Assessment of the oxidant–antioxidant blood balance in a field exercise test in Standardbred and eventing horses

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Abstract
The aim of this study was to determine which oxidant-antioxidant blood markers are of interest for a field exercise test (ET) performed on a racetrack. Healthy Standardbred horses (S: n = 12) and healthy eventing horses (E: n = 12) were investigated. Exercise was monitored by measuring velocity (V), heart rate (HR), and plasma lactate (LA). Whilst maximal LA did not differ (11.8 ± 0.88 mmol l⁻¹), maximal V (S: 12.3 ± 0.17 m s⁻¹ versus E: 11.1 ± 0.24 m s⁻¹, P < 0.05) and final HR (S: 222 ± 1 versus E: 203 ± 8 beats min⁻¹, P < 0.05) were significantly different between groups. Venous blood was collected at rest (R) prior to ET and the following oxidant–antioxidant markers were determined: uric acid (UA), ascorbic acid (AA), α-tocopherol (Vit E), vitamin A (Vit A), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione (reduced: GSH and oxidized: GSSG), glutathione redox ratio (GRR), copper (Cu), zinc (Zn) and selenium (Se), oxidized proteins (Protox), lipid peroxides (Pool), antioxidant capacity of water-soluble components (ACW) and antioxidant capacity of lipid-soluble components (ACL). The following markers were further determined 15 min (E15) after the ET: UA, ACW, AA, GSH, Protox, Pool, ACL. Standardbreds had significantly higher concentrations of ACW, GSH, ACL and Protox, whilst Se, Zn and SOD were significantly lower than in eventing horses. Exercise induced a significant increase in ACW and UA. GSH decreased in eventing horses and Pool significantly decreased in both horse groups. This study describes a field ET of high intensity for Standardbred and eventing horses, which could be performed by all animals tested. By sampling blood at rest and at E15, changes of the hydrophilic antioxidant defence were partially assessed, whereas no interpretable changes of the lipophilic antioxidants and of oxidation markers (Protox, Pool) could be detected.

Keywords: oxidative stress; exercise; field study; horses

Introduction
Oxidative phenomena are part of physiological processes, which occur naturally in plants, micro-organisms and living species¹,². Endogenous and exogenous antioxidants prevent the appearance of oxidative stress that is defined as an imbalance between antioxidants and pro-oxidants in favour of pro-oxidants³. During the last decade, research has provided evidence that oxidative stress might be harmful for the organism by damaging its biological components, such as DNA, lipids and proteins (for review, see Clarkson and Thompson⁴). Among the factors inducing oxidative processes are inflammation, degenerative diseases, ageing and intense exercise⁵–⁸.

In the equine species, oxidative stress has been intensively investigated in inflammatory lower airway disease, such as recurrent airway obstruction (RAO) or heaves⁹–¹² and ozone-induced irritation¹³. An oxidant/antioxidant imbalance also seems to favour the development...
of exercise-induced pulmonary haemorrhage\textsuperscript{14}, myopathy\textsuperscript{15}, neurological disorders\textsuperscript{16,17} and joint diseases\textsuperscript{18}. Exercise has also been shown to dramatically increase oxidative processes in horses. Given that oxygen consumption increases by 20–30 times during exercise\textsuperscript{19,20}, the amount of reactive oxygen species (ROS) released during the mitochondrial electron transfer (2–5\% of the mitochondrial oxygen consumption) disturbs the oxidant/antioxidant balance\textsuperscript{12,21,22}. The antioxidant enzyme superoxide dismutase (SOD) is positively correlated with the maximal oxygen consumption (VO\textsubscript{2max}) in Thoroughbreds, indicating that an appropriate antioxidant defence system might favour performance\textsuperscript{21}. Consequently, the maintenance of an optimal oxidant/antioxidant ratio has gained considerable importance for training and competition in man and horses\textsuperscript{23–25}.

Studies investigating exercise-induced oxidative stress in horses have most often been performed under laboratory conditions\textsuperscript{9,11,21,26,27}, but field studies on endurance horses are available\textsuperscript{28–31}. Most of these studies demonstrate that exercise-induced oxidative stress occurs, but also suggest that the oxidant/antioxidant imbalance is specific and depends on the type and duration of exercise as well as on the breed or type of horses tested (Saddlebred, Thoroughbred, endurance, etc.)\textsuperscript{21,32–34}.

Given the increasing interest and practice for investigating and controlling exercise-induced oxidative stress in horses in veterinary research, there might be a future need for field exercise tests (ETs). These tests need to be adapted to different disciplines and should be feasible under field conditions, which ideally implies an exercise test of relatively short duration and an assessment of the oxidant-antioxidant status by one pre- and one post-exercise blood sample. The objective of this study was to determine in healthy Standardbred and eventing horses the blood oxidant-antioxidant markers that might be of interest in a field ET.

Material and methods

Horses

Twelve clinically healthy Standardbred horses [S: 6 geldings, 6 mares; age [mean ± standard deviation (SD)]: 4.37 ± 1.6 years] and 12 clinically healthy warmblood eventing horses [E: 6 geldings, 6 mares; age (mean ± SD): 10.3 ± 3.5 years] were enrolled in this field study. All horses performed regularly in national competitions.

The Standardbred horses belonged to a single owner and underwent the same training regimen. The horses had daily access to a paddock. Training consisted of a twice-weekly trotting of c. 8 km at low speed (5–6 m s\textsuperscript{−1}) and three times weekly at a low speed trotting of 4 km followed by three 500 m steps of incremental speed (ranging from 8 to 12 m s\textsuperscript{−1}). Diet consisted of good quality hay (3–5 kg day\textsuperscript{−1}) and a commercial cereal blend for Standardbred horses (3–5 kg day\textsuperscript{−1}; 3.2\% lipids, 12\% proteins, 10\% starch, 8000 IU kg\textsuperscript{−1} Vit A, 1500 IU kg\textsuperscript{−1} Vit D, 120 mg kg\textsuperscript{−1} Vit E).

Eventing horses were kept altogether in another stable and were also trained under similar conditions by a professional rider. Each horse was trained for 1 h for five or six times per week (c. 20 min walk, c. 30 min trot, c. 10 min gallop). An additional canter of 15 min was performed twice weekly by controlling heart rate (HR), which ranged between 140 and 150 beats min\textsuperscript{−1}. The weeks during which the horses did not compete, both canter steps were prolonged by 1 min where the horses reached a HR higher than 180 beats min\textsuperscript{−1}. Diet consisted of good quality hay (3–5 kg day\textsuperscript{−1}) and a commercial cereal blend for eventing horses (3–5 kg day\textsuperscript{−1}; 2.75\% lipids, 10\% proteins, 8\% starch, 8000 IU kg\textsuperscript{−1} Vit A, 1000 IU kg\textsuperscript{−1} Vit D, 75 mg kg\textsuperscript{−1} Vit E).

At the time of the investigation (May 2004), all horses had been intensively trained for 3 months and were competing twice monthly.

Study design

Two blood samples were taken in each horse to assess its oxidant-antioxidant status, respectively, 30 min before and 15 min after a standardized field ET performed on a racetrack. During this test, HR, speed and plasma lactate were monitored. The ET was designed for each horse group; Standardbred horses were trotted tracking a sulky, whereas eventing horses were ridden by a professional rider and allowed to gallop. Both groups were tested within the same calendar month (May 2004). Outside temperature was recorded each day of investigation and ranged from 17 to 22°C.

Field exercise test

The ET was performed on the racetrack of Ghlin (Mons, Belgium). The ET was adapted to the discipline; it comprised, a warm-up walk (c. 5 min) and trot (3000 m at low speed) followed by three steps of determined speed and length. Standardbred horses were intended to exercise as follows: 1500 m at 8.3 m s\textsuperscript{−1}, 1500 m at 9.7 m s\textsuperscript{−1} and 1500 m at 11.1 m s\textsuperscript{−1}, whereas eventing horses had to perform the following test: 1000 m at 6.9 m s\textsuperscript{−1}, 1000 m at 8.3 m s\textsuperscript{−1} and 1000 m at 9.7 m s\textsuperscript{−1}. A final step of 1000 m was performed by each horse at its individual maximal speed. A cool-down walk of 15 min finished the ET.

Exercise monitoring

Measurement and control of speed

During the different exercise steps of the Standardbred horses, the driver controlled race speed using a
dry ice and kept at
whereas the second part was immediately frozen on
be analysed for ascorbic acid within 6 h of collection,
acid (MPA, 1:1 plasma:MPA) and was kept on dry ice to
of the plasma was stabilized with 10% metaphosphoric
trifugation of EDTA blood (15 min, 900 £
£

b

2

80

m

2

50

nium-trifluoro-methane-sulphonate for GSSG analysis;
oxidant capacity of lipid components in plasma (ACL),
components in plasma (ACW), ascorbic acid (AA), anti-
quotted into EDTA tubes [antioxidant capacity of water
horses were further equipped with the Equipilot6 GPS
(Fidelak, Germany) system, which allowed a posteriori
and continuous calculation of mean speed during each
race step.

Heart rate monitoring
HR was determined during the last 20–30 s of each exercise step using a telemetric electro-cardiogram recorder
(Life Scope 8, Nihon Kohden Corp., Tokyo, Japan). The recorder was placed along the racetrack in order to allow monitoring of HR over a distance of 300 m.

Venous blood lactate determination
After each exercise step, the horses were stopped for 1 min and venous blood (1 ml) was sampled by jugular venipuncture for analysis of plasma lactate (LA)
(Accusport6, Boehringer Mannheim, Germany).

Blood sample collection and processing for oxidant/antioxidant markers
Venous blood collection (maximally 55 ml) was performed by jugular venipuncture for all antioxidant marker analysis when the horses were at rest 30 min before the ET, and for markers known to be influenced by exercise21 15 min after the end of the ET. Blood was aliquoted into EDTA tubes [antioxidant capacity of water components in plasma (ACW), ascorbic acid (AA), anti-
oxidant capacity of lipid components in plasma (ACL), α-tocopherol, β-carotene (β-car), glutathione peroxidase
(GPx), superoxide dismutase (SOD), glutathione (GSH), oxidized protein (Protox), lipid peroxide (Pool) and haemoglobin (Hb)], into trace-element-free vacutainer NH143 IU tubes [Selenium (Se), copper (Cu), zinc (Zn)] and into heparin tubes [cholesterol, uric acid (UA), packed cell volume (PCV), total protein].

The blood samples were processed immediately after collection as follows. One part of whole blood (EDTA) was kept on ice for analysis of GPx, SOD and Hb within 8 h of collection. Further samples of whole blood (100 µl + 10 µl of 1-methyl-2-vinyl-pyridi
nium-trifluoro-methane-sulphonate for GSSG analysis; 50 µl for GSH analysis) were immediately frozen on dry ice and stored at −80°C until analysis. After centrifugation of EDTA blood (15 min, 900 × g), one part of the plasma was stabilized with 10% metaphosphoric acid (MPA, 1:1 plasma:MPA) and was kept on dry ice to be analysed for ascorbic acid within 6 h of collection, whereas the second part was immediately frozen on dry ice and kept at c. 80°C (ACW, ACL, α-tocopherol, β-car, prot-ox, Pool). Blood sampled into NH143 IU and heparin tubes was centrifuged (15 min, 900 × g) and plasma was frozen on dry ice and stored at −80°C until analysis of Se, Cu, Zn, uric acid and cholesterol. Analyses of frozen samples were performed within 7 days of collection.

Blood marker analysis
Blood marker analyses were performed by a laboratory specialized for oxidant-antioxidant markers and that was also certified to apply good laboratory practices.

Total antioxidant capacities
Plasma ACW and ACL were determined by photochem-
iluminescence detection using the Photochem assay (Photochem Analytic) according to the methods described by Popov and Lewin55,56. Intra-assay coefficient of variation (CV) of ACW was 5% and intra- and inter-assay CVs of ACL were 0.5 and 0.68%, respectively. Sample stability had been tested in preliminary trials and indicated at −20°C that decrease in ACW was more or less 50% within 24 h, but at 80°C, if analysis was performed within 48 h after collection, decrease in ACW was lower than 3%.

Ascorbic acid (AA)
Plasma concentrations of AA were spectrophotometrically determined according to the method described by Omaye et al.37 by using the reduction of 2,6-
dichloro-phenol-indophenol (Merck no. 1.03 028). The intra-assay CV was 4% and the inter-assay CV was 6%. As there were some concerns about sample stability at −20°C, it had been shown in a preliminary test that plasma concentrations of AA remained unchanged after immediate stabilization with MPA and storage at −20°C between 4 and 48 h after collection (data not shown).

α-Tocopherol and β-carotene
Plasma concentrations of α-tocopherol and β-carotene were determined by high-pressure liquid chromatography (HPLC) on reversed phase columns (C18 120, 100 × 45 mm) with an isocratic elution (methanol:water 98:2) and ultraviolet (UV) detection at 280 and 450 nm, respectively, for α-tocopherol and β-carotene.38 Intra- and inter-assay CVs were <3.7 and 9.3%, respectively.

Selenium, copper, zinc
Plasma concentrations of trace elements were determined by coupled mass spectrometry (Agilent 7500a ICP-MS, Palo Alto, CA, USA) according to the method described by Chappuis et al.39 The intra- and inter-assay CVs were 2.7–4.3% and 3.8–6.5%, respectively.

Superoxide dismutase (SOD) and glutathione peroxidase (GPx)
Whole blood was analyzed spectrophotometrically using RANSOD and RANSEL kits, (Randox Laboratories,
Antrim, UK). The intra- and inter-assay CVs were lower than 5 and 7%, respectively.

**Glutathione**
Whole blood glutathione (GSH and GSSG) was determined spectrophotometrically using the Bioxytech® kit GSH/GSSG 412 Assay (Oxis, Portland, OR, USA). The intra- and inter-assay CVs for GSH were 1 and 3%, respectively, and 6.5 and 7.6% for GSSG.

**Uric acid**
Plasma concentration of uric acid was analyzed spectrophotometrically using an automatic analyzer (Roche Hitachi, Roche Diagnostic, Vilvorde, Belgium). The intra- and inter-assay CVs were 2.1 and 4.2%, respectively.

**Pool**
Plasma concentration of lipid peroxides was analyzed spectrophotometrically using OxyStat Kit (Biomedica, Vienna, Austria) according to the method described by Hildebrandt et al. The intra- and inter-assay CVs were 7.2 and 11%, respectively.

**Protox**
Plasma concentration of oxidized proteins was analyzed spectrophotometrically allowing determination of carbonyl structures according to the method described by Reznick and Packer. The intra- and inter-assay CVs were 7.2 and 11%, respectively.

**Standardization of blood markers’ concentration**
α-Tocopherol was standardized by calculating the α-tocopherol/cholesterol ratio, GPx and SOD by expressing their activities per gram of haemoglobin (Hb) and Protox by expressing per gram of protein. Concentration of hydrophilic plasma markers (AA, UA) and trace elements was standardized by total protein concentration, whereas lipophilic plasma markers (α-tocopherol and β-car) were standardized by cholesterol. As GSH and GSSG were determined in whole blood, their concentrations were adjusted for changes of PCV. Adjustment was performed using the following equation:

$$X_{\text{adj}} = X_{\text{obs}} + \left( X_{\text{obs}} \times \frac{M_R - M_{\text{obs}}}{M_R} \right)$$

where $X_{\text{adj}}$ is adjusted marker concentration, $X_{\text{obs}}$ is measured marker concentration at (ti), $M_R$ is concentration of standardizing element determined at rest and $M_{\text{obs}}$ is concentration of standardizing element determined at (ti). ti is the time point at which $X_{\text{obs}}$ and $M_{\text{obs}}$ are measured.

**Statistical analysis**
Blood antioxidant concentrations measured at rest and 15 min after the ET were normally distributed and were analyzed by a mixed linear model for repeated measures (SAS®) allowing analysis of the effect of the sampling time, discipline and their interactions. Physiological variables and blood antioxidant concentration determined at rest were analyzed by a general linear model allowing analysis of the difference between disciplines. Differences were considered as significant when $P < 0.05$. Data are shown as least square means with associated standard error of mean (SEM).

**Results**

**Physiological variables**
The initially scheduled speed for each horse group and exercise step was verified a posteriori using data recorded by the GPS system. Differences between expected and effective race speed were higher for eventing horses than for Standardbred horses (Table 1).

Velocities at different HR for each group are shown in Table 2, as well as velocity at which LA equalled 4 mmol l⁻¹ (VLA4). At an HR of 180 (V180) and 200 (V200), velocity of Standardbred horses was significantly lower than that of eventing horses. In contrast, at VLA4, the velocity of Standardbred horses was higher than that of eventing horses. The HR reached

<table>
<thead>
<tr>
<th>Exercise step</th>
<th>Discipline</th>
<th>Expected velocity (m s⁻¹)</th>
<th>Effective velocity (m s⁻¹)</th>
<th>Relative difference from expected velocity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>S</td>
<td>8.3</td>
<td>8.2 ± 0.3</td>
<td>−1</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>6.9</td>
<td>7.7 ± 1.1</td>
<td>+10</td>
</tr>
<tr>
<td>Step 2</td>
<td>S</td>
<td>9.7</td>
<td>9.3 ± 0.4</td>
<td>−4</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>8.3</td>
<td>8.5 ± 0.3</td>
<td>+3</td>
</tr>
<tr>
<td>Step 3</td>
<td>S</td>
<td>11.1</td>
<td>10.6 ± 0.6</td>
<td>−4</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>9.7</td>
<td>9.6 ± 0.3</td>
<td>−1</td>
</tr>
<tr>
<td>Step 4</td>
<td>S</td>
<td>Maximum</td>
<td>12.3 ± 0.5</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>Maximum</td>
<td>11.1 ± 0.8</td>
<td>−</td>
</tr>
</tbody>
</table>
at the last exercise step (HR\textsubscript{end}) and maximal velocity (V\textsubscript{max}) was significantly higher in Standardbred horses than in eventing horses, whereas no significant difference was noted for LA\textsubscript{max}.

**Blood oxidant/antioxidant markers**

Among the oxidant/antioxidant markers examined before and after exercise, an exercise-induced increase was found for ACW, AA and UA in Standardbreds, whereas only ACW and UA increased in eventing horses (Fig. 1a–1c). Post-exercise concentrations of ACW, AA and UA were lower in eventing horses than in Standardbreds. Reduced glutathione remained unchanged in Standardbreds, whilst a significant decrease was observed in eventing horses, which further showed lower GSH concentrations than Standardbred trotters (Fig. 2a). GSSG concentrations remained unchanged in both groups, but GRR significantly increased at E15 in eventing horses (Fig. 2b). Oxidized proteins did not change by exercise but were significantly higher in eventing horses than in Standardbred trotters (Fig. 3a). Lipid peroxides were similar in both horse groups at rest and significantly decreased after exercise (Fig. 3b). The ACL-values determined in Standardbreds were significantly higher than those in eventing horses (8.8 ± 0.74 \textit{versus} 4.0 ± 0.76 \textmu mol eq TROLOX ml\textsuperscript{-1}) and were not affected by exercise.

For markers known to be unchanged by a single and intense exercise\textsuperscript{21}, and which were only determined in blood sampled before exercise at rest, discipline-related changes were found for Se (S: 160 ± 63 \textit{versus} E: 190 ± 7 \textmu g l\textsuperscript{-1}, P < 0.05), Zn (S: 0.49 ± 0.02 \textit{versus} E: 0.59 ± 0.028 mg l\textsuperscript{-1}, P < 0.05) and SOD (S: 1097 ± 60 \textit{versus} E: 1323 ± 66 UI g Hb\textsuperscript{-1}, P < 0.05). Concentration of Vit E (3.46 ± 0.2 mg g chol\textsuperscript{-1} or 6.6 ± 0.34 \mu mol l\textsuperscript{-1}) and the activity of GPx (252 ± 6.6 UI g Hb\textsuperscript{-1}) were similar in both groups.

**Discussion**

The assessment of the blood oxidant–antioxidant balance at rest and during or after exercise is gaining increasing interest in equine sport medicine. The objective of this study was to test the oxidant–antioxidant markers that might be of use for establishing under field conditions an oxidant–antioxidant status in exercising horses. Healthy Standardbred trotters and healthy eventing horses performed an adapted ET on a racetrack, which allowed monitoring of physiological variables such as HR and LA.

The present study investigated two groups of horses of very different disciplines and whose exercise physiology differs importantly. Indeed, competing Standardbred trotters are generally aged between 2 and 5 years and perform intense exercise bouts of a very short duration. Eventing horses are older once they start competing, they are trained to gallop over longer time periods but must also be able to perform more intense exercise of short duration.

Field ETs in Standardbred horses have been widely investigated and their interest for training follow-up and early detection of subclinical diseases is well known\textsuperscript{15}. The ET the Standardbred horses performed in our study was similar to the middle category test described by Leleu et al.\textsuperscript{44}, but our test included a last step at maximal velocity. The test we designed for eventing horses is less well adapted with regard to their training regimen and performance requirements. A test of longer duration at reduced velocity and including fences might have been more appropriate to mimic a competition, but our objective was to use the same test for all horses, independent of their competition level. Our test was feasible for all horses, and the expected velocity could be reached during the last three exercise steps, although this is much more difficult when a horse is ridden than when tracking a sulky. The GPS system used for \textit{a posteriori} velocity control appeared to be an interesting tool for calculation of effective mean speed at each step.
Even if it might be argued that the ET the eventing horses performed was too intense with regard to velocity, the final HR they reached (203 ± 8 beats min⁻¹) was similar to that recorded in eventing horses during their cross country day (195 ± 8 beats min⁻¹). As plasma LA max recorded in these horses (10.2 ± 4.2 mmol l⁻¹) was also very similar to the values obtained in our study (11.8 ± 0.88 mmol l⁻¹), the exercise intensity of our test seemed to be comparable to that of a competition.

The comparison of the physiological exercise variables shown in Table 1 reflects, on the one hand, that both horse groups performed an ET of high intensity where a final HR higher than 200 beats min⁻¹ and a LA max higher than 10 mmol l⁻¹ were reached. Between-discipline comparisons are difficult because different ETs had been performed. Furthermore, age, weight, gait, training, and possibly feeding-related differences between horse groups might play a role in these results. The exercise monitoring did not intend to evaluate the athletic capacity or performance of the horses, but essentially aimed at controlling whether a standardized ET of high intensity could be realized under field conditions.
Post-exercise blood sampling for oxidant-antioxidant markers was performed 15 min after the end of the ET. The decision for sampling relatively late after the exercise took into account (1) that a relatively large blood volume had to be sampled carefully and without risk for horse, rider or driver and the investigator, which implied that the horse had recovered from the ET; (2) that LA determination immediately after the exercise steps was not a prerequisite for the assessment of the blood oxidant-antioxidant status (meaning that the possibility for safe sampling immediately after maximal exercise was not considered); and (3) that earlier studies performed under laboratory conditions had shown that 15 min post-exercise was an appropriate moment for several markers. Nevertheless, we were aware that the time point selected carried the risk that some modifications would not be optimally evidenced and that repeated sampling would have been preferable.

Among the blood oxidant/antioxidant markers sampled at rest and at E15, most exercise-related changes were similar to those reported in a study performed in Standardbred horses on a treadmill under laboratory conditions. In Standardbred horses as well as in eventing horses, ACW and UA significantly increased after exercise, even if modifications in eventing horses were less pronounced than in Standardbred horses. These differences are most likely due to a higher workload during exercise in Standardbred horses, UA being known to depend on exercise intensity. As UA significantly contributes to ACW in exercising individuals, the increase in ACW might be attributed to UA rather than to AA, which only significantly increased in Standardbreds.

The concentration in red blood cell GSH was expected to decrease, but this was only observed in eventing horses where an increase of GRR was also noted. As GSH concentration has been shown to reach its minimum at peak exercise and to return back to baseline values within 15–30 min, it is possible that the maximal effect of exercise on GSH could not be assessed.

Antioxidant capacity of lipid-soluble components (ACL) remained unchanged by exercise in both groups, as well as Protox. Protein carbonyls, or Pool, have been shown to be increased only at 4 and 24 h post-exercise in trained Standardbred trotters, and at 24 h post-exercise in eventing horses, indicating that this marker is suitable for delayed assessment of exercise-induced oxidative stress but is of no interest as a short-term marker after exercise.

Pool was significantly decreased at E15 in both groups. A similar trend of lipid peroxidation had been shown using the lipid peroxidation marker 8-iso-PGF$_{2\alpha}$ in plasma of horses submitted to a standardized treadmill exercise. A significant increase in 8-iso-PGF$_{2\alpha}$ was observed at peak exercise, whereas at 60 min post-exercise, 8-iso-PGF$_{2\alpha}$ tended to be decreased. Mills et al. assessed malondialdehyde as a marker of lipid peroxidation in Thoroughbred horses and demonstrated a significant increase of urinary clearance during recovery, which would explain the decrease of Pool at E15 observed in our study. However, this hypothesis needs further investigations to be confirmed.

Pre-exercise differences of oxidant-antioxidant markers between Standardbred and eventing horses were likely to be influenced by differences in management and feeding regimens. Although the lack of feeding standardization presents a drawback for this study and makes interpretation hazardous, several observations are worth mentioning. Standardbred horses had significantly lower concentrations of Zn, which might have contributed to a decreased SOD activity in these animals, Zn being an important catalyzing trace element of this enzyme. Although Se was also lower in Standardbred horses, GPx activity did not differ between groups. Increased GSH concentration in Standardbreds might have contributed to an improved antioxidant defence against protein oxidation, which was higher in eventing horses. Similarly, a higher lipophilic antioxidant defence in Standardbreds, reflected by ACL, might have reduced lipid peroxidation in this horse group, whereas low ACL and increased Pool values were observed in eventing horses.

By comparing the oxidant-antioxidant marker results of this field study with those obtained during laboratory tests, it appears that E15 post-exercise sampling provided only partial evidence for an increased oxidative burden. Indeed, the hydrophilic antioxidants UA and ACW were increased in both horse groups, whereas AA only increased in Standardbred trotters and decrease of GSH only occurred in eventing horses. Changes of the lipophilic antioxidant capacity were not detected by ACL. The oxidation marker Protox was unchanged at E15, whereas Pool significantly decreased in both horse groups. More information might be obtained if the post-exercise sample is taken as soon as possible after the end of the last exercise step. Furthermore, it would be interesting to perform later sampling points, for example 24 h post-exercise, for Protox, SOD and GPx, but they are poorly compatible with a field investigation.

**Conclusion**

This study describes a specific field ET of short duration and relatively high intensity designed for Standardbred trotters and eventing horses. Oxidant-antioxidant blood makers determined at rest prior to exercise showed differences between horse groups.
that might have been influenced by feeding and training of the horses. Fifteen minutes post-exercise blood sampling was of interest for the hydrophilic antioxidants UA, AA and ACW. However, variations of lipophilic antioxidants and oxidation markers could only be poorly assessed or interpreted.

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