Changes in plasma cortisol and ascorbic acid in horses with and without recurrent airway obstruction upon exercise and ascorbic acid supplementation

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Abstract

Diminished basal plasma cortisol concentrations and a blunted cortisol response to exercise have been observed in human asthmatics. In horses with recurrent airway obstruction (RAO), plasma concentrations of cortisol at rest are not significantly different from those of healthy horses, but the effect of exercise on endogenous cortisol concentrations has not been described. Ascorbic acid is a non-enzymatic antioxidant with proposed immune-modulating properties. In man, supplementation with ascorbic acid has been shown to attenuate the exercise-induced increase in plasma cortisol following prolonged, submaximal exercise. The relationship between cortisol and ascorbic acid has not previously been investigated in the horse. In a blinded cross-over design, five horses with RAO and six healthy non-RAO controls performed a standard exercise test following 4 weeks of supplementation with either an antioxidant (providing 10 mg ascorbic acid kg\(^{-1}\) day\(^{-1}\)) or a placebo (<1 mg ascorbic acid kg\(^{-1}\) day\(^{-1}\)). Venous blood samples were obtained 1 h prior to exercise and at 0, 15, 60 min and 24 h thereafter. Exercise resulted in a significant increase in plasma cortisol concentrations in both groups of horses (\(P<0.05\)). Basal and post-exercise concentrations of plasma cortisol in the RAO group (136 ± 16 and 210 ± 16 \(\mu\)mol l\(^{-1}\), respectively) were not significantly different from those in the non-RAO group (129 ± 43 and 218 ± 30 \(\mu\)mol l\(^{-1}\), respectively). Antioxidant supplementation increased basal and post-exercise concentrations of plasma ascorbic acid in RAO and non-RAO horses (\(P<0.05\)) but had no effect on plasma cortisol concentration in either group, before or after exercise (RAO: rest 157 ± 27 \(\mu\)mol l\(^{-1}\), post-exercise 222 ± 21 \(\mu\)mol l\(^{-1}\); non-RAO: rest 140 ± 11 \(\mu\)mol l\(^{-1}\), post-exercise 227 ± 35 \(\mu\)mol l\(^{-1}\)). In conclusion, RAO-affected horses in remission demonstrate the same cortisol response to exercise as healthy controls. Antioxidant supplementation had no impact on post-exercise concentrations of plasma cortisol in either healthy or RAO-affected horses in remission.

Keywords: recurrent airway obstruction; chronic obstructive pulmonary disease; heaves; respiratory disease; vitamin C; antioxidant; hydrocortisone
Introduction

Cortisol is the primary glucocorticoid hormone secreted by the adrenal gland, and demonstrates potent anti-inflammatory and immunosuppressive properties. During exercise, cortisol plays a central role in substrate mobilisation by enhancing gluconeogenesis, increasing glycogen deposition and stimulating lipolysis. Furthermore, a glucocorticoid deficiency is known to impair muscle performance. Cortisol is often used as an index of physiological and/or psychological stress. In man, changes in cortisol concentrations and rhythms as a result of exercise and disease have been extensively described. In contrast, while the cortisol response to exercise in the horse has been reported, few references to non-adrenal disease have received little attention.

In relation to respiratory disease, there is conflicting evidence as to whether asthmatics have normal hypothalamus–pituitary–adrenal (HPA) axis function. This controversy also applies to the cortisol response to exercise in asthmatics. Untrained human asthmatic patients have been shown to demonstrate a diminished cortisol response to exercise and psychological stress compared with non-asthmatics. However, in contrast, Sly et al. found no significant difference in the cortisol response to exercise between healthy and asymptomatic asthmatic children, while Jaffe et al. reported an increased cortisol response to exercise in asthmatics in remission. In horses, while acute stress has been shown to result in increased plasma cortisol concentrations, chronic stress has been more commonly shown to be associated with depressed cortisol concentrations. Equine recurrent airway obstruction (RAO) is a chronic inflammatory disease of the small airways exacerbated by exposure to the stable environment and resulting in neutrophilic infiltration of the airways, bronchoconstriction, mucus hyper-secretion and airway remodelling. The disease has been proposed as an animal model of human asthma. Systemic cortisol concentrations of RAO-affected horses at rest have been described and do not appear to differ from those of non-RAO controls; however, there do not appear to have been any reports of the cortisol response to exercise in RAO-affected horses.

Ascorbic acid is an important hydrophilic antioxidant that has also been proposed to have effects on the immune system, and its concentration in plasma has been shown to be increased by exercise in man. The adrenal cortex contains high concentrations of ascorbic acid in both non-synthesising species, including man, and synthesising species such as the rat and co-release with cortisol has been suggested. Gleeson et al. showed a strong positive correlation between plasma ascorbic acid and cortisol increase in human athletes as a result of a 21-km race. These authors hypothesised that the adrenal gland was a major source of ascorbic acid efflux during exercise. More recent studies have reported a similar relationship between cortisol and ascorbic acid following ultramarathon competition.

Further evidence that the adrenal gland may be an important modulator of systemic ascorbic acid concentrations comes from studies of high-dose ascorbic acid supplementation (~20 mg kg$^{-1}$ day$^{-1}$) which have demonstrated transient suppression of cortisol release in human athletes following ultramarathon running. In addition, in guinea-pigs, elevated cortisol concentrations have been observed in association with reduced dietary ascorbic acid intake; conversely, high supplementary doses of ascorbic acid have been associated with a reduction in adrenal cortisol production. Suppression of basal cortisol concentrations following ascorbic acid supplementation has also been reported in the rat.

A reduced incidence of post-race upper respiratory tract infection has been reported in relation to the observed attenuation of the exercise-induced increase in circulating cortisol following vitamin C supplementation. The marked immunosuppressive action of cortisol is well documented and this apparent effect of high-dose vitamin C supplementation may be of interest in the horse, for example, following exercise and/or transportation. For example, with regard to the endurance horse, Robson et al. demonstrated that endurance exercise in horses has a negative impact on the function of the innate immune system which lasts for several days. This phenomenon is likely to be attributable to the significant elevations in plasma cortisol concentrations observed post-race.

While humans and guinea-pigs require a dietary source of ascorbic acid, as they are unable to synthesise ascorbic acid due to the absence of L-gulonolactone oxidase, the horse is able to synthesise ascorbic acid. As far as we are aware, the relationship between ascorbic acid and cortisol has not been investigated in the horse. Of further interest is the observation that plasma ascorbic acid at rest is significantly reduced in RAO-affected horses compared with non-RAO controls.

The aims of the present study were therefore to: (1) describe the cortisol responses to exercise in non-RAO and RAO-affected horses; and (2) determine the effect of ascorbic acid supplementation on the cortisol response to exercise in non-RAO and RAO-affected horses.

Materials and methods

Animals

Six Thoroughbred non-RAO horses (four geldings and two mares; age 7.5 ± 2.7 years, weight 466 ± 16 kg,
mean ± standard deviation (SD)), free of respiratory disease as determined by resting clinical examination, tracheal wash (cytology and bacteriology), routine haematology and serology, were studied. Five RAO-affected horses in remission (four geldings and one mare; age 16 ± 4 years, weight 460 ± 51 kg, mean ± SD) were also studied. Remission was defined as less than 20 neutrophils per µl bronchoalveolar lavage (BAL) fluid. The horses were diagnosed as having RAO on the basis of developing marked neutrophilic airway inflammation when stabled on hay and straw. One of the horses in the RAO group showed clinical signs of Cushing’s syndrome and a dexamethasone suppression test was therefore undertaken, but was negative. None of the horses in either group had been treated with oral or inhaled corticosteroids for at least 6 months or 6 weeks, respectively, prior to the start of the study or during the study period. The experimental protocol was approved by the Ethical Review Committee of the Animal Health Trust and conformed to the Home Office Animals (Scientific Procedures) Act 1986.

Both groups of horses were stabled continuously throughout the study, except during exercise. They were bedded on shredded paper and fed 9 kg haylage (Marksway Horsehage Ryegrass; Marksway and Son, Paignton, Devon, UK) and 3 kg mixed feed (Winery® Balanced Energy; Effem Equine Ltd, Old Wolverton, Milton Keynes, UK) per day in two feeds. Water was provided ad libitum.

Study design

The study design is similar to that previously described by Deaton et al.43. Briefly, the horses were treadmill-trained for at least 16 weeks prior to the start of the study. One week before the study commenced each horse performed an incremental exercise test to determine maximum oxygen uptake (VO₂ max). The horses were studied in a blinded cross-over design which was preceded by a 4-week lead-in period whilst the horses were fed the basal diet. Following the lead-in period the non-RAO group was divided into two groups of three, while the RAO group was divided into a group of two and a group of three. Each group was fed an oral antioxidant supplement containing ascorbic acid, α-tocopherol and selenium (Winery VENTIL-ATE®; Effem Equine Ltd) or a placebo for 4 weeks. The daily intake of ascorbic acid, α-tocopherol and selenium, per kg body weight, were < 0.95 mg, 1.1 mg and 1.9 µg, respectively, on the placebo diet; and 10 mg, 6 mg and 5.1 µg, respectively, on the antioxidant diet. After a 4-week washout period the groups were reversed. For the duration of the study the horses were exercised three days per week (Mon, Wed and Fri) on a treadmill (SÄTO I; SÄTO AB, Sweden) at a 3° (5.2%) incline (5 min at 1.7 m s⁻¹, 5 min at 3.7 m s⁻¹, 2 min at 90% VO₂ max, 5 min at 1.7 m s⁻¹). For the remaining four days (Tues, Thurs, Sat, Sun) they walked for 30 min on a horse walker.

On the final day of each 4-week period, both groups of horses performed a standard exercise test consisting of 10 min at 1.7 m s⁻¹ and 5 min at 3.7 m s⁻¹, followed by three 2-min periods at 70% VO₂ max, 80% VO₂ max and 90% VO₂ max separated by 5 min at 1.7 m s⁻¹. The entire test was carried out on a 3° (5.2%) incline. Venous blood samples (20 ml) were collected from an indwelling catheter at rest and at 0 and 15, 30, 60 min and 24 h after exercise. Exercise tests were conducted between 10:00 and 14:00 hours.

Sample processing and analysis

Venous blood samples were placed in separate tubes containing either lithium-heparin or ethylenediaminetetraacetic acid (EDTA) on ice and centrifuged at 400 g for 10 min at 4°C within 5 min of collection. Samples for ascorbic acid analysis were processed as described by Deaton et al.43. Plasma for cortisol analysis was obtained from the lithium-heparin samples which were then snap-frozen in liquid nitrogen and stored at −80°C. Plasma ascorbic acid was analysed in EDTA plasma by high-performance liquid chromatography with ultraviolet detection as described previously45. Concentrations of plasma cortisol were determined in duplicate by radioimmunoassay (Cortisol Solid Phase Component System; ICN Pharmaceuticals, Orangeburg, NY). Intra- and inter-assay coefficients of variance were 6.0% and 8.8%, respectively.

Statistics

A nested analysis of variance (ANOVA) for repeated measures was used to determine the significance of the effect of the antioxidant and placebo supplement and the effect of time (resting vs. 0 min vs. 15 min vs. 30 min vs. 60 min vs. 24 h post-exercise). A nested ANOVA design for repeated measures was also used to study the effect of disease status, exercise and ascorbic acid supplementation on the ratio of ascorbic acid to cortisol. If significance was attained, further analyses were performed with the Newman–Keuls test for multiple comparisons. Data are presented as mean ± SD, except where stated otherwise. Statistical analysis was performed using StatMost v3.6 for Windows (DataMost Corporation, Salt Lake City, UT).

Results

Concentrations of cortisol and ascorbic acid in plasma before and after exercise in non-RAO and RAO-affected horses prior to and following dietary ascorbic acid supplementation are shown in Fig. 1. Resting cortisol concentrations were not different between non-RAO and RAO-affected horses, nor was the magnitude or
time course of the response to exercise different. Exercise resulted in significant increases in plasma cortisol concentrations at 0, 15 and 60 min post-exercise in both non-RAO and RAO groups compared with the pre-exercise concentration. In addition, there was no effect of supplementation on plasma cortisol concentrations either at rest or following exercise in either group.

At rest on the placebo diet, RAO horses had a significantly lower plasma ascorbic acid concentrations (10.6 ± 3.0 \( \mu \)mol L\(^{-1} \)) than non-RAO affected horses (16.2 ± 4.7 \( \mu \)mol L\(^{-1} \); \( P < 0.05 \)), but following supplementation they were no longer significantly different (Fig. 1). Antioxidant supplementation significantly increased ascorbic acid concentrations at rest (pre) and following exercise (Fig. 1) in both non-RAO and RAO groups compared with the placebo diet (\( P < 0.05 \)). Despite the lower initial plasma ascorbic acid concentration in RAO, there was no significant difference between non-RAO and RAO groups in the increase in plasma ascorbic acid from rest to end of exercise. Neither was there any difference following supplementation.

The change in cortisol and change in ascorbic acid with exercise were not significantly correlated in either the non-RAO or RAO group before or following supplementation. Even when the data from both non-RAO and RAO groups were combined, there was still no significant correlation between the increase in cortisol and the increase in plasma ascorbic acid with exercise.

The ratio of ascorbic acid to cortisol at rest and following exercise in non-RAO and RAO-affected horses before and after antioxidant supplementation is shown in Fig. 2. There was a trend towards a higher ratio of ascorbic acid to cortisol in the non-RAO group following antioxidant supplementation, which reached significance in the RAO group (\( P < 0.05 \)). There was no effect of exercise on the ratio of ascorbic acid to cortisol in either the non-RAO or RAO horses for either placebo or treatment (\( P < 0.05 \)). After antioxidant supplementation the ratio of ascorbic acid to cortisol in the RAO-affected horses was not different from that of non-RAO horses.

**Discussion**

A potential criticism of the present study is that the number of animals studied is relatively low. However, a cross-over design was used so each animal acted as
its own control. Furthermore, variance in both non-RAO and RAO groups was similar. Another potential limitation of this study is that total cortisol was measured, whereas the biologically active fraction is defined by the pool of free cortisol.  

Cortisol secretion in the horse is governed by a circadian rhythm; however, the timing of peak and nadir cortisol concentrations appears to be a matter of some contention. For example, times cited in the literature include 06:00 hours and 09:00 hours for peak concentrations, and 16:00 hours and 21:00–23:00 hours for the minimum daily concentration. In the present study, venous blood samples were collected between 10:00 and 14:00 hours. It is conceivable that there was a small component of diurnal variation that contributed to the changes observed in the present study; however, exercise induced an increase in cortisol while diurnal rhythm consistently shows a decrease from morning to evening. Since all horses in the present study were exercised at approximately the same time of day, we believe the contribution of diurnal variation to our data will be small.

The existence of impaired adrenocortical function in asthmatics has long been the subject of speculation. Plasma cortisol concentrations at rest and following exercise in non-RAO horses in the present study are similar to those reported for healthy horses in the literature. To the best of our knowledge, concentrations of plasma cortisol have not been previously described in RAO-affected horses in response to exercise. The results of the present study suggest that RAO-affected horses in remission appear to demonstrate a normal cortisol response to exercise.

In untrained human asthmatics, diminished HPA axis function has been proposed, evidenced by significantly lower concentrations of plasma cortisol compared with healthy patients following exercise. Although the asthmatic subjects were considered to be asymptomatic, they were pre-medicated before exercise with salbutamol and sodium cromoglycate. In contrast, in the present study, plasma cortisol concentrations of RAO-affected horses in clinical remission (and in the absence of any medication) did not differ significantly from the non-RAO horses either at rest or at any of the four time points following exercise. In addition, the horses in the present study had been in training for more than 20 weeks. Training significantly reduced the cortisol response to exercise in asthmatic children, however, there was no comparison with normal children in this particular study. In addition, the RAO horses in the present study had been maintained at pasture for at least 6 months prior to the study and were maintained on a minimum dust regimen for the duration of the study. None of the RAO group experienced a clinical exacerbation in the 6 months prior to or during the study. As such, they would be reasonably considered to be in a relatively high degree of remission (BAL fluid neutrophil count <20 cells µl⁻¹), a prerequisite for an exercise study of this nature, since RAO-affected animals with airway inflammation are often exercise-intolerant.

Ascorbic acid concentrations in plasma of RAO-affected horses at rest were significantly lower than in the non-RAO group, consistent with a previous publication by our group. Prior to antioxidant supplementation, both non-RAO and RAO-affected horses showed similar time courses for changes in ascorbic acid in response to exercise. Supplementation resulted in significant increases in plasma ascorbic acid before and after exercise in both non-RAO and RAO-affected groups; however, the increase was greater in the RAO group such that, following supplementation, non-RAO and RAO groups were not different. While there was a difference in the ascorbic acid response to exercise following antioxidant supplementation in both non-RAO and RAO groups, the lack of an overall significant increase in ascorbic acid with exercise on the placebo is consistent with a previous study by White et al. in Thoroughbreds following a 1000-m sprint. In addition to ascorbic acid, the supplement used in the present study contained α-tocopherol and selenium. It is possible that this may have influenced our results since interactions are known to exist between the non-enzymatic antioxidants. In supplemented sled dogs, endurance exercise resulted in a significant increase in plasma ascorbic acid concurrent with a decrease in α-tocopherol. This suggests that ascorbic acid may have been spared at the expense of α-tocopherol consumption. Data from the same horses as used in the present study, published elsewhere, showed no significant change in...
plasma α-tocopherol concentrations with exercise following supplementation with the same antioxidant cocktail. Moreover, α-tocopherol supplementation has been shown to have no effect on post-exercise plasma cortisol concentrations in female athletes39, and there do not appear to be any published data concerning an interaction between selenium and cortisol.

There are conflicting reports in the literature concerning the relationship between ascorbic acid and cortisol during exercise (see review by Peake60). For example, Gleeson et al.20 reported a significant positive correlation between cortisol and ascorbic acid in human athletes following a 21-km race, while another group have reported weak negative correlations50,61. In the present study there was no clear relationship between ascorbic acid and cortisol following exercise, although this may be due to relatively low power owing to low numbers, even when the two groups were combined. Furthermore, the exercise protocol used in the present study consisted of short, intermittent bouts of medium to high-intensity exercise, whereas prolonged submaximal exercise was used in studies where an attenuating effect of dietary ascorbic acid supplementation upon exercise-induced increases in plasma cortisol have been reported. At rest, however, the ratio of ascorbic acid to cortisol was lower in RAO horses compared with the non-RAO group, while after exercise it was increased and not different from the non-RAO group. This implies that releases of ascorbic acid and cortisol are proportional during exercise of the type used in the present study, both in RAO and non-RAO horses.

In guinea-pigs administered adrenocorticotropic hormone (ACTH), there was no change in plasma ascorbic acid concentration despite an increase in plasma cortisol concentration62. Laney et al.63 concluded that dietary ascorbic acid intake had a strong influence on the ascorbic acid content of the adrenal glands in guinea-pigs, but no effect on either the basal or ACTH-stimulated cortisol concentrations was observed. Following supplementation with ~10 mg ascorbic acid kg−1 day−1, Palmer et al.25 found no impact of supplementation on post-exercise plasma cortisol concentrations. This is in agreement with the findings of Thompson et al.64, where subjects were supplemented with ~5.5 mg ascorbic acid kg−1 day−1. The supplement used in the present study provided approximately 10 mg ascorbic acid kg−1 day−1. In all three instances this is considerably lower than the dose used in studies where an attenuating effect of ascorbic acid on plasma cortisol has been reported. For example, Peters et al.26 supplemented athletes with 1500 mg vitamin C daily (~25.5 mg kg−1 day−1). These authors reported significantly lower cortisol concentrations immediately post-exercise than in those athletes supplemented with a lesser amount of vitamin C or a placebo.

In conclusion, trained RAO-affected horses in a high degree of clinical remission show the same plasma (total) cortisol response to exercise as non-RAO controls, which is unchanged by supplementation with ~10 mg ascorbic acid kg−1 day−1. The ratio of ascorbic acid to cortisol was unchanged by exercise, supporting the suggestion that they are co-released or released in proportion to one another. The mean ratio of ascorbic acid to cortisol was increased by moderate antioxidant supplementation (providing ~10 mg ascorbic acid kg−1 day−1), but only significantly so in RAO-affected horses. There was no correlation between ascorbic acid increase and cortisol increase in either the non-RAO or RAO group, even when combined.

References

Plasma cortisol and ascorbic acid in non-RAO and RAO-affected horses


