was subject to a standard training (ST) (20 minutes): after a warm up of 3 laps horses went at a steady trot for two laps.

Laboratory Analysis

From each subject, at every ST, blood samples were collected from the jugular vein:

- before ST (T1)
- immediately after the end of ST (T2)
- 60 minutes after ST (T3)
- 120 minutes after ST (T4)
- 180 minutes after ST (T5)
- 240 minutes after ST (T6).

At each time, 20 ml blood samples were collected and divided into three evacuated tubes, without and with anticoagulant (sodium heparin and K-EDTA). For β-endorphin assay, 0.05 ml of aprotinine was added for avoiding protein denaturation in K-EDTA tubes (Immunoassay, Peninsula Laboratories, USA). Heart rate was also recorded using an automatic heart rate recorder device (Polar Accurex Plus, Finland) at T1 and T2. Blood samples were placed immediately in crushed ice and transported to the laboratory within 2 hours. Samples were divided into total blood (for GPx activity determination) and plasma aliquots (blood centrifuged at 2500 rpm x for 5 min at 4°C) then stored at –20°C until analysis. Plasma was assayed for cortisol by radioimmunoassay (Kit RIA Cortisol, Orthoclinical Diagnostics), for packed cell volume (PCV) by micromethod, and for glucose and total proteins by colorimetric method (Analyser Express Plus 560, Bayer Diagnostics). Glutathione Peroxidase activity (expressed in U/haematocrit: U/Ht) was determined by spectrophotometry with the use of Ransel kits (RS 505, Randox Laboratories Ltd, UK), following the method described by Paglia and Valentine (1967).

Statistical analysis

General Linear Model (GLM) for repeated measures (MINITAB® Release 14) was used to analyse the data for sampling time, individual and sex. Least square means were evaluated by t-test (SAS/GLM, PDIFF option). Relationship among adrenal cortex and opiod response, PCV and GPx were further evaluated by Correlation Analysis (MINITAB® Release 14).

RESULTS

No significant difference were recorded, in all parameters, for sex. Immediately after the end of the ST (T2), a significant increase ($P<0.001$) of cortisol, PCV and HR ($P<0.05$) was observed (Table 1; Graph.3, 4 and 5). However, whereas PCV and HR returned to baseline within 1 hour (T3) (Table 1; Graph. 4 and 5), plasma cortisol concentrations decreased within T4 (Table 1; Graph. 3). Plasma β-endorphin response showed a similar trend, but changes over the time where not significant (Table 1; Graph. 2). Total blood GPx activity showed no significant changes at T2, but progressively increased during recovery time (from T3 up T6) ($P<0.001$)

(Table 1; Graph. 1). GPx peak values were observed at T4 (149 ± 6.5 U/Ht) but values remained elevated up to T6 (Graph. 1). No significant differences in GPx activities were recorded for individuals (Graph. 7).
A large inter-individual variability (P<0.001) was recorded for cortisolo, β-endorphin and PCV, and total protein (Graph. 6). However, plasma glucose and total proteins concentrations always ranged within normal values (Meyer and Harvey, 1998).
A positive correlation was found between GPx activity and PCV (R = 0.1289; P<0.02).

Table 1: Estimated Means ± Std Err. of the endocrine-metabolic parameters and heart rate in relation to sampling time.

PARAMETERS	T1	T2	T3	T4	T5	T6	P<
GPx (U/Ht)	112 6.5 **Aa**	116 6.5 **Aa**	135 6.5 **b**	149 6.5 **B**	137 6.5 **b**	140 6.5 **b**	**0.01**
β-endorphin (ng/dl)	1.05 0.37 a	2.01 0.37 a	0.88 0.37 b	0.92 0.37 b	0.90 0.42 b	0.85 0.42 b	**NS**
Cortisol (μg/ml)	7.51 0.67 **aAb**	13.23 0.67 **D**	8.98 0.67 **aA**	5.87 0.67 **ab**	5.11 0.67 **aBc**	3.75 0.67 **Bc**	**0.01**
PCV (%)	40.3 1.2 **A**	47.4 1.2 **B**	39.7 1.2 **A**	39.4 1.2 **A**	38.9 1.2 **A**	38.3 1.2 **A**	**0.01**
Heart Rate (beats/min)	71 10 **A**	111 10 **B**					**0.05**
Glucose (mmol/L)	6.5 0.7	6.2 0.7	4.3 0.7	4.6 0.7	5.7 0.7	4.9 0.7	**NS**
Total protein (g/L)	62.5 1.3	64.6 1.3	64.8 1.3	62.7 1.3	61.3 1.3	60.9 1.3	**NS**

Legend: A,B,C,D: P<0.01; a,b,c,d: P<0.05.

DISCUSSION

The ST acted as a mild stress to the trotters, always inducing a transient increase of the circulating cortisol (P<0.001) and β-endorphin (Table 1; Graph. 3 and 2). Other studies reported circulating glucocorticoid level increases as effect of exercise in horses (Kurosawa et al., 1998; Nagata et al., 1999). This indicates the constant activation of the pituitary response to stress following exercise. There was no a significant correlation between plasma cortisol and β-endorphin concentrations in relation to sampling time. A large variation in the β-endorphin response after maximal exercise has been already observed (Art et al., 1994). However, no relation between these two parameters has been reported in another study (Art et al., 1994). Since after exercise, the peaks of cortisol are delayed respect those of ACTH (Church et al., 1987) this could explain the lack of significant parallelism between the increase in cortisol and β-endorphin concentrations observed in the present work. An additional factor, could be the large inter-individual differences recorded in these parameters.
Cortisol values at T1 were higher than those recorded during recovery, probably because a state of excitement related to learned signals advising the animals the onset of training (i.e., wearing the training equipment). This could also explain the moderate increase of plasma glucose concentrations recorded immediately before the ST (T1) (Table 1). Similar observations on training induced glucose changes have been already reported by other Authors (Art et al., 1994; Freestone et al., 1991). The higher PCV and total plasma protein concentration at T2 (total protein also at T3), although the increases are no significant, are a

common findings that coupled the elevation of cortisol as effect of exercise in horses (White et al., 1991).
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). In this study the opiod response seems to follow a similar trend, but this increase was only moderate and limited to T2, so no significant differences were recorded (Table 1).
The physical exercise involved in the ST seem to have an effect also on the antioxidant systems of trotters, as shown by the progressive and significant ($P<0.001$) increases of GPX (Table 1; Graph. 1). In particular, a 33% increase of whole-blood GPx activity was observed from T4 up to T6 respecting the baseline values (T1). A similar trend, in particular a 27% increase in RBC GPx was recorded in the last two stages (after 56-80 km) of an 80-km endurance rice (Williams et al., 2004).
The increase of GPx recorded in our study seems to suggest that, notwithstanding the short duration of the ST (20 minutes) compare to an endurance race, the entity of such an exercise was enough to evocate a perturbation in the antioxidant status (Hargreaves et al., 2002). However, the increase of GPx activity was detectable only two hours after the beginning of the ST (Table 1; Graph. 1). In other equine studies (Chiaradia et al., 1998; Marlin et al., 2002), plasma thiobarbituric acid-reactive substances (TBARS), which are an index of lipid peroxidation, increased only at the end of exercise and remained high for hours after recovery. Trombetta and Falaschini, (2003) did not observe a rise in GPx activity following a standardized exercise test in trotters. This results, apparently in contrast with our findings, may be related to the short sampling interval used by these Authors, considering the delayed increase of GPx activity respect to the beginning of exercise/increased oxygen consumption that we have observed.
The positive correlation between GPx and PCV is in agreement with previous studies (Lindner et al., 1992). The lack of correlations between endocrine variables and GPx activities could be due to the delay of the antioxidant change, here evaluated by GPx assay, compared with the activation of the pituitary response.

CONCLUSIONS

This study confirms that the physical exercise involved in the ST acted as a mild stress in trotters. Such an homeostatic response is well assessed by the variation of the endocrine-metabolic parameters investigated. However, the significant and progressive increases of the GPx activity recorded may give a deeper insight of the study of the physiological response to physical stress in trotters.

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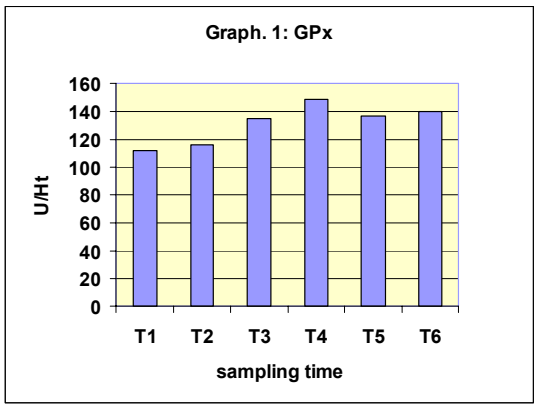
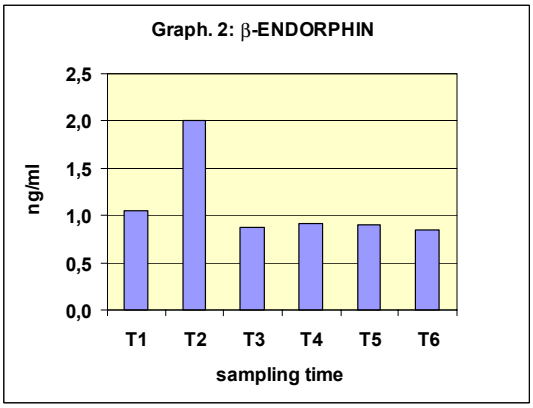
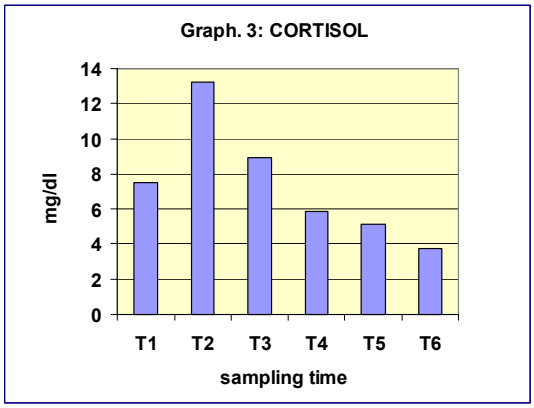
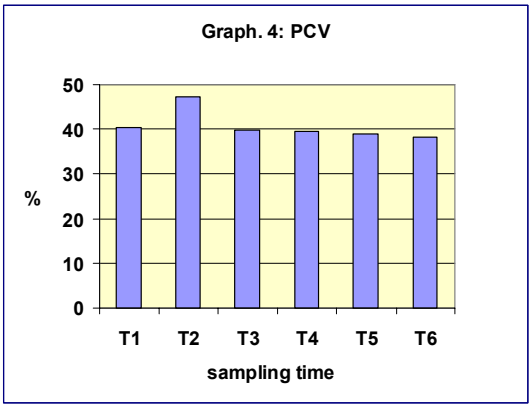
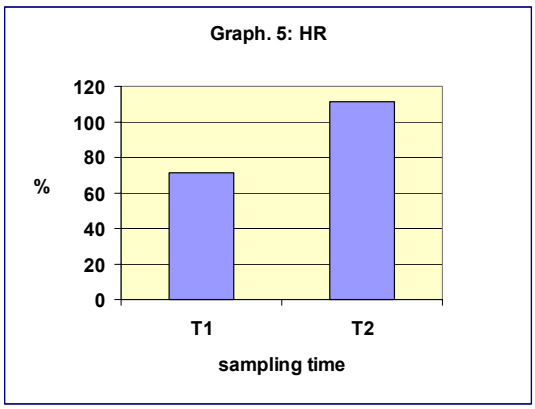
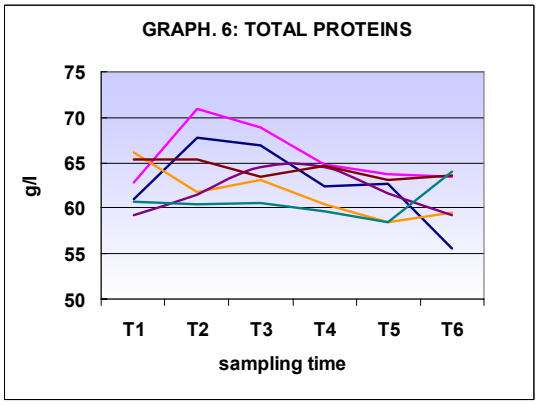

Graph. 1: GPx
U/Ht
160
140
120
100
80
60
40
20
0
T1
T2
T3
T4
T5
T6
sampling time

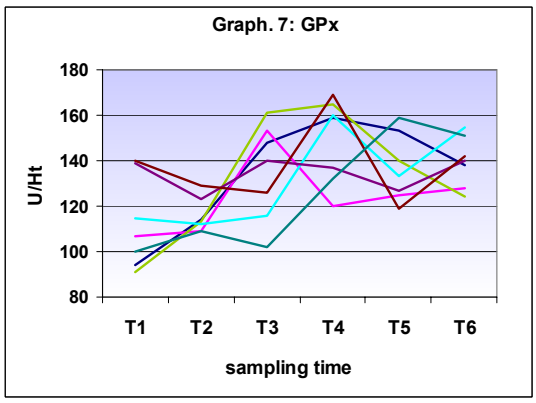

Graph. 2: β-ENDORPHIN
ng/ml
2,5
2,0
1,5
1,0
0,5
0,0
T1
T2
T3
T4
T5
T6
sampling time


Graph. 3: CORTISOL
mg/dl
14
12
10
8
6
4
2
0
T1
T2
T3
T4
T5
T6
sampling time


Graph. 4: PCV
%
50
40
30
20
10
0
T1
T2
T3
T4
T5
T6
sampling time


Graph. 5: HR
%
120
100
80
60
40
20
0
T1
T2
sampling time


GRAPH. 6: TOTAL PROTEINS
g/l
75
70
65
60
55
50
T1
T2
T3
T4
T5
T6
sampling time


Graph. 7: GPx
U/Ht
180
160
140
120
100
80
T1
T2
T3
T4
T5
T6
sampling time