Vitamin E is a potent antioxidant that is able to diminish the effects of free radicals, the production of which can cause severe damage to cell membranes and intracellular components. Vitamin E scavenges free radicals and converts them to relatively stable compounds, thereby disrupting damaging free radical–induced chain reactions. Free radical production in neurologic tissue becomes excessive following neurologic injury or disease. Increased free radical production accentuates or causes further neuronal injury. Vitamin E also has antiapoptotic actions via inhibition of protein kinase C, which can be beneficial in diseased or injured neural tissue. It is also known that vitamin E enhances humoral and cell-mediated immunity. 

### Objective

To determine concentrations of α-tocopherol in serum and CSF of healthy horses following administration of supplemental vitamin E in feed.

### Animals

10 healthy adult horses.

### Procedures

Horses were allocated to receive supplemental d-α-tocopherol (1,000 U/d [group A; n = 5] or 10,000 U/d [group B; 5]) in feed for 10 days. Blood samples were collected before (baseline), during, and at intervals for 10 days after discontinuation of vitamin E administration for assessment of serum α-tocopherol concentration. Cerebrospinal fluid samples were collected prior to and 24 hours after cessation of vitamin E administration. α-Tocopherol concentrations in serum and CSF samples were analyzed via high-performance liquid chromatography; changes in those values during the treatment period were compared between groups, and the relationship of serum and CSF α-tocopherol concentrations was evaluated.

### Results

In both groups, serum α-tocopherol concentration increased significantly from baseline during vitamin E administration; values in group B were significantly greater than those in group A during and after treatment. At the end of vitamin E administration, CSF α-tocopherol concentration was not significantly greater than the baseline value in either group; however, the increase in CSF concentration was significant when the group data were combined and analyzed. Serum and CSF α-tocopherol concentrations were significantly correlated at baseline for all horses, but were not strongly correlated after 10 days of vitamin E administration.

### Conclusions and Clinical Relevance

In healthy horses, daily oral administration of supplemental vitamin E in feed resulted in increases in serum and CSF α-tocopherol concentrations. (Am J Vet Res 2008;69:785–790)
concentration, whereas grass hay contains the lowest. Absorption of orally administered vitamin E is maximized when consumed with a small amount of grain.

In animals, natural sources of vitamin E have greater biological activity and are retained for longer duration in tissues than synthetic supplemental sources. Therefore, diet and source of supplemental vitamin E can dramatically affect concentrations of vitamin E in tissues and fluids of an animal.

Horses are capable of vitamin E absorption following oral administration, resulting in a corresponding increase in serum α-tocopherol concentration. However, it has not been proven that vitamin E will cross an intact blood-brain barrier or that concentrations in CSF and CNS parenchyma will increase following oral administration. In horses with central neurologic diseases, the CNS is the desired target area for the antioxidant activity of vitamin E. Cerebrospinal fluid can be analyzed to monitor concentrations of α-tocopherol within the CNS, including the brain and spinal cord. To the authors’ knowledge, the exact mechanism by which vitamin E enters the CSF has yet to be determined in any species. However, because vitamin E is a lipid-soluble vitamin, it likely requires a carrier-mediated process rather than simple diffusion for transport across the blood-brain barrier. Therefore, the rate of uptake into the CSF in an animal with an intact blood-brain barrier would be limited by this carrier process. Despite this, concentrations of lipid-soluble vitamins within the CSF would still be controlled in large part by the respective concentrations in serum. This suggests that increases in serum concentrations of α-tocopherol achieved via oral administration of the vitamin would consequently result in increases in concentrations of α-tocopherol in the CSF.

The purpose of the study reported here was to assess concentrations of α-tocopherol in serum and CSF of healthy horses following administration of supplemental vitamin E in feed. The question of interest was whether oral administration of supplemental vitamin E in horses would result in increased concentrations of α-tocopherol in the CSF, because the CNS is the target site of action for treatment of most neurologic diseases. The veterinary medical literature contains widely variable dose ranges for vitamin E treatments from 600 to 12,000 U/d. To our knowledge, there are no published data that indicate the dose necessary to achieve clinically important increases in α-tocopherol concentration within the CSF. Therefore, the study was also conducted to evaluate whether α-tocopherol concentrations in CSF vary in relation to the dosage of supplemental vitamin E administered orally in horses.

Materials and Methods

Animals—Ten healthy adult horses were included in the study. Prior to study commencement, the horses were evaluated for any signs of ill health or neurologic abnormalities by means of a full physical examination and gait assessment. A CBC was performed for each horse, and all results were within reference intervals. The horses were randomly assigned to 1 of 2 groups (5 horses/group). Group A consisted of 2 geldings and 3 mares. Group B consisted of 3 mares and 2 geldings. The age of horses in group A ranged from 15 to 23 years; the age of horses in group B ranged from 9 to 17 years. Groups A and B had the same breed distribution: 2 Thoroughbreds, 1 Arabian, 1 Standardbred, and 1 Quarter Horse. The weights of the 10 horses ranged from 480 to 610 kg. All horses belonged to the research herd at the Center for Equine Health, University of California, Davis. All procedures were approved by the Institutional Animal Care and Use Committee of the University of California.

Study design and sample collection—At the onset of the study, the horses were receiving alfalfa hay fed twice daily. Blood was collected (day −7) from each horse to provide sera for α-tocopherol analysis. To evaluate effects of diet change, horses were then fed grass hay twice daily for 7 days, at which time blood was again collected (day 0) to provide sera for analysis. Samples from the alfalfa and grass hay sources, as well as samples of the sweet feed that was to be used as the vehicle for supplemental vitamin E administration later in the study, were also collected. α-Tocopherol concentrations in serum samples and feed samples were analyzed via high-performance liquid chromatography. The alfalfa diet provided approximately 633 mg of α-tocopherol/kg of dry matter/d, whereas the grass hay provided approximately 362 mg of α-tocopherol/kg of dry matter/d. The sweet feed provided 872 mg of α-tocopherol/kg of dry matter/d.

Baseline samples of serum and CSF were obtained from all horses immediately prior to the first administration of supplemental vitamin E (day 0). Each horse was anesthetized with xylazine hydrochloride (1.1 mg/kg, IV) and ketamine hydrochloride (2.2 mg/kg, IV). Cerebrospinal fluid was collected into a tube that did not contain anticoagulant via puncture of the atlanto-occipital space. The atlanto-occipital site was used to minimize blood contamination of the CSF. All CSF samples were submitted for cytologic evaluation; any sample containing >300 RBCs/µL was not included for analysis. Because light, contact with rubber, hemolysis, or heat can decrease α-tocopherol concentrations in equine sera, the serum and CSF samples were immediately protected from light and placed on ice. Samples were centrifuged and transferred to glass vials within 1 hour after collection, then frozen and kept at −80°C until analysis. From results of a previous study, it is known that the stability of α-tocopherol is maintained for several years when samples are handled in this manner.

The horses then received daily oral administration of supplemental vitamin E in the form of natural micellized d-α-tocopherol. The supplementation was provided as 1 of 2 doses: 1,000 or 10,000 U/d/horse. The dose of 1,000 U of d-α-tocopherol/d/horse is within the range of daily vitamin E requirement for healthy adult horses recommended in the NRC guidelines. The dose of 10,000 U of d-α-tocopherol/d/horse was based on a dosage that is commonly used clinically for horses with neurologic disease.

Horses in group A received 1,000 units of d-α-tocopherol mixed in 0.227 kg of sweet feed every 24 hours for 10 days (days 0 through 9). It was confirmed that each horse consumed its full dose. All horses were kept separated in covered pipe pens and fed a strict diet of grass hay alone twice daily.
Statistical data analysis was performed by use of Wilcoxon-Mann-Whitney tests to assess differences in age and weight of horses and actual dosages of vitamin E (U/kg) between groups A and B. The Friedman test was used to assess changes in serum and CSF α-tocopherol concentrations throughout the treatment period. When significant changes were identified, the Wilcoxon signed rank test was used to make pairwise comparisons of serum and CSF α-tocopherol concentrations at different time points throughout the study; the test was also used to compare horses’ weights before and after the study. A Spearman rank correlation test was used to assess the relationship between α-tocopherol concentrations in serum and CSF A value of $P \leq 0.05$ was considered significant.

Results

With regard to weight, sex, or breed, there was no significant difference between the treatment groups. The weight of each horse did not change significantly ($P = 0.17$) during the study period. Within each group, the coefficient of variation for actual dosages of vitamin E (U/kg) was not significantly ($P = 0.09$) different among horses. Horses in group A received 1.6 to 2.0 U of α-tocopherol/kg. Horses in group B received 17.2 to 20.8 U of d-α-tocopherol/kg.

Serum α-tocopherol concentrations in all horses during the periods in which they were fed alfalfa hay or grass hay diets were compared. On the alfalfa hay diet, median serum α-tocopherol concentration was 1.4 µg/mL (range, 0.8 to 2.5 µg/mL; interquartile range, 1.2 to 1.9 µg/mL). On the grass hay diet, median serum α-tocopherol concentration was 1.15 µg/mL (range, 0.7 to 1.8 µg/mL; interquartile range, 0.9 to 1.7 µg/mL). These values were significantly ($P = 0.03$) different, indicating an important dietary contribution of vitamin E to serum α-tocopherol concentrations in the study horses. Vitamin E concentrations in the alfalfa hay, grass hay, and sweet feed were 63.3 µg/g (63.3 ppm), 36.2 µg/g (36.2 ppm), and 87.2 µg/g (87.2 ppm), respectively.

Compared with baseline values at day 0, serum α-tocopherol concentrations in groups A and B increased significantly throughout the period of vitamin E administration and reached a plateau by day 10 (Table 1). Baseline serum α-tocopherol concentrations did not differ significantly between the 2 groups. However, during and after administration of supplemental vitamin E, serum α-tocopherol concentrations differed significantly between the 2 groups; values in group B horses were significantly higher than those in group A horses at each time point.

Compared with day 0 values, α-tocopherol concentrations in CSF at day 10 were increased, albeit not significantly, in groups A and B. The CSF data for horses in both groups at each time point were combined for comparison. The median α-tocopherol concentration in CSF at day 0 for all horses was 9.5 ng/mL (range, 2 to 19 ng/mL; interquartile range, 8 to 12 ng/mL). The median α-tocopherol concentration in CSF at day 10 for all horses was 17.5 ng/mL (range, 11 to 41 ng/mL; interquartile range, 13 to 24 ng/mL). The combined CSF concentration at day 10 was significantly ($P = 0.004$) greater than that at day 0. Evaluation of the day 10 value with respect to the day 0 value within each group did not reveal a significant difference because of the small size of each treatment group. Following oral administration of vitamin E, there was a 1.3- to 3.4-fold increase in α-tocopherol concentration in CSF in 9 of 10 horses.

A significant correlation between serum and CSF α-tocopherol concentrations for all horses was determined on day 0 ($P = 0.05$) but not on day 10 ($P = 0.30$). Although median and maximum CSF concentrations of
α-tocopherol in group B were higher than those values in group A, there was no significant difference in α-tocopherol concentration in CSF between the 2 treatment groups prior to or at the end of the period of vitamin E administration.

In all horses, serum α-tocopherol concentrations decreased within a few days after discontinuation of vitamin E administration (Table 1). Within 10 days after discontinuation of treatment, median and mean serum α-tocopherol concentrations in both treatment groups had almost returned to baseline values (no significant difference between day 0 and day 20 values in either group). However, at days 13, 16, and 20, horses in group B had significantly higher serum concentrations of α-tocopherol, compared with findings in horses in group A. The higher mean and median values in group B horses at day 20 were likely a reflection of the higher peak serum concentration achieved in those horses at day 10.

**Discussion**

According to the NRC, the daily vitamin E requirement of horses is 50 to 80 U/kg of dry matter or 0.75 to 1.0 U/kg of body weight. Growing, pregnant or lactating, and performance horses require 1.5 to 2.0 U/kg of body weight. Horses that are fed a low-vitamin E diet (ie, horses that have no access to pasture) should receive 600 to 800 U/d or 1.5 to 4.4 U/kg of body weight/d.4 In the present study, horses in group A were given 1.6 to 2.0 U of d-α-tocopherol/kg/d (mean, 1.9 U/kg/d), which is within the NRC recommendation; horses in group B were given 17.2 to 20.8 U of d-α-tocopherol/kg/d (mean, 18.1 U/kg/d), which is approximately 10 times the NRC recommendation. Serum α-tocopherol concentrations > 4 μg/mL are considered adequate and concentrations < 2 μg/mL are considered inadequate in clinically normal horses.16,17

In the present study, the atlanto-occipital space was used for collection of CSF samples from anesthetized horses to minimize blood contamination; also, this site was selected because α-tocopherol concentrations in CSF collected from the cervical vertebrae and cisterna magna are 3 to 5 times as great as the concentration in CSF collected from the lumbosacral region in humans and many other animal species. There appears to be a decreasing rostral-to-caudal gradient of α-tocopherol concentrations in CSF from brain to spinal cord in those species.5,18 To our knowledge, this gradient has not been definitively identified in horses, but similar findings would be expected. Therefore, samples of CSF collected from the atlanto-occipital space presumably provided accurate values of α-tocopherol concentrations around the brain and cervical portion of the spinal cord, which is where the most damage to the CNS is detected in horses.

At the end of treatment with supplemental vitamin E, the correlation of serum and CSF α-tocopherol concentrations in the study horses was poor. This finding suggests that the duration of treatment with vitamin E supplementation required to achieve a steady-state (plateau) concentration of α-tocopherol in CSF is likely longer than that required to achieve a steady-state concentration of α-tocopherol in serum. In the horses of the present study, serum α-tocopherol concentration peaked and reached a plateau within 10 days after initiating oral administration of supplemental vitamin E. In a study19 in humans, α-tocopherol concentration in CSF had not reached equilibrium values and continued to slowly increase even after 600 days of treatment with 2,000 U of vitamin/d.

In the present study, serum α-tocopherol concentrations decreased rapidly following cessation of daily administration of supplemental vitamin E in both groups of horses; within 10 days, median values had almost returned to pretreatment baseline values, especially in the group of horses that were treated with 1,000 U of d-α-tocopherol/d. Results of the study by Ronen et al16 indicated that a period of 1 month would have to elapse for serum α-tocopherol concentrations to decrease to a minimum steady state in horses fed a vitamin E–deficient diet. Similar to findings in our study, those authors also reported rapid increases in serum α-tocopherol concentrations as a result of oral administration of supplemental vitamin E and achievement of a steady-state serum concentration within a

**Table 1—Concentrations of α-tocopherol in serum and CSF samples obtained from horses before (day 0; baseline), during (days 3 and 6), and after (days 10 through 20) oral administration of supplemental vitamin E (1,000 U of d-α-tocopherol/d [group A] or 10,000 U of d-α-tocopherol/d [group B]). Prior to day 7, horses had been fed alfalfa hay only; grass hay was provided during the remainder of the study. Vitamin E was administered in 0.227 kg of sweet feed beginning on day 0 (after sample collection) and ending on day 9.***

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A (n = 5)</th>
<th>Group B (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (μg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>Mean ± SD</td>
<td>Median (range)</td>
</tr>
<tr>
<td>–7</td>
<td>1.44 ± 0.39</td>
<td>1.4 (1.2–2.1)</td>
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<tr>
<td>0</td>
<td>1.10 ± 0.25</td>
<td>1.0 (0.8–1.7)</td>
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<td>3</td>
<td>2.16 ± 1.28</td>
<td>1.6 (1.2–4.4)</td>
</tr>
<tr>
<td>6</td>
<td>2.98 ± 1.33</td>
<td>2.4 (2.1–5.3)</td>
</tr>
<tr>
<td>10</td>
<td>2.96 ± 1.05</td>
<td>2.5 (2.3–4.8)</td>
</tr>
<tr>
<td>13</td>
<td>2.54 ± 1.01</td>
<td>2.1 (1.6–4.3)</td>
</tr>
<tr>
<td>16</td>
<td>1.80 ± 0.67</td>
<td>1.8 (1.2–2.9)</td>
</tr>
<tr>
<td>20</td>
<td>1.70 ± 0.51</td>
<td>1.5 (1.4–2.6)</td>
</tr>
<tr>
<td>CSF (ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>Mean ± SD</td>
<td>Median (range)</td>
</tr>
<tr>
<td>0</td>
<td>8.8 ± 4.66</td>
<td>9 (2–15)</td>
</tr>
<tr>
<td>10</td>
<td>17.4 ± 8.02</td>
<td>16 (11–31)</td>
</tr>
</tbody>
</table>

*For this variable, value was significantly (P ≤ 0.05) different from mean value for group A at this time point.

IQR = Interquartile range.
few days after commencement of treatment. Following discontinuation of treatment, \( \alpha \)-tocopherol concentrations in serum decreased rapidly during the first week and then more slowly during the subsequent 2 weeks. In addition, that same study revealed that \( \alpha \)-tocopherol concentrations in muscle increased more slowly and remained increased for a much longer period (> 7 weeks) following cessation of vitamin E administration, compared with concentrations in serum. Those findings suggest that vitamin E concentration is more stable in muscle and responds more slowly to changes in concentrations of vitamin E in feed. A similar situation could apply to CSF if passage of vitamin E across the blood-brain barrier is rate limited by carrier molecules, resulting in slower changes in CSF concentration relative to changes in serum concentration. This was not confirmed in our study because CSF \( \alpha \)-tocopherol concentrations in horses were not reevaluated later than 24 hours after discontinuation of vitamin E treatment. However, a rate-limiting step in entry of vitamin E into the CSF could explain the proposed finding that \( \alpha \)-tocopherol concentrations are slower to reach a steady state after administration of supplemental vitamin E in CSF than in serum.

In another study in horses, it was determined that serum \( \alpha \)-tocopherol concentration within an individual could vary widely during a period of several days without any change in diet or administration of supplemental vitamin E. The coefficient of variation within an individual in that study was 12%. Such individual variation has been attributed to differences in metabolism, which could account for some of the variation among individual horses in the present study. However, regardless of the absolute serum \( \alpha \)-tocopherol concentrations measured in our study, concentrations increased significantly in response to oral administration of vitamin E in each horse. Also, results of the previous study indicated that serum values reflect only 1% of total body amounts of \( \alpha \)-tocopherol and that fluctuations in serum \( \alpha \)-tocopherol concentration do not affect tissue concentrations.

Nine of the 10 horses in the present study had 1.3- to 3.4-fold increases in CSF \( \alpha \)-tocopherol concentrations as a result of oral administration of supplemental vitamin E. However, there was 1 horse that did not develop a significant increase in CSF \( \alpha \)-tocopherol concentration in response to treatment. In horses, there is considerable individual variation in tissue vitamin E concentrations following intake of high doses of vitamin E (ie, > 1,800 mg/d or > 2,600 U/d). Because vitamin E is a fat-soluble vitamin, the percentage of body fat in an individual horse may favor the uptake of vitamin E into adipose tissue, thereby decreasing concentrations that accumulate in the CSF and other body tissues. Although all horses in the present study were considered healthy on the basis of results of a physical examination and CBC, older horses are likely to have higher antioxidant demand than younger horses because of greater age-related oxidative stress. Tissue cells are exposed to ongoing endogenous and exogenous oxidative reactions over time, which results in cumulative oxidative damage as part of the aging process. Therefore, it could be postulated that older horses may have lower \( \alpha \)-tocopherol concentrations within the CSF because of increased utilization rather than decreased entry of vitamin E into the CSF. Large individual variation in serum \( \alpha \)-tocopherol concentrations was not detected in the horses of our study, suggesting that the variation in CSF \( \alpha \)-tocopherol concentrations was not attributable to individual differences in intestinal absorption of orally administered vitamin E.

The findings of the study of this report indicated that serum \( \alpha \)-tocopherol concentration in a horse increases rapidly in a nonlinear manner if 10,000 units of supplemental \( \delta \)-\( \alpha \)-tocopherol is administered orally each day, compared with the effects of 1,000 units of supplemental \( \delta \)-\( \alpha \)-tocopherol. Establishment of steady-state concentrations of \( \alpha \)-tocopherol in the CSF is likely necessary to thoroughly compare the 2 treatment doses and determine the minimum dose needed for a beneficial antioxidant effect. However, median and maximum CSF \( \alpha \)-tocopherol concentrations were higher in horses treated with 10,000 U of \( \delta \)-\( \alpha \)-tocopherol/d, compared with findings in horses treated with the lower dose, suggesting that high doses of supplemental vitamin E are likely of greater antioxidant benefit. Additional clinical studies to further evaluate the efficacy of daily oral administration of supplemental vitamin E in horses could include investigation of \( \alpha \)-tocopherol concentrations in the CSF of horses with clinical signs of neurologic disease before and after vitamin E treatment. Such an investigation could evaluate the effects of similar treatment protocols in horses with inflammatory or degenerative neurologic diseases, including equine protozoal myeloencephalitis, equine herpesvirus-1 myeloencephalopathy, equine degenerative myeloencephalopathy, equine motor neuron disease, cervical vertebral malformation, West Nile virus encephalitis, or traumatic injury to the CNS. Results of that research would help to further evaluate the clinical application of vitamin E treatment in affected horses, particularly as those data could be compared with findings in the healthy, neurologically normal horses that were evaluated in the present study. Horses with neurologic disease may respond somewhat differently to vitamin E treatment because of possible disruption of the blood-brain barrier or increased oxidant damage associated with the underlying neurologic disease. However, results of oral administration of supplemental vitamin E in neurologically affected horses would likely be similar to those achieved in the healthy horses of the present study, potentially with higher CSF concentrations of \( \alpha \)-tocopherol in horses with a compromised blood-brain barrier. It is possible that vitamin E in CSF might be more rapidly metabolized in horses with neurologic disease and increased oxidative stress within neural tissue. Therefore, it can be postulated that, compared with findings in healthy horses, measurements of CSF \( \alpha \)-tocopherol concentrations in horses with neurologic disease may not increase to the same extent if vitamin E is being used more rapidly at the desired site of action.

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a. Emcelle-Tocopherol, Stuart Products, Bedford, Tex.
d. Waters 2475 multiﬂuorescence detector, Waters Corp, Milford, Mass.

References