Oral supplementation with superoxide dismutase in Standardbred trotters in training: a double-blind placebo-controlled study

C. NOTIN, **L. VALLON**, **F. DESBORDES**[†] and **C. LELEU**[‡]*

SEPPIC, Puteaux (92); [†]Clinique St Herbot, Chateaulin (29); and [‡]EQUI-TEST, Villiers Charlemagne (53), France.

Summary

Reasons for performing study: **Intense physical exercise produces an excess of reactive oxygen species which can disturb the antioxidant/oxidant balance of the horse in training. Several classes of antioxidant dietary compounds have been suggested to provide health benefits and there is evidence that consumption of these products leads to a reduction in the expression of various pro-inflammatory and/or oxidative stress biomarkers. The recent development of a new galenic system allows the oral delivery of the antioxidant enzyme: superoxide dismutase (SOD). This has been developed from a specific melon variety with a particularly high SOD activity.**

Objectives: **To study the influence of an oral supplementation with an encapsulated melon rich in SOD on muscular and antioxidant balance variables in a population of Standardbreds in training.**

Methods: **Twenty-four Standardbreds in training were paired by age, sex and training level. They were randomly split into 2 groups: SOD group (520 iu/day) and placebo group. At the beginning of the study (T0) and after 30 days (T30) and 60 days (T60) of supplementation, physiological response during a standardised exercise test, plasma muscular enzymes at rest and post exercise (creatine kinase), oxidative stress markers (erythrocyte SOD) and blood resistance to haemolysis (KRL test) were assessed. Analysis of variance of time, treatment and interaction time x treatment was calculated.**

Results: **Between T0 and T60, in contrast with placebo group, a significant increase in the plasma resistance to haemolysis in the SOD group was observed and it was significantly higher (P<0.05) in the SOD group than in the placebo group on T60. Between T0 and T60, resting CK remained constant in SOD group whereas a significant increase in plasma CK in the placebo group was observed. On T60, the CK level was significantly lower (P<0.05) in SOD group than in the placebo group.**

Conclusions: **These results suggest that oral SOD supplementation might increase blood resistance to haemolysis and reduce the increase in muscular membrane permeability induced by training.**

Introduction

Many classes of antioxidant dietary compounds have been suggested to present health benefits for athletic horses and there is evidence that consumption of these products leads to a reduction of the expression of different oxidative stress biomarkers. In athletic horses, various antioxidant supplementation trials have provided evidence that exercise-induced disturbances could be partially prevented. McMeniman and Hintz (1992) studied the oral vitamin E supplementation in polo ponies and demonstrated a negative correlation between blood vitamin E concentration and some markers of lipid peroxidation. Avellini *et al*. (1999) also investigated the influence of vitamin E and selenium supplementation in racehorses and described an increase of antioxidant capacity. The influence of vitamin C supplementation has been studied in Thoroughbreds (White *et al*. 2001) and endurance horses (Williams *et al*. 2004a). White *et al*. (2001) found that vitamin C supplementation could prevent exercise-induced increase of some markers of lipid peroxidation and could maintain the plasma antioxidant capacity. On the other hand, Williams *et al*. (2004a) observed no treatment effect on muscle enzymes and markers of oxidative stress. Williams *et al*. (2004b) studied the effect of a supplementation with lipoic acid in endurance horses. The authors reported an increase of antioxidant status in supplemented horses. Those finding were confirmed by Kinnunen *et al*. (2005) in Standardbred trotters. Finally, in a field study, De Moffarts *et al*. (2005) investigated the effect of a mixture of vitamins and trace elements on 40 Thoroughbreds during a 3 month period. The authors concluded that oral antioxidant supplementation could improve the hydrophilic, lipophilic and enzymatic antioxidant blood capacity.

The major role of antioxidants is to inactivate or to transform oxidants, which can be transformed by antioxidant enzymes into less reactive forms or which can react with antioxidant molecules that are chemically stable. With catalase and gluthation-peroxidase (GPX), superoxide dismutase (SOD) is one of the most important antioxidant enzymes. Superoxide dismutase is an endogenously produced metalloenzyme present in every aerobic cell. It catalyses the dismutation of superoxide (O_2^-) into oxygen and hydrogen peroxide.

In a specific variety of cantaloupe melon (*Cucumis melo L*), it was demonstrated that correlations exist between the maintenance

of cellular integrity (i.e. a delayed senescence) and a particularly high level of SOD activity (Lacan and Baccou 1998). Following this finding, an encapsulated extract of this specific variety of cantaloupe melon has been developed and the effect of oral administration of this cantaloupe melon extract (CME) rich in SOD activity was studied in different experimental models. Vouldoukis *et al*. (2004a) confirmed the antioxidant and anti-inflammatory properties of CME *in vitro*, by measuring the effects of CME on redox status and production of pro- and anti-inflammatory cytokines by peritoneal macrophages. The anti-inflammatory properties of CME were principally related to its capacity to induce the production of anti-inflammatory cytokines by peritoneal macrophages. They also confirmed these properties by *in vivo* experimentation with a CME oral supplementation of mice. Moreover, it was demonstrated that an oral supplementation in CME could increase the resistance of red blood cells to oxidative stress-induced haemolysis in mice (Vouldoukis *et al*. 2004b). Muth *et al*. (2004) concluded to the efficacy of an orally effective SOD on hyperbaric oxygen-related cell damage. Naito *et al*. (2005) showed that a gastroprotected form of SOD could reduce the diabetes-induced renal oxidative stress in mice. The antioxidative effect of pumpkin seed protein isolate was also demonstrated in CCL4-induced acute liver injury in low-protein fed rats (Nkosi *et al*. 2006). Finally, Kick *et al*. (2007) concluded that oral CME may be a therapeutic option to reduce oxidative cell injury with a model of induced ischaemia/reperfusion (aortic cross-clamping) injury in pigs.

So far, no data are available on the influence of supplementation with CME selected for its high SOD activity on antioxidant equilibrium in athletic horses. The aim of this study was to evaluate the effects of a 60 day oral supplementation with a CME on muscular and antioxidant balance variables in a population of trotter horses in training.

Materials and methods

Horses and study design

Twenty-four clinically healthy Standardbreds in training for a minimum of 4 months were recruited for the experiment in 3 different stables. The population was composed of 10 females, 10 males and 4 geldings. The age distribution of horses was six 2-year-olds, eight 3-year-olds, six 4-year-olds and four 6-year-olds. In each stable, horses were trained and fed by the same individuals. All horses were stabled in individual straw boxes from 07.00–18.00 h and kept on a sand paddock during the night. They were fed 3 times daily with hay (approximately 6 kg/day) and a complete industrial feed (approximately 6 kg/day) providing an average intake of 2500 mg vitamin E, 2.5 mg of selenium. No oral supplements were administrated before and during the protocol.

All horses were exercised according to a traditional training schedule: 2 high intensity training sessions per week completed by 2 or 3 low intensity training sessions. High intensity workouts were usually composed of 2 bouts of 2500 or 3000 m at medium speed (i.e. 9 m/s for 2-year-olds to 11 m/s for older horses) ending with 500 m at maximal speed. Low-intensity workouts usually consisted of 30 to 45 min at an average speed of 7.5–8 m/s. Two-year-olds were not yet involved in competition but were in preparation for qualification. Older horses were in competition and raced regularly.

Twelve pairs were constituted on the basis of same sex, same age and same level of physical condition. Then horses of each pair

were randomly assigned either in the initially called '*group A*' corresponding to the supplemented group (SOD group), or in the initially called '*group B*' corresponding to the placebo group (PLACEBO group). In each pair of horses, the training and nutrition schedules were exactly equivalent during the whole trial. The experiment was double blind in that neither operator nor trainer knew which of the *A* or *B* groups received the SOD supplementation or the placebo.

SOD supplementation and placebo

The SOD supplementation was based on the consumption of a manufactured product (Promutase 200)[1]. The manufacturing process consists in concentrating and freeze-drying of the juice of a specific melon variety with a guaranteed SOD activity. It is then coated with a fat matter authorised in animal feed, adsorbed on a mineral carrier and diluted in a vegetal excipient. The coating secures the protection of SOD activity from gastric juices and high temperature and makes its blending in animal feed easy. The daily dose of SOD was 520 iu per horse on recommendations of the manufacturer, based on unpublished field studies in horses. The placebo product was composed of the same excipient with no active substance. The supplement or the placebo was given once daily mixed in the ration by the same feeder. The correct ingestion of the supplement or the placebo was systematically verified by the feeder. Both supplement and placebo were well tolerated by horses and no adverse reactions were reported.

Sampling

On Days 0, 30 and 60, respectively, noted T0, T30 and T60, a standardised protocol of measurements was followed. Total bodyweight was measured with an electronic weighting scale[2] and body composition evaluated by measuring the rump fat thickness with an ultrasound methodology (Westervelt *et al*. 1976). A clinical examination and blood collection by jugular puncture were performed at rest before training. All horses had not been racing or training very intensely in the last 48 h before sampling. Blood was collected on EDTA tubes (basic haematology, blood resistance to haemolysis) and heparin tubes (biochemistry, erythrocytes SOD and GPX activities). All samples were kept at 4°C for analysis within 6 h of collection for haematology and biochemistry analysis and within 24 h of collection for erythrocytes SOD and GPX activities and KRL test.

Blood haematology and biochemistry

Haematology: Basic resting haematology data: packed cell volume (PVC), haemoglobin concentration (Hb), red blood cells (RBC), white blood cells (WBC) were evaluated with automated analyser (Scil Vet abc)[3].

Biochemistry: Plasma creatine kinase (CK) activity was measured at rest and 3 h after the standardised exercise test.

Erythrocyte SOD and GPX activities: Erythrocytes SOD and GPX activities were assessed by spectrophotometry using Ransod and Ransel kits[4]. Intra and inter-assays CVs were lower than 5 and 7%, respectively.

Blood resistance to haemolysis

The antiradical potential of each horse's blood was evaluated using a KRL test which is a biological test of blood resistance based on free radical induced haemolysis (Blache and Prost 1992; Stocker *et al.* 2003; Lesgards *et al.* 2005). After sampling on EDTA tubes, the blood, diluted to 1/50 in isotonic saline solution, was submitted to organic free radicals produced at 37°C under air atmosphere from the thermal decomposition of a 27 mmol/l solution of 2,2′ azobis (2-aminido-propane) dihydrochloride (Spiral, Dijon, France). The extracellular and intracellular antioxidant defences contribute in maintaining blood cell membrane integrity and function until cell lysis. Haemolysis is recorded using a 96 well microplate reader by measuring the optical density decay at 450 nm. Results are expressed as the time that is required to reach 50% of maximal haemolysis. Half haemolysis time for total blood cells (HT50 WB), expressed in minutes, refers to the whole blood resistance to free radical attack. Half haemolysis time for red blood cells (HT50 RC), expressed in minutes, refers to the red cell resistance to free radical attack. The measurement of HT50 has been shown to be representative of the overall defence against free radicals in humans and animal models. The plasma resistance to haemolysis (HT50 P), also expressed in minutes, is calculated by deducting HT50 RC from HT50 WB. Intra and inter-assays CVs of KRL test were of 2.5 and 4%, respectively.

Standardised exercise test and physiological measurements

Exercise tests were performed on training sand tracks under defined environmental conditions: dry track, temperature of 10–20°C, little or no wind to avoid any climatic influence. Each horse started with a 10 min warm-up at about 6 m/s and then did a 3step test at increasing speed. The duration of each step was 3 min, with a 1 min rest between 2 steps. The velocity of the 3 steps was defined in accordance with the age of each pair. For 2-year-old horses, the velocities of each step were 8, 9 and 10 m/s; for the 3-year-olds, the velocities were 8.5, 9.3 and 10.5 m/s; finally for older horses, the velocities were 8.5, 10 and 11 m/s. At the end of the test, each horse completed a 10 min recovery period at the speed of 6 m/s preceding a passive recovery.

Trotting speeds and heart rates were measured and recorded using a polar cs600[5]. The driver used information on the screen to keep the speed as constant as possible during each step. The same device recorded heart rate during exercise. Following the test, the data from tachymeter and heart rate meter were downloaded to a laptop computer to determine the mean speed and heart rate during each step. Jugular venous blood samples were collected within one minute of the end of each step. The blood was collected into tubes containing fluoride oxalate for later determination of whole blood lactate concentration by the enzymatic method of Boehringer.

We calculated 2 physiological variables for each horse from the information on speed, heart rate and blood lactate concentration at the 3 steps:

V_4: velocity of a 4 mmol/l blood lactate concentration,

V_{200}: velocity for a 200 beats/min heart rate.

Statistical analysis

All variables measured on T0, T30 and T60 were analysed using statistical software[6]. Analysis of variance (GLM) was calculated to assess effects of time, treatment and time-treatment interaction. A level of significance of P<0.05 was used throughout this study for all the tests.

Results

Weight and body composition

There was no evolution during the experiment and no difference between groups in weight and body composition of the horses.

Haematology and biochemistry

Table 1 shows the mean resting PVC, Hb, RBC, WBC and post exercise plasma CK in the SOD group and the placebo group between T0 and T60. No change in any of the basic haematological data was observed in time and between the 2 groups.

Figure 1 represents the mean and s.e. of resting plasma CK activity in both groups on T0, T30 and T60. Resting plasma CK activity remained constant in SOD group whereas plasma CK activity increased significantly in placebo group. At T30 and T60, plasma CK activity in placebo group was significantly higher than in SOD group (P<0.05). Post exercise CK activities were not different between the 2 groups at any time of the study.

Resting erythrocyte SOD and GPX activities

Table 2 shows the mean erythrocytes SOD and GPX activities in the SOD group and the placebo group between T0 and T60. In the

TABLE 1: Haematological and biochemical variables in the SOD group (n = 12) and placebo group (n = 12) before supplementation (T0) and 30 (T30) and 60 (T60) days after oral supplementation

Variables	Groups	T0	T30	T60	Treatment effect	Time effect
PVC	SOD group	38.3 ± 1.1	38.8 ± 1.3	38.5 ± 1.3	ns	ns
(%)	Placebo group	38.1 ± 1.3	37.8 ± 1	38.5 ± 1.2		
Hb	SOD group	12.7 ± 0.4	12.9 ± 0.5	13.1 ± 0.4	ns	ns
(g/dl)	Placebo group	12.7 ± 0.4	12.4 ± 0.3	12.9 ± 0.4		
RBC	SOD group	9.1 ± 0.4	9.1 ± 0.4	9.2 ± 0.3	ns	ns
(10^6/mm³)	Placebo group	9.2 ± 0.3	9.2 ± 0.3	9.1 ± 0.3		
WBC	SOD group	8.46 ± 0.73	8.69 ± 0.55	9.45 ± 0.41	ns	ns
(10^3/mm³)	Placebo group	8.06 ± 0.33	8.34 ± 0.44	8.8 ± 0.41		
Post exe CK IU/l	SOD group	230 ± 20	263 ± 32	247 ± 21	0.007	ns
	Placebo group	290 ± 26	325 ± 33	347 ± 56		

Data are shown as mean ± s.e. PCV, packed cell volume; Hb, haemoglobin concentration; RBC, red blood cells count; WBC, white blood cells count; post exe CK, creatine kinase post exercise; ns, nonsignificant.
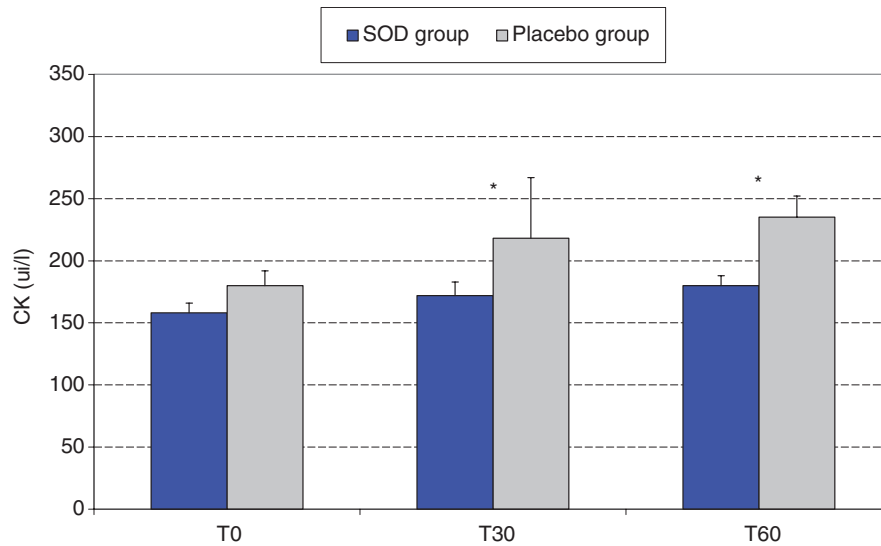
*Fig 1: Evolution of the resting plasma creatine kinase activity in the SOD group (n = 12) and placebo group (n = 12) before supplementation (T0) and 30 (T30) and 60 (T60) days after oral supplementation. Data are shown as mean ± s.e. *: SOD group different from placebo group (P<0.05).*

TABLE 2: Antioxidant enzymes, blood resistance to haemolysis and physiological variables in the SOD group (n = 12) and placebo group (n = 12) before supplementation (T0) and 30 (T30) and 60 (T60) days after oral supplementation

Variables	Groups	T0	T30	T60	Treatment effect	Time effect
SOD	SOD group	1214 ± 51	1026 ± 40	1137 ± 40	ns	0.000
(iu/g Hg)	Placebo group	1147 ± 46	900 ± 54	1049 ± 47		
GPX	SOD group	174 ± 13	188 ± 12	193 ± 15	ns	ns
(iu/g hg)	Placebo group	185 ± 15	198 ± 12	188 ± 15		
HT50WB	SOD group	76.9 ± 1.8	75.5 ± 1.5	79.8 ± 1.9	ns	ns
(min)	Placebo group	76 ± 0.8	73.8 ± 1.2	76 ± 1.4		
HT50RC	SOD group	58.6 ± 0.6	56.1 ± 0.7	55.3 ± 0.8	ns	ns
(min)	Placebo group	58.7 ± 0.7	56.03 ± 0.7	56.6 ± 0.5		
V_4	SOD group	619 ± 12	621 ± 10	637 ± 12	ns	ns
(m/min)	Placebo group	602 ± 8	610 ± 6	621 ± 10		
V_{200}	SOD group	602 ± 18	606 ± 16	613 ± 18	ns	ns
(m/min)	Placebo group	583 ± 11	582 ± 9	585 ± 9		

Data are shown as mean ± s.e. SOD, erythrocytes superoxide dismutase activity; GPX, erythrocytes glutathione peroxidise activity; HT50WB, half haemolysis time for total blood cells; HT50RC, half haemolysis time for red blood cells; V_4, velocity for a blood lactate concentration of 4 mmol/l; V_{200}, velocity for a heart rate of 200 beats/min. a: SOD group different from placebo group (P<0.05). ns, nonsignificant.

2 groups, we observed a decrease in SOD activity between T0 and T30 and a significant increase between T30 and T60.

There was no significant difference in GPX activity between the 2 groups at any time of the experiment nor any significant evolution over the time of the study.

Blood resistance to haemolysis

Table 2 shows the mean HT50WB and HT50RC in the SOD group and the placebo group between T0 and T60. Figure 2 represents the means and sem of plasma resistance to haemolysis (HT50 P) in both groups on T0, T30 and T60. Between T0 and T60, a significant increase in the plasma resistance to haemolysis is observed in the SOD group but not in the placebo group. On T60, the plasma resistance to haemolysis was significantly higher in the SOD group compared to the placebo group (P<0.05).

Standardised exercise test and physiological measurements

Table 2 shows the mean V_4 and V_{200} in the SOD group and the placebo group between T0 and T60. There was no

significant difference between the 2 groups at any time of the experiment.

Discussion

Changes in plasma CK

As a blood marker of muscle integrity, CK activity was determined in plasma both at rest and post exercise. We observed that resting plasma CK activity remained constant during the 60 day period in SOD group whereas plasma CK activity increased significantly in placebo group. On T30 and T60, plasma CK activity in placebo group was significantly higher than in SOD group. Those findings were similar to those observed in a supplementation trial with an antioxidant mixture (De Moffarts *et al*. 2005). During the 12 week period of supplementation, resting plasma CK remained stable in the supplemented group whereas a significant increase was observed in the control group. However, it would be anticipative to conclude that SOD supplementation improved muscle integrity as no treatment effect was observed on post exercise CK activity. Moreover, many factors including age, gender, physical fitness,
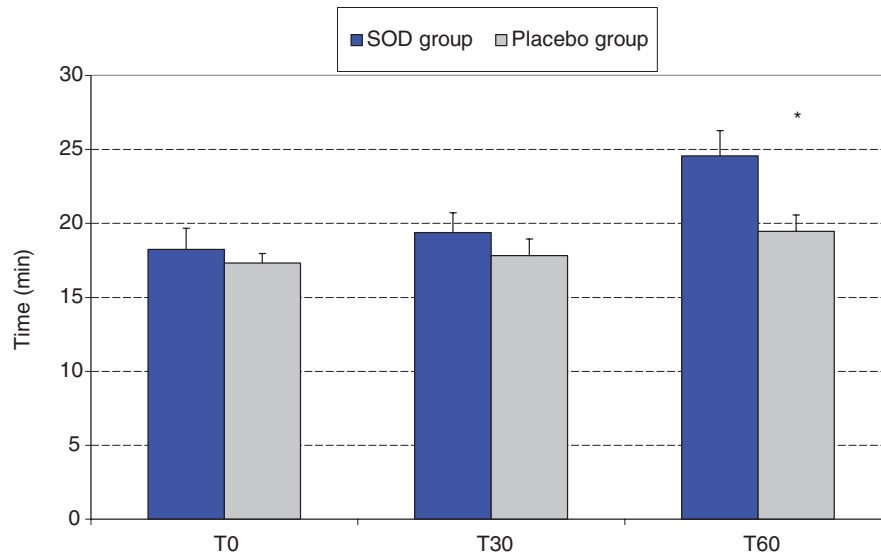
Fig 2: Evolution of plasma resistance to haemolysis in the SOD group (n = 12) and placebo group (n = 12) before supplementation (T0) and 30 (T30) and 60 (T60) days after oral supplementation. Data are shown as means ± SEM. *: SOD group different from placebo group (P<0.05).

season of year and training can contribute to increase fluctuation in plasma CK activities, even if some of them had been controlled in the experimental design.

Some positive correlations of plasma CK with various measures of antioxidant status (principally lipid hydroperoxides) have been described in antioxidant supplementation trials (Williams *et al.* 2004a,b; De Moffarts *et al.* 2005; Kinnunen *et al.* 2005). Williams *et al.* (2004a) working on 46 endurance horses observed a positive correlation between oxidative plasma lipid hydroperoxides and blood CK activity, showing that muscle cells membrane leakage could be associated with oxidative stress. Kinnunen *et al.* (2005) studied the effect of a supplementation with lipoic acid on exercise induced oxidative stress markers and muscle enzymes. They found smaller increase of plasma CK and AST in supplemented group compared to nonsupplemented group. Those changes were associated to lower markers of lipids peroxidation in supplemented group.

However, a clear relationship between free radicals production during exercise and changes in membrane permeability of muscle cells in horses is not established. Siciliano *et al.* (1997) studied the influence of 3 different vitamin E level intakes on the period of 90 days period in a population of 19 horses. They observed no influence of the treatment on muscle enzymes activity. A trial on 44 nonsupplemented Thoroughbred horses showed that i.v. administration of ascorbate increased basal and post exercise blood antioxidant capacity as compared to the nontreated horses but this supplementation failed to prevent muscular damages evaluated by plasma CK activity (White *et al.* 2001).

In their review Kirschvink *et al.* (2008) indicate that so far supplementation in commonly used dietary antioxidant (vitamin E and vitamin C) enhances an antioxidant response, but no significant effect on CK, with the exception of lipoic acid supplementation. In the current study, we observed that the CME antioxidant supplementation prevented the increase in resting plasma CK activity induced by training. However, further work is needed to verify if this effect on CK could be linked to a specific protection of muscle cells membrane against oxidation phenomenon. The measurements of other markers of lipid peroxidation such as blood

isoprostane levels or TBARS (thiobarbituric acid reactive substances) pre- and post exercise might have given interesting information.

Changes in erythrocytes SOD

In this study, both groups also present a drop in erythrocyte SOD activity during the first part of the exercise period (from T0 to T30), which could be considered as one of the markers of oxidative stress caused by intense training. On the other hand, the increase in erythrocyte SOD activity observed at T60 could be the characteristic biological response of the endogenous antioxidant defence system to training as demonstrated by De Moffarts *et al.* (2004). These results highlight once again that erythrocyte SOD activity may not be the most adequate blood marker to evaluate the oxidant/antioxidant balance in horses and that getting a comprehensive view of this equilibrium requires several biomarkers but probably also new approaches.

Even if at T30, a trend for a higher SOD activity in the SOD group compared to placebo group was noticed (P = 0.07), the global difference in erythrocytes SOD activity between the 2 groups was not significant. As erythrocytes have a life span of about 140 days in horses, a total ingestion period of that duration might have been required to achieve an increased circulating SOD activity. The impact of the duration of the feeding period was also shown for the CME supplementation of mice, in which the red blood cell life is about 30 days, circulating antioxidant activity peaked between 3 and 4 weeks of feeding (Vouldoukis *et al.* 2004b).

Blood resistance to haemolysis

To that extent we have also evaluated the global and dynamic blood resistance to a free radical attack through the KRL test. Lesgards *et al.* (2002) used that dynamic test to demonstrate that lifestyle determinants of cancer and cardiovascular risks were associated with a decreased overall antioxidant status as dynamically measured by the KRL test. That biological test was also used to assess the antioxidative activity of lipid and water-soluble vitamins

in human whole blood (Stocker *et al*. 2003; Lesgards *et al*. 2005). Whereas no effect of the training period on blood resistance capacity was observed, the supplementation with CME rich in SOD significantly increased the plasma resistance to haemolysis capacity. This result is in accordance with those obtained in study of oral supplementation with CME in mice by Vouldoukis *et al*. (2004b). Each group of 10 animals randomly received either a normal diet or a supplementation with excipient, or a supplementation with nonprotected SOD melon extract or supplementation with a protected CME for 28 days. The effects of this supplementation for 28 days were a significant elevation in antioxidant enzymes activities (SOD, GPX and catalase), correlated with an increased resistance of red blood cells to oxidative stress-induced haemolysis. The authors also demonstrated that the effect was dose-dependent and that SOD activity began to increase after 7 days of supplementation. However, unlike this study, we did not observe a change in GPX activity.

The supplementation studied here is a novel antioxidant source, as it is characterised by a high and protected enzymatic antioxidant activity. Its detailed mode of action is not yet very well understood.

Vouldoukis *et al*. (2004a) confirmed the antioxidant and anti-inflammatory properties of CME *in vitro*, by measuring the effects of CME on redox status and production of pro-inflammatory cytokines (TNFα) and anti-inflammatory cytokines (Il 10) by peritoneal macrophages. The anti-inflammatory properties of CME were principally related to its capacity to induce the production of Il 10 by peritoneal macrophages. However, recent work on other animal models unveiled a mode of action which is much more complex than common antioxidant supplementation. Superoxide dismutase is well known for its role in the dismutation of superoxide anion into hydrogen peroxide (McCord and Fridovich 1969). Indeed, Décordé *et al*. (2009) worked on a diet-induced atherosclerosis model in hamsters known to be associated with oxidative stress. This study evaluated the antioxidant mechanism of a CME rich in SOD. The findings opened a very complex biological pathway as the supplementation induced both a prevention of oxidant generation through a reduction of NADPH oxidase expression and an increase in oxidant transformation into less reactive species through an increase in SOD activity.

Conclusion

As intense training can lead to disturbances of the oxidant/antioxidant equilibrium, appropriate antioxidant supplementation may have beneficial effects on equine health. The improvement of antioxidant defences is a biological key event in the health-promoting effects of antioxidants nutrients. This pilot field study performed on 24 Standardbreds over a 60 day training period demonstrated that oral supplementation with CME could increase the blood resistance to haemolysis and limit the increase of plasma muscular enzymes. Although this might reflect a reduction of the muscular membrane permeability induced by training, further research is needed to verify that this functional antioxidant enzyme supplementation can promote cellular resistance to stress by strengthening the endogenous antioxidant defences and to understand the mechanism by which it exerts its biological effect.

Conflict of interest

Manufacturers' addresses

References

Avellini, L., Chiaradia, E. and Gaiti, A. (1999) Effect of exercise training, selenium and vitamin E on some free radical scavengers in horses. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **123**, 147-154.

Blache, D. and Prost, M. (1992) Free radical attack: biological test for human resistance capability. In: *Proceedings of the IX College Park Colloquium on Chemical Evolution: A Lunar-Based Chemical Analysis Laboratory (LBCAL)*, Eds: C. Ponnamperuma and C.W. Gehrke, NASA, Washington, DC. pp 82-98.

De Moffarts, B., Kirschvink, N., Art, T., Pincemail, J. and Lekeux, P. (2005) Effect of oral antioxidant supplementation on blood antioxidant status in trained thoroughbred horses. *Vet. J.* **169**, 7-9.

De Moffarts, B., Kirschvink, N., Art, T., Pincemail, J., Michaux, C., Cayeux, K., Defraigne, J.O. and Lekeux, P. (2004) Impact of training and exercise intensity on blood antioxidant markers in healthy Standardbred horses. *Equine Comp. Exerc. Physiol.* **1**, 211-220.

Décordé, K., Ventura, E., Lacan, D., Ramos, J., Cristol, J.P. and Rouanet, J.M. (2009) An SOD rich melon extract Extramel prevents aortic lipids and liver steatosis in diet-induced model of atherosclerosis. *Nutr. Metab. Cardiovasc. Dis.* **18**, 1-7.

Kick, J., Hauser, B., Bracht, H., Albicini, M., Öter, S., Simon, F., Ehrmann, U., Garrel, C., Sträter, J., Brückner, U., Leverve, X., Schelzig, H., Speit, G., Radermacher, P. and Muth, C.M. (2007) Effects of a cantaloupe melon extract/wheat gliadin biopolymer during aortic cross-clamping. *Intensive Care Med.* **33**, 694-702.

Kinnunen, S., Hyyppä, S., Lappalainen, J., Oksala, N., Venojärvi, M., Nakao, C., Hänninen, O., Sen, C.K. and Atalay, M. (2005) Exercise-induced oxidative stress and muscle stress protein responses in trotters. *Eur. J. appl. Physiol.* **93**, 496-501.

Kirschvink, N., de Moffarts, B. and Lekeux, P. (2008) The oxidant/antioxidant equilibrium in horses. *Vet. J.* **177**, 178-191.

Lacan, D. and Baccou, J.C. (1998) High levels of antioxidant enzymes correlate with delayed senescence in nonnetted muskmelon fruits. *Planta* **204**, 377-382.

Lesgards, J.F., Durand, P., Lassarre, M., Stocker, P., Lesguard, G., Lanteaume, A., Prost, M. and Lehucher-Michel, M.P. (2002) Assessment of lifestyle effects on the overall antioxidant capacity of heathly subjects. *Environ. Health Perspect.* **110**, 1-9.

Lesgards, J.F., Lehucher-Michel, M.P., Vidal, N., Prost, M. and Stocker, P. (2005) Assessment of antioxidative activity of lipid- and water-soluble vitamins in human whole blood. Comparative analysis between a biological test and chemical methods. *Int. J. Vitam Nutr. Res.* **75**, 11-18.

McCord, J.M. and Fridovich, I. (1969) Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J. biol. Chem.* **25;244**, 6049-6055.

McMeniman, N.P. and Hintz, H.F. (1992) Effect of vitamin E status on lipid peroxidation in exercised horses. *Equine vet. J.* **24**, 482-484.

Muth, C.M., Glenz, Y., Klaus, M., Radermacher, P., Speit, G. and Leverve, X. (2004) Influence of an orally effective SOD on hyperbaric oxygen-related cell damage. *Free Radic. Res.* **38**, 927-932.

Naito, Y., Akagiri, S., Uchiyama, K., Kokura, S., Yoshida, N., Hasegawa, G., Nakamura, N., Ichikawa, H., Toyokuni, S., Ijichi, T. and Yoshikawa, T. (2005) Reduction of diabetes-induced renal oxidative stress by a cantaloupe melon extract/gliadin biopolymers, oxykine, in mice. *Biofactors* **23**, 85-95.

Nkosi, C.Z., Opoku, A.R. and Terblanche, S.E. (2006) Antioxidative effects of pumpkin seed (Cucurbita pepo) protein isolate in CCl4-induced liver injury in low-protein fed rats. *Phytother. Res.* **20**, 935-940.

Siciliano, P.D., Parker, A.L., hawrence, L.M. (1997) Effect of dietary vitamin E Supplementation on the integrity of skeletal muscle in exercise horse. *J Anim Sci.* **75**, 1553-1560.

Stocker, P., Lesgards, J.F., Vidal, N., Chalier, F. and Prost, M. (2003) ESR study of a biological assay on whole blood: antioxidant efficiency of various vitamins. *Biochim. Biophys. Acta* **1621**, 1-8.

Vouldoukis, I., Conti, M., Krauss, P., Kamaté, C., Blazquez, S., Tefit, M., Mazier, D., Calenda, A. and Dugas, B. (2004b) Supplementation with gliadin-combined plant superoxide dismutase extract promotes antioxidant defences and protects against oxidative stress. *Phytother. Res.* **18**, 957-962.

Vouldoukis, I., Lacan, D., Kamate, C., Coste, P., Calenda, A., Mazier, D., Conti, M. and Dugas, B. (2004a) Antioxidant and anti-inflammatory properties of a Cucumis melo LC. Extract rich in superoxide dismutase activity. *J. Ethnopharmacol.* **94**, 67-75.

Westervelt, R.G., Stouffer, J.R., Hintz, H.F. and Schryver, H.F. (1976) Estimating fatness in horses and ponies. *J. anim. Sci.* **143**, 781-785.

White, A., Estrada, M., Walker, K., Wisnia, P., Filgueira, G., Valdés, F., Araneda, O., Behn, C. and Martínez, R. (2001) Role of exercise and ascorbate on plasma antioxidant capacity in thoroughbred race horses. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **128**, 99-104.

Williams, C.A., Kronfeld, D.S., Hess, T.M., Saker, K.E. and Harris, P. (2004b) Lipoic acid and vitamin E supplementation to horses diminishes endurance exercise induced oxidative stress, muscle enzyme leakage and aptotosis. *Proceeding of CESMAS (Conference on Equine Sports Medicine and Science) 'The elite race and endurance horse'*, Ed: A. Lindner. pp 105-119.

Williams, C.A., Kronfeldt, D.S., Hess, T.M., Saker, K.E., Waldron, J.N., Crandell, K.M., Hoffman, R.M. and Harris, P.A. (2004a) Antioxidant supplementation and subsequent oxidative stress of horses during an 80-km endurance race. *J. anim. Sci.* **82**, 588-594.