Review
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A Comparative Review of Vitamin E and Associated Equine Disorders

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Vitamin E is a primary chain-breaking antioxidant that prevents cyclic propagation of lipid peroxidation. Across species, vitamin E is essential for normal neuromuscular function by acting as a potent antioxidant, as well as by modulating the expression of certain genes, inhibiting platelet aggregation and stabilizing plasma membranes. This review focuses on vitamin E structure, absorption, metabolism, current equine dietary recommendations, the interplay between antioxidants and exercise, a discussion of the necessity of vitamin E supplementation in the horse above the Nutritional Research Council (NRC) 2007 requirements, and a review of equine diseases that are associated with a vitamin E deficiency. Particular emphasis is placed on the proteins involved in vitamin E absorption, transport, and metabolism as potential candidates for vitamin E-associated diseases across species.

Key words: Genetics; Neurology; Neuromuscular disorders; Nutrition; Spinal cord disease.

Vitamin E was discovered in 1922 as an element essential for normal reproductive function in rats.[1] Over the next 50 years, evidence emerged that vitamin E was also important for normal immune, neurologic, and muscular system function. This review includes comparative information on the function, absorption, metabolism, and tissue concentrations of vitamin E, with emphasis on the horse. Additionally, diseases associated with vitamin E deficiency and current recommendations for dietary and supplemental vitamin E in the horse are reviewed.

Chemical Structure and Properties

Vitamin E describes a closely related family of 8 fat-soluble naturally occurring compounds.[2] The family consists of 2 subgroups: tocopherols (saturated) and tocotrienols (unsaturated). Within each subgroup, there are 4 individual isoforms (α, β, γ, and δ) (Table 1). Although γ-tocopherol is the most abundant isoform in the diet, constituting 70% of the vitamin E in a human diet,[3] α-tocopherol is the most potent antioxidant, the most biologically available, and therefore the most well-researched isoform.[4] There are 8 different stereoisomers of α-tocopherol (Table 1). Naturally occurring α-tocopherol (D-α-tocopherol) has the R-configuration at the 3 positions (2, 4, and 8') in the chromanol ring and is therefore termed RRR-α-tocopherol. Because of preferential hepatic uptake and subsequent secretion into plasma for transport to the peripheral tissues, RRR-α-tocopherol is the most biologically active isoform of vitamin E and RRR-α-tocopherol also possesses the most potent antioxidant properties.[4]

Abbreviations:

ABCA1	ATP-binding cassette transporter 1
α-TTP	α-tocopherol transfer protein
ALS	amyotrophic lateral sclerosis
AVED	ataxia with vitamin E deficiency
BBB	blood–brain barrier
BW	body weight
CEHC	carboxyethyl hydroxychroman
CNS	central nervous system
CSF	cerebrospinal fluid
CYP	cytochrome
DMI	dry matter intake
EDM	equine degenerative myeloencephalopathy
EMND	equine motor neuron disease
GSH-Px	glutathione peroxidase
HDL	high-density lipoprotein
IgG	immunoglobulin G
LDL	low-density lipoprotein
LPL	lipoprotein lipase
MDA	malondialdehyde
MDR2	multi-drug resistance 2
NAD	neuroaxonal dystrophy
NPV	negative predictive value
NRC	Nutritional Research Council
PLTP	phospholipid transfer protein
PPV	positive predictive value
PXR	pregnane X receptor
ROS	reactive oxygen species
SC	sacrocaudalis
SR-BI	scavenger receptor class BI
SOD	superoxide dismutase
TAP	tocopherol-associated protein
VLDL	very low-density lipoprotein

Historically, the role of γ-tocopherol in human health was deemed nominal.[5] However, more recent evidence in humans suggests that α- and γ-tocopherol are not differentially absorbed across the blood–brain barrier and both could be important for brain function.[6] Furthermore, in humans, the tocotrienol subfamily of vitamin E possesses powerful neuroprotective and anticancer effects not often exhibited by tocopherols and have potent antioxidant activity.[7] In

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Table 1. Components of vitamin E: Vitamin E consists of 2 subgroups: tocopherols (saturated) and tocotrienols (unsaturated). Within each subgroup, there are 4 individual isoforms (α, β, γ, and δ). There are 8 different stereoisomers of α-tocopherol. Naturally occurring α-tocopherol has the R-configuration at the 3 positions (2, 4, 8') in the chromanol ring and is therefore termed RRR-α-tocopherol.

Components of Vitamin E	
Tocopherols	Tocotrienols
α-tocopherol (8 stereoisomers)	α-tocotrienol
RRR	
SRR	
RRS	
RSS	
RSR	
SSR	
RSS	
SSS	
β-tocopherol	β-tocotrienol
γ-tocopherol	γ-tocotrienol
δ-tocopherol	δ-tocotrienol

the horse, γ -tocopherol and the tocotrienol isoforms have not been studied and therefore, for the purposes of this review, the focus will remain primarily on α-tocopherol.

Function

Of the 8 isoforms, RRR-α-tocopherol is the most potent antioxidant. Peroxyl radicals react 1,000× more favorably with α-tocopherol than with polyunsaturated fatty acids,[4] and the order of reactivity toward peroxyl radicals is $\alpha > \beta = \gamma > \delta$.[8] Vitamin E's role in peroxyl radical scavenging is as follows:

$$ROO\cdot + VitE - OH \rightarrow ROOH + VitE - O\cdot$$

The tocopherol radical (VitE–O·) then reacts with vitamin C [AH: hydrogen donors] to return to a reduced state:

$$VitE - O\cdot + AH \rightarrow VitE - OH + A\cdot$$

In the absence of vitamin E, reactive oxygen species (ROS) are repeatedly generated and oxidative damage ensues:

$$ROO\cdot + RH \rightarrow ROOH + R\cdot$$
$$R\cdot + O_2 \rightarrow ROO\cdot$$

Reactive oxygen species are a double-edged sword—they serve as key signal molecules in physiological processes and also have a role in pathologic processes. ROS are implicated in more than 100 diseases.[9]

The Role of Vitamin E

In vivo studies in humans[10,11] and horses[12,13] have largely evaluated the effect of supplemental vitamin E in individuals with baseline normal vitamin E status. The information ascertained in such studies does not always identify the basal role of vitamin E. In contrast, a limited number of studies, mainly in rats, have evaluated the perturbations in various body systems arising from vitamin E deficiency.[14]

Immune Function. High polyunsaturated fatty acid content of immune cells puts them at increased risk for oxidative damage. In many species, T- and B-cell functions are impaired by vitamin E deficiency.[15] Furthermore, vitamin E supplementation in rats repairs some of the age-related changes in the immune system.[16]

In most domestic animals, there is evidence that α-tocopherol supplementation can improve immune function in immunocompromised animals. For example, the supplementation of α-tocopherol-deficient goats and cows with selenium and/or α-tocopherol improved neutrophil function.[17,18] Furthermore, α-tocopherol-deficient donkeys had decreased serum immunoglobulin concentrations and α-tocopherol supplementation increased the IgG_T concentrations.[19] However, there is little evidence that a deficiency of selenium, vitamin E or both affects resistance to disease or the total or specific immune response.[20]

In horses fed diets low in vitamin E and selenium, the role of α-tocopherol in the humoral immune response was demonstrated by vaccination against tetanus toxoid and equine influenza virus.[21] An increased IgG response occurred in horses receiving either vitamin E or vitamin E and selenium. This finding was recently confirmed where, in addition to increased IgG_a and IgG_T, horses supplemented with synthetic α-tocopherol (15 IU all-rac α-tocopherol [racemic mix consisting of an equal mix of the 8 stereoisomers]/kg body weight [BW] for 16 weeks) demonstrated increased bacterial killing capacity of monocytes and neutrophils.[22] These studies prompted the Nutritional Research Council (NRC) to increase the recommended dietary level of α-tocopherol from 15 IU/kg DMI (maintenance and growth) in 1978 to 50 IU/kg DMI and 80 IU/kg DMI (maintenance and growth, respectively) in 1989.[23]

Reproduction. Reactive oxygen species affect multiple reproductive processes from oocyte maturation to fertilization, embryo development, and pregnancy, as well as normal spermatozoal function.[24,25] There is, however, no clear evidence that vitamin E supplementation improves reproductive function in humans or horses.[26,27] This is of note since vitamin E was originally discovered for its effects on reproduction in rats.[1]

Gene Transcription and Cellular Functions. In addition to its antioxidant effects, RRR-α-tocopherol affects the rate of transcription of certain genes in humans and mice, including CD36,[28] α-tropomyosin,[29] and collagenase.[30] Other functions attributed to this vitamin are inhibition of cell proliferation,[31] nuclear factor (NF-κB) activation,[32] platelet aggregation,[33]

monocyte adhesion,[34] and stabilization of plasma membranes.[8] These effects appear unrelated to the antioxidant properties and instead result from specific interactions of α-tocopherol with components of the cell, including proteins, enzymes, and membranes.[8]

Neuromuscular Function. Early studies in rats revealed that both ataxia and muscle dysfunction result from prolonged dietary vitamin-E deficiency.[35] Electrophysiologic studies of deficient rats determined that muscle degeneration preceded degeneration of peripheral nerves.[36] In these rats, necrosis of type I muscle fibers was observed in addition to increased spheroid (axonal swelling) formation in the gracilis and cuneate nuclei of the brainstem. The role of vitamin E in the maintenance of normal nervous tissue is highlighted by human patients with mutations in specific genes causing fat absorption disorders, including abetalipoproteinemia, and from patients suffering from ataxia with vitamin E deficiency (AVED). Accumulation of spheroids in the gracilis and cuneate nuclei occur in AVED,[37] which have ultrastructural features of tubulovesicular structures, as well as accumulations of smooth membranes and dense core vesicles.[38] This accumulation probably occurs because of interruption of active anterograde and retrograde transport along the axon, which is crucial for neuronal survival. Cargos such as synaptic vesicle precursors, neurotransmitters, neurotropic factor receptors, signaling endosomes, and mRNAs are actively transported within axons by molecular motors, which also remove damaged or misfolded proteins.[39] Alpha-tocopherol is probably involved in maintaining normal axonal transport,[40] and α-tocopherol deficiency could cause a defect of fast retrograde axonal transport, vesicular "turnaround", or both, causing an accumulation of normal and abnormal cytoplasmic organelles at the axon terminal and subsequent blockage of normal axonal flow.[41] The underlying molecular and cellular mechanisms that result in neuromuscular dysfunction after α-tocopherol deficiency have yet to be defined.

Vitamin E deficiency has also been implicated in the pathogenesis of motor neuron diseases. Lipopigment deposits in the spinal cord, along with predilection for denervation of the highly oxidative type I muscle fibers, suggest that oxidative injury plays a role in several motor neuron diseases. In humans, free radical damage to the motor neurons has been incriminated in the pathogenesis of amyotrophic lateral sclerosis (ALS) where several distinct familial ALS mutations in the copper/zinc superoxide dismutase gene (SOD1), a critical component of cellular antioxidant defense mechanisms, have been identified.[42] Vitamin E supplementation slowed progression and delayed the onset of clinical disease in a transgenic mouse model of ALS.[43] Recently, a pooled analysis from 5 prospective cohort studies determined that long-term vitamin E supplementation was associated with decreased risk of ALS.[44] Vitamin E deficiency also plays a contributory role in equine motor neuron disease as discussed later in this review.

Vitamin E in Exercise. Vitamin E deficiency is rare in human athletes performing at peak levels,[45] and exercise performance is unimpaired in deficient individuals.[46] In 2000, insufficient information was available to recommend antioxidant supplementation in human athletes[47] and recent evidence suggests that it could actually be detrimental during exercise. A daily high dose of vitamins C and E ameliorated the transient oxidative stress associated with exercise; however, this transient oxidative stress was considered beneficial because it at least partially induced the increase in insulin sensitivity that occurs with physical exercise.[48]

Early studies in horses showed no apparent increase in measures of oxidative stress with exercise.[49] More recent equine studies using measures of oxidative stress, such as plasma thiobarbituric reactive substances, plasma lipid hydroperoxides, total glutathione and glutathione peroxidase, and malondialdehyde (MDA), have demonstrated oxidative stress with intense exercise.[50,51] Some studies of α-tocopherol supplementation have shown no effect on exercise-induced oxidative stress,[12,49,50,52] whereas others have shown a minimizing effect.[13,51] Plasma MDA levels and vitamin E concentrations in one study were significantly inversely correlated in fit horses.[13] In response to intense exercise, elite 3-day event horses at the 2- and 3-star level showed no change in plasma α-tocopherol concentrations.[53] Additional research is needed to determine if there are any detrimental effects of high antioxidant supplementation in exercising horses.

Current Equine Dietary Recommendations for Vitamin E

Vitamin E content varies markedly among equine dietary constituents, with the highest levels in fresh grass and declining concentrations with processing and storage.[54] A significant decrease in vitamin E occurs between the 1st and 5th cutting of alfalfa hay[55] and storage losses can reach 50% in 1 month.[54] A seasonal variation in plasma vitamin E concentrations occurs with increased plasma vitamin E in the summer in

Table 2. National Research Council, Nutrient Requirements of Horses (2007): The recommended dietary amount has been provided in IU/kg body weight of horse based upon an assumed total daily dietary forage intake of 2–2.5% of the horse's body weight.

Classification	Vitamin E (IU/kg)	Assumed Feed Intake (%)
Adult (sedentary)	1	2
Adult light exercise	1.6	2
Adult moderate exercise	1.8	2.25
Adult heavy exercise	2	2.5
Stallions and breeding mares	1	2
Pregnant mares	1	2
Lactating mares	2	2.5
Growing horses	2	2.5

grazing horses fed fresh hay compared with the winter when horses were fed dried hay and oats.[56,57]

The 2007 NRC equine dietary requirements for vitamin E are provided in Table 2. It is important to account for the 2 : 1 ratio (RRR-α-tocopherol to all-rac-α-tocopherol acetate, Table 3), which is described further in the section on vitamin E supplementation, when considering supplementation. For example, if pure RRR-α-tocopherol is the supplement provided to a 500-kg adult sedentary horse (recommended dose of 1 IU/kg), only 250 IU should be administered, whereas if a synthetic all-rac-α-tocopherol supplement is provided, 500 IU should be administered. These respective dosages will provide the same active amount of α-tocopherol.

Absorption and Metabolism

Baseline serum or plasma vitamin E concentrations vary widely among healthy horses[58,59] as does the individual response to supplementation, especially at higher dosages.[60–62] In humans, polymorphisms in many of the genes involved in vitamin E uptake, distribution, and metabolism can contribute to individual differences in response to vitamin E supplementation.[63] As the proteins involved in vitamin E metabolism appear to play a pivotal role in overall vitamin E concentrations, the pathways are discussed in detail in the following section.

Table 3. Types of α-tocopherol supplementation: The various forms of α-tocopherol supplementation are provided with their components and relative potency (IU/mg) as compared with the standard, all-rac-α-tocopherol acetate.

Supplement	Component(s)	International Units Per Milligram
All-rac-alpha-tocopherol acetate	α-tocopherol: equivalent proportions of 4 pairs of diasteresoisomers RRR and SRR RRS and RSS RSR and SSR RSS and SSS	1
All-rac-alpha-tocopherol	α-tocopherol: equivalent proportions of 4 pairs of diasteresoisomers RRR and SRR RRS and RSS RSR and SSR RSS and SSS	1.1
DL-alpha-tocopherol acetate	α-tocopherol: equivalent proportions of one pair of diastereoisomers: RRR and SRR	1
DL-alpha-tocopherol	α-tocopherol: equivalent proportions of one pair of diastereoisomers: RRR and SRR	1.1
RRR-alpha-tocopherol acetate	α-tocopherol: one stereoisomer: RRR	1.36 or 2[148,149]
RRR-alpha tocopherol	α-tocopherol: one stereoisomer: RRR	1.49
RRR-γ-tocopherol	γ-tocopherol: one stereoisomer: RRR	0.15

D-α-tocopherol = RRR-α-tocopherol.
L-α-tocopherol = SRR-α-tocopherol.
All references from Weber et al[147] unless specified.

Most research on vitamin E absorption is based in human studies and canine and rat models. The pathways appear to be similar across species and involve intestinal absorption, prehepatic transport, hepatic uptake and transfer, posthepatic transport, and cellular uptake (Fig 1).

Intestinal Absorption. Passive uptake of vitamin E by enterocytes is considered the major pathway for intestinal absorption; however, the scavenger-receptor class B type I (SR-BI) could also partially mediate intestinal vitamin E absorption.[64] This receptor also plays an important overall role in vitamin E transport and tissue uptake as discussed in the following sections. Vitamin E absorption and transport require normal fat absorption and metabolism and, in the absence of dietary fat, little absorption occurs.[4] Secretion of pancreatic esterases and bile acids results in the micellarization of dietary fats and the hydrolysis of triglycerides that release free fatty acids. Micelles are taken up by the intestinal enterocytes and vitamin E is incorporated into chylomicrons formed within the enterocyte that are then secreted into the lymphatic system and ultimately into the bloodstream. In humans, the intestinal absorption and secretion into chylomicrons of various vitamin E isoforms, such as α- and γ-tocopherols, occur at similar rates.[65] Therefore, the discrimination between the vitamin E isoforms does not occur during intestinal absorption.

Prehepatic Transport. Circulating chylomicrons can undergo triglyceride lipolysis by lipoprotein lipase (LPL). This process also delivers vitamin E into the cell[66] and mice genetically engineered to overexpress LPL have enhanced transfer of vitamin E to muscle cells.[67] LPL is the primary vitamin E delivery pathway to the lungs, liver, spleen, kidney, and erythrocytes as demonstrated in rats.[68] During the process of LPL lipolysis, some tocopherols are also transferred to circulating lipoproteins, such as high-density lipoprotein (HDL), while others remain with chylomicrons. Vitamin E isoforms can be readily transferred between circulating HDL and other lipoproteins, a feature that is catalyzed by phospholipid transfer protein (PLTP).[69] PLTP is also required to achieve normal concentrations of vitamin E in the brain[70] and spermatozoa[71] as shown in mice.

Hepatic Uptake and Transfer. The liver takes up chylomicrons via endocytosis mediated by the SR-BI receptor.[4] The cytosolic α-tocopherol transfer protein (α-TTP) then selectively binds RRR-α-tocopherol and packages it into very low-density lipoproteins (VLDL) and/or HDL for secretion into plasma. It is important to note that it is the liver, not the intestine, in which the strict discrimination among isoforms of vitamin E occurs. Binding to the RRR stereoisomer is 25× higher than the SRR counterpart because of the effect

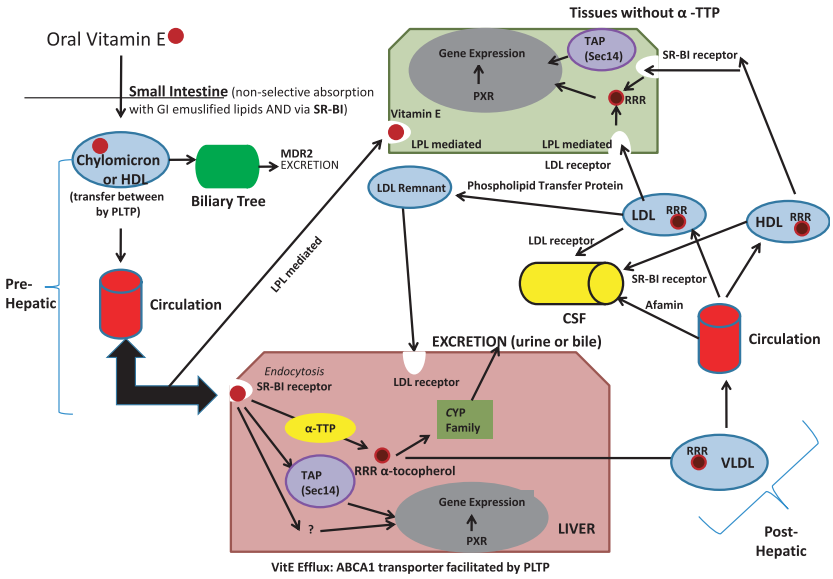

Fig 1. Diagram of vitamin E transport. Vitamin E, containing all 8 isoforms, is represented by the red circle, while RRR-α-tocopherol, an isoform of vitamin E, is represented by the dark red outlined circle. Abbreviations can be found in the abbreviation list.

of ligand geometry on binding.[72] As α-TTP selectively binds RRR-α-tocopherol, this isoform has a 3× greater half-life (mean ± SD, 57 ± 19 hours) in plasma as compared with the other isoforms (range 1.2–4 hours).[4] Alpha-TTP and its ligand, RRR-α-tocopherol, co-localize to the same compartment in the hepatocyte, specifically to endosomes and lysosomes.[73] The ligand-bound state of α-TTP must undergo a conformational change, where, in the closed state, it probably acts as a chaperone for α-tocopherol, ferrying the lipophilic molecule through the cytoplasm for eventual secretion to peripheral tissues.[74] Alpha-tocopherol secretion by hepatocytes into the bloodstream is dependent on the ATP-binding cassette transporter 1 (ABCA1).[75] Targeted mutations of *ABCA1* lead to plasma vitamin E deficiencies in mice, and decreased hepatic lipid uptake was observed, although hepatic α-tocopherol concentrations were not measured.[76] In addition to the liver, α-TTP has been found in rat brain[77] and pregnant mouse uterus.[78]

PostHepatic Transport. Once bound to lipoproteins in circulation, RRR-α-tocopherol is transferred between HDL and low-density lipoprotein (LDL), a reaction that is catalyzed by PLTP.

Cellular Uptake. Various receptors are utilized in the cellular uptake of RRR-α-tocopherol in peripheral tissues. The lipoprotein receptor SR-BI that is involved in intestinal absorption of vitamin E is also present in various tissues. As a transmembrane receptor for α-tocopherol, SR-BI appears to be the key determinant of vitamin E uptake and subsequent concentrations in various tissues.[79] Importantly, central nervous system (CNS) uptake appears to be dependent on SR-BI, with SR-BI-deficient mice exhibiting a 70% reduction in brain α-tocopherol content.[79] Scavenger receptor class BI is a member of the cellular retinaldehyde-binding protein-trio guanine exchange factor (CRAL-TRIO) family, which also includes yeast phosphatidylinositol transfer protein (Sec14p) and tocopherol-associated protein (TAP). Phosphatidylinositol transfer protein (Sec14p) and TAP appear to exhibit only a weak, non-selective binding affinity for tocopherols (25× less than α-TTP for α-tocopherol)[72,80] and are most likely not essential for vitamin E homeostasis. Vitamin E has been demonstrated, in vitro, to bind to and activate the pregnane X receptor, a nuclear receptor involved in regulating a constellation of genes involved in drug detoxification.[81]

Uptake across Blood–Brain Barrier (BBB). An in vitro model of the BBB was used to show that the LDL receptor is involved in transcytosis-dependent transport of RRR-α-tocopherol from the bloodstream into the cerebrospinal fluid (CSF) in porcine and murine models[82]; however, loss of the LDL receptor does not result in decreased brain RRR-α-tocopherol,[79,83] and therefore this pathway does not appear to be essential for vitamin E homeostasis, but could play a minor role. There appears to be no discrimination for isoforms at the BBB. Although RRR-α-tocopherol is present in higher circulating concentrations because of the selectivity of α-TTP, both RRR-α-tocopherol and γ-tocopherol can be found in CSF, suggesting a shared transport mechanism, most likely HDL-mediated by means of SR-BI, across the BBB.[6,84] Most recently, afamin has been demonstrated in vitro to play a role in transporting vitamin E across the BBB.[85] Transporters involved in distributing α-tocopherol into neurons include apolipoprotein E, the LDL-receptor-related protein (LRP) and SR-BI.[86]

Metabolism and Excretion of Vitamin E

Major stores of vitamin E include adipose, liver, and muscle tissue, with 90% of vitamin E storage

occurring in adipose tissue.[87] Vitamin E metabolites are termed 2′-carboxyethyl-6-hydroxychroman (CEHC) products and each respective form of vitamin E has its specific metabolite, namely α-, β-,γ- and δ-CEHCs. The metabolism of vitamin E is modulated by the cytochrome P450 (CYP) system and high level of supplementation with α-tocopherol increases metabolism of vitamin E by modulating a subset of CYP enzymes.[88] Before excretion in bile or urine, most CEHCs undergo enzymatic conjugation. In addition to α-CEHC, α-tocopherol itself is excreted into bile by multidrug-resistance 2 or *p*-glycoprotein (MDR2).[89] As many other drugs are excreted through the CYP system, caution should be used when combining vitamin E supplementation with therapeutic drugs. Animals and humans receiving higher dosages of α-tocopherol could suffer from adverse effects caused by increased drug bioavailability.[90]

Reference Range for Alpha-Tocopherol Concentrations in the Horse

Plasma/Serum. The suggested reference range for normal plasma/serum concentrations of α-tocopherol in the horse can be found in Table 4. Other recommendations cite inadequate concentrations at <3 μg/mL,[91,92] which would place many healthy horses in the deficient range (Table 5). Therefore, the reference range described in Table 4 could be more clinically appropriate.

Originally, it was reported that plasma/serum α-tocopherol concentrations in humans are only 1% of total body stores and blood concentrations can fluctuate without appreciably affecting tissue concentrations.[93] For that reason, it has been suggested that tissue α-tocopherol concentrations could be a better marker of whole body α-tocopherol status than plasma/serum concentrations. In healthy horses, plasma and adipose tissue concentrations[94] and plasma and hepatic and muscle[60] concentrations are linearly correlated. This correlation is also evident in deficient horses suffering from neuroaxonal dystrophy/equine degenerative myeloencephalopathy (NAD/EDM) (Finno CJ, unpublished data), but not in horses with vitamin E-deficient myopathy.[95] Additionally, although adipose α-tocopherol concentrations could be more representative of nervous tissue concentrations than plasma/serum α-tocopherol concentrations,[87] there is a large variability of α-tocopherol concentrations in adipose tissue of clinically normal adult horses.[94] The ease of obtaining a blood sample compared with tissue sampling has resulted in most veterinarians using plasma/serum concentrations of α-tocopherol to estimate overall α-tocopherol status. Factors to consider when evaluating equine plasma or serum α-tocopherol concentrations include breed, age, number of samples, diet, sampling time in relation to feeding, physical conditioning, collection method, and sample storage before analysis (Table 5).

Table 4. Concentrations of α-tocopherol in equine serum and associated status.[99–100,162]

Serum Concentration of α-Tocopherol (μg/mL)	α-Tocopherol Status
>2	Adequate
1.5–2	Marginal
<1.5	Deficient

Influence of age or breed. Although within the reference range (>2 μg/mL as adequate), statistically lower plasma α-tocopherol concentrations have been reported in Thoroughbreds compared with other breeds (Quarter horses, Arabians, Percherons, Paints, Appaloosa) regardless of gender.[94] Foals (<1 week of age),[96] weanlings,[97] and yearlings[57,58] appear to have significantly lower plasma/serum α-tocopherol concentrations compared with adults (>1 year of age). Using a cut-off value of 1.2 μg/mL, 80% of weanlings were α-tocopherol-deficient as compared to 20% of adults, regardless of diet or season with no neuromuscular abnormalities apparent.[57] In neonates, this is likely because less than 2% of the dam's α-tocopherol is transferred into the milk,[54] and a significant increase in colostral α-tocopherol levels at foaling is not apparent.[98] Interestingly, α-tocopherol concentrations in mares and their foals are not well correlated, with some mares having a low serum/plasma α-tocopherol concentration while their foals were high and vice versa.[59] Age is an important variable to consider in cases of NAD/EDM, which typically affect horses during the first year of life and is associated with vitamin E deficiency. Based upon current research, horses $\leq$1 year of age might require a different reference range from that of adults.

Influence of season. A seasonal effect on serum α-tocopherol concentrations occurs with lower values in the winter months,[56,59] but this is most likely because of changes in diet, including pasture and fresh hay in summer versus stored hay in the winter. No effect of season was noted in a group of horses on Prince Edward Island.[99]

Sampling time point. There is wide variability in individual serum α-tocopherol concentrations over a 72-hour period with no apparent relation to feeding times or diurnal variation.[58] There was more variation in the yearlings (13%) than the adults (10%) in this study (combined average of 12% mean coefficient of variation). Based on these results, multiple samples (ie, 3 in 1 horse) were recommended to assess vitamin E status.[58] Deficient horses have larger fluctuations in serum α-tocopherol (mean coefficient of variation 41%) than nondeficient horses (mean coefficient of variation 14%).[100] The deficient horses fluctuated between deficient and normal α-tocopherol concentrations within a 24-hour period. This fluctuation could be caused by the rapid recirculation of α-tocopherol between the liver and plasma. This results in the daily replacement of nearly the entire circulating α-tocopherol pool.[101] Of note, in the horses with normal concentration (>2 μg/mL), serum α-tocopherol never fell below 1.5 μg/mL.[100] The sensitivity of a single α-tocopherol concentration was 91%, specificity was 82%, positive predictive value

Table 5. Concentrations of vitamin E in serum, plasma, and various tissues as measured in normal horses.

Sample Category	α-Tocopherol (μg/mL) (mean ± SD)	Sample Size (n)	Age Group	Breed and Reference	Diet
Plasma	4.04 ± 0.31[a]	6	3–5 years	Standardbreds[102]	Grass silage and oats
	3.03 ± 0.18	40	2–3 years	Thoroughbred[163]	Grass hay and oats
	3.15 ± 0.33	116	2–16 years	Saddlebreds[164]	N/A
	5.1 ± 0.43	337	Not specified	Thoroughbred[164]	N/A
	4.2 ± 1.6	90	Not specified	Warmbloods[165]	Grass hay and concentrates
	4.74 ± 1.4	3	≤ 10 days	Free ranging Przewalski horses[96]	Optimal Forage
	6.58 ± 1.14	16	5–19 years	Free ranging Przewalski horses[96]	Optimal Forage
	2.8 ± 0.9	25	4–23 years	Thoroughbred, Quarter horse, Arabian, Percheron, Paint, Appaloosa, mixed[94]	High quality coastal hay and alfalfa pellets
Serum	1.44 ± 0.66	5	9–23 years	Thoroughbred, Arabian, Standardbred, Quarter horse[61]	Alfalfa hay
	1.15 ± 0.43	5	9–23 years	Thoroughbred, Arabian, Standardbred, Quarter horse[61]	Grass hay
	1.94 ± 0.14	47	3–15 years	Finnish trotters[56]	Dried hay and oats
	2.64 ± 0.14	47	3–15 years	Finnish trotters[56]	Fresh hay, grass and oats
	3.07 ± 0.79	151	≥ 2 years	Mixed Breeds and Standardbreds[99]	Pasture, hay, complete feeds: various
	4.08 ± 1.57	50	<72 hours	Standardbreds[99]	Mare's milk
	2.6 ± 0.34	6	1 year	Thoroughbred, Quarter horse, Arabian, Appaloosa, grade horses[58]	Grass/alfalfa plus custom grain mix
	3.59 ± 0.34	6	2–18 years	Thoroughbred, Quarter horse, Arabian, Appaloosa, grade horses[58]	Grass/alfalfa plus custom grain mix
	2.62 ± 0.01	29	1–20 years	Various breeds[103]	Unknown
	Range 2.86–3.51	11	3–12 years	Thoroughbreds[146]	Grass hay, unfortified sweet feed and vitamin/mineral premix deficient in vitE
Muscle	4.2 ± 0.8 ug/g	12	Adult	Standardbred[60]	Hay and oats deficient in vitE
Liver	3.5 ± 1.2 ug/g	12	Adult	Standardbred[60]	Hay and oats deficient in vitE
Adipose	22 ± 15 ng/mg weight	25	4–23 years	Mixed[94]	Grass hay and alfalfa pellets
CSF	9.5 ± 5.54 ng/mL	10	9–23 years	Thoroughbred, Arabian, Standardbred, Quarter horse[61]	Grass hay

N/A, not available; vitE, vitamin E.
[a]Results given as mean ± SEM.

(PPV) was 85%, and negative predictive value (NPV) was 90% in this study.

Intraindividual consistency in horses, whether toward a high α-tocopherol or low α-tocopherol concentration, was maintained in 12 broodmares over a 13-month period sampled monthly.[59] The same individual consistency is true, such that horses that have a normal α-tocopherol serum concentration remain consistently within the normal range despite multiple samplings over time. Therefore, although multiple sampling over a 24-hour period would be ideal to definitively determine plasma/serum α-tocopherol concentrations, one sample could be sufficient to gain insight into the animal's overall status (adequate, marginal, or deficient). We recommend that, if a sample is marginal (α-tocopherol between 1.5 and 2 μg/mL), an additional sample should be taken and an average calculated to determine true status. Evaluating 2 samples increased the sensitivity to 97%, specificity to 91%, PPV to 90%, and NPV to 98%.[100] Horses with a high suspicion of α-tocopherol deficiency based on clinical signs, but with marginal or normal serum α-tocopherol concentrations, should have at least 2 samples evaluated at different time points.

Diet and sample collection with relation to feeding. Concentrations of α-tocopherol in the body are reflec-

tive of dietary intake. Fasting does not appear to be necessary before obtaining a serum/plasma sample.[58]

Physical conditioning. When unsupplemented Standardbreds were trained for 12 weeks, plasma α-tocopherol significantly decreased as compared with baseline values[102]; however, all values remained with the reference range of >2 μg/mL. In examining over 450 competition horses, plasma α-tocopherol concentrations were within the reference range (Table 5). Overall, physical conditioning does not appear to play a significant role in plasma/serum α-tocopherol concentrations.

Collection method. Hemolysis results in the greatest change in serum α-tocopherol values, with a mean decrease of 33%, thus hemolyzed samples should be discarded.[103] Blood left in contact with the rubber stopper (stored horizontally) for 72 hours (decrease of 10%), storage at room temperature for 72 hours (5% increase), or repeatedly (by 3rd cycle) freezing and storing samples (3% decrease), all impact serum α-tocopherol. In the same group of horses, serum α-tocopherol values are significantly higher (4%) than those in plasma (EDTA-treated or heparinized).[103] Based on these findings, blood samples for α-tocopherol analysis should be stored upright in a refrigerator for up to 72 hours and, if a longer period is necessary, serum or plasma separated from blood. Light decreases α-tocopherol concentrations in human samples[104,105] and protection from light might be warranted.

Use of α-tocopherol ratios. In humans, a ratio of α-tocopherol to serum lipid (serum total lipid or cholesterol) was proposed as a more accurate method of determining α-tocopherol status because hyperlipemic patients had high or false-normal serum vitamin E concentrations.[106] In horses, this is not recommended as the values do not appear to be correlated and demonstrate wide fluctuations.[58,94] A ratio of α-tocopherol to albumin might be necessary in exercising horses to account for changes in fluid redistribution during exercise.[50,51]

Tissue and Cerebrospinal Fluid (CSF). Baseline reference ranges for tissue and CSF α-tocopherol concentrations are provided in Table 5. Significant correlations between plasma/serum α-tocopherol concentrations and adipose tissue,[94] liver,[60] muscle,[60] and CSF[62] occur in healthy horses. Muscle, nervous, and adipose tissue concentrations are the most stable and slowest to change upon dietary alteration.[60,107,108] In states of depletion, adipose tissue α-tocopherol is mobilized extremely slowly.[107] In humans, before the measurement of CSF α-tocopherol, adipose tissue was most closely correlated with brain tissue concentrations.[105] Alpha-tocopherol CSF concentrations are significantly correlated with striatal brain tissue concentrations.[109] It is necessary to consider the total protein concentration in CSF as α-tocopherol concentrations in CSF appear to be strongly correlated with total protein and albumin concentrations.[110]

Human Vitamin E Deficiencies

Many of the pathways involved in vitamin E absorption were uncovered through studying specific defects in fat and/or vitamin E metabolism. Abetalipoproteinemia, an inborn error of metabolism involving a defect in the microsomal triglyceride transfer protein,[111] results in undetectable serum concentrations of vitamin E from birth.[112] The low serum vitamin E is attributable to the absence of chylomicra, which are necessary for vitamin E absorption, and result from the lack of LDL and VLDL, which are necessary for vitamin E transport. By adulthood, patients suffering from abetalipoproteinemia develop progressive ataxic neuropathy and retinal pigmentation.[41]

Ataxia with vitamin E deficiency is a severe deficiency of vitamin E in patients with normal fat absorption. Neurologic and retinal lesions develop similar to abetalipoproteinemia. AVED is caused by missense mutations (single nucleotide change resulting in coding for a different amino acid) and splice-site mutations in the *α-TTP* gene.[113,114] Many of these mutated proteins cannot discriminate between the RRR and SRR configurations of α-tocopherol,[115] which results in clinical disease due to RRR-α-tocopherol deficiency.

Equine Diseases Associated with Vitamin E Deficiency

Vitamin E deficiency, in and of itself, does not appear to reliably cause disease in horses. Studies examining the effect of vitamin E deficiencies in exercising or resting horses have revealed no apparent clinical signs resulting from vitamin E deficiency.[49,52,57,60] There are, however, 3 specific diseases that consistently have been associated with α-tocopherol deficiency: EMND, NAD/EDM, and vitamin E-deficient myopathy.

Equine Motor Neuron Disease (EMND). Equine motor neuron disease is a worldwide acquired neurodegenerative disorder of the somatic lower motor neurons in the ventral horns of the spinal cord and selected brain stem nuclei.[116] Clinical signs include weight loss because of muscle wasting, muscle fasciculations, and prolonged recumbency.[117] A definitive diagnosis is based upon postmortem demonstration of degeneration and loss of motor neurons from the ventral horns of the spinal cord.[116] Antemortem diagnosis of EMND is based upon either histologic evidence of the degeneration of myelinated axons upon biopsy of the ventral branch of the spinal accessory nerve or the finding of neurogenic atrophy of predominantly type I muscle fibers in sacrocaudalis dorsalis medialis muscle biopsy (sensitivity of approximately 90%).[118,119]

Equine motor neuron disease affects neurons supplying highly oxidative type I muscle fibers, and this oxidative disorder is associated with a dietary deficiency of vitamin E[120] and low plasma concentrations of vitamin E.[117] Horses with naturally occurring EMND require at least 18 months of a vitamin E deficiency before developing clinical signs,[121] and in an experimental model, a 21-month interval of vitamin E deficiency was required before the development of clinical disease.[120] In addition, excessive dietary copper, a potential pro-oxidant, is a risk factor for EMND development.[120]

In the experimental model of EMND, although all horses (n = 8) developed a vitamin E deficiency during the 30-month study, only 4 developed clinical signs of EMND.[120] In naturally occurring EMND, there appears to be an individual susceptibility to oxidative stress in at-risk horses, with clinical signs developing in only a subset of horses maintained in high-risk environments, such as no access to pasture and no vitamin E supplementation.[117] Clinically unaffected horses could suffer from subclinical disease as histologic lesions can be found in vitamin E-deficient apparently unaffected horses,[120] or as discussed previously, it could be that specific polymorphisms in genes involved in vitamin E metabolism determine individual susceptibilities to EMND under the same conditions of deficiency. Indeed, genetic factors outside the major histocompatibility complex were suggested to influence susceptibility to EMND.[122] This effect could also be indirect, in that the α-tocopherol deficiency directly impairs BBB integrity, thereby potentially allowing neurotoxins access to the CNS.[123]

Equine motor neuron disease shares commonalities to the sporadic form of human ALS. Sporadic ALS constitutes 90–95% of all ALS cases, with 5–10% considered familial. The majority of familial ALS cases are associated with mutations in various genes, including Cu/Zn superoxide dismutase (SOD1),[124] a potent free radical scavenger. SOD1 helps to prevent oxidative damage in metabolically active cells, such as neurons. Sequencing of cDNA from SOD1 in EMND affected and unaffected horses did not reveal any putative mutations.[125]

Horses without access to green forage should be supplemented with 1 U/kg BW/day of vitamin E to prevent against EMND development.[120] This dosage is similar to NRC requirements for horses without pasture access (600–800 IU/500 kg horse/day). For EMND-affected cases, 5,000–7,000 IU α-tocopherol/day is recommended. With this treatment, approximately 40% of cases demonstrate clinical improvement within 6 weeks, and some could appear normal within 3 months.[95] It should be noted, however, that return to performance could result in deterioration. Divers et al report that approximately 40% of cases will stabilize, but remain permanently disfigured, while 20% will have continual progression of clinical signs.[95]

Neuroaxonal Dystrophy/Equine Degenerative Myeloencephalopathy. Equine NAD is clinically indistinguishable from EDM. Neuroaxonal dystrophy is a morphologic abnormality of select neurons and their axonal processes in the nervous system. Equine NAD is considered the underlying basis of EDM, with a high likelihood that the pathophysiology of the two diseases is similar. Histologic lesions in both NAD and EDM consist of dystrophic neurons and axons, vacuolization, and spheroid formation,[126] with the only difference being the distribution of the lesions. In previous case reports, the disease was classified as NAD if the lesions were confined to the lateral (accessory) cuneate, medial cuneate, and gracilis nuclei,[126,127] whereas a diagnosis of EDM was assigned when axonal necrosis and demyelination extended into the dorsal and ventral spinocerebellar tracts and ventromedial funiculi of the cervicothoracic spinal cord.[92,128–131] Histologic lesions consistent with both NAD and EDM can occur in the same animal.[132]

Clinical cases of NAD/EDM have been reported in several breeds, including Standardbreds,[133] Paso Finos,[133] Quarter horses,[128,132] Mongolian horses,[92] Appaloosas,[130] Haflingers,[127] Arabians,[134] Morgans,[135] Lusitanos,[136] Thoroughbreds,[134,137] Paint horses,[138] Tennessee Walking Horses,[138] Norwegian Fjord Horses,[138] a Welsh Pony,[138] and various mixed breeds.[129,131] There is no sex predilection[135] and age of onset ranges from birth[134,139] to 36 months,[135] although most cases demonstrate clinical signs by 6–12 months of age. There is strong evidence of a genetic component, in that many of the clusters of case reports involve related horses.[127,129,130,135,136] The mode of inheritance appears to be autosomal dominant with variable expression or polygenic.[135] A study of risk factors associated with the development of EDM found that foals from dams that had an EDM-affected foal were at a significantly higher risk (25× more likely) of developing EDM than foals from other dams.[140]

Clinical signs in all cases include symmetric ataxia that is often more severe in the pelvic limbs than in the thoracic limbs, abnormal base-wide stance at rest, and proprioceptive deficits. In some reports, hyporeflexia of the cervicofacial and cutaneous trunci is described in addition to an absence of laryngeal adductor reflex.[128,133] Horses with NAD/EDM that survive to 2–3 years of age commonly exhibit lifelong, stable neurologic deficits.[138]

The developing nervous system is dependent on adequate vitamin E for normal development[141] and vitamin E appears to play a role in the pathophysiology of NAD/EDM. Although vitamin E deficiency occurs in some cases of equine NAD/EDM,[128,130,133] low α-tocopherol levels are not present consistently in all cases. Serum α-tocopherol concentrations were not significantly different between EDM-affected horses and control horses in some studies.[131,140] It does appear that vitamin E supplementation of susceptible horses, such as those on the same farm as previously diagnosed horses, does lower the severity and overall incidence of NAD/EDM.[133,140] Foals from an EDM-affected stallion compared with controls from an unaffected stallion had significantly lower plasma α-tocopherol concentrations, and it was concluded that vitamin E is a factor in the development of EDM in the first year of life in genetically predisposed foals.[130] Blythe et al determined that foals with EDM do not demonstrate significant differences in oral vitamin E absorption as compared with controls.[142] Overall, there is very strong evidence that NAD/EDM is an inherited disorder, and it could be that the serum concentration of α-tocopherol acts as an environmental modifier to determine the overall severity of the phenotype of horses affected with NAD/EDM.

An antemortem diagnosis of NAD/EDM is based solely upon clinical signs, the elimination of other causes of neurologic disease, and a possible association with a low serum α-tocopherol concentration. At this

time, a definitive diagnosis is only available upon histopathologic evaluation of spinal cord and brainstem tissue at postmortem. There is no treatment for NAD/EDM, and there have been no reports of spontaneous resolution.[140] Suspected cases are often treated empirically with vitamin E supplementation because of the association of low serum vitamin E concentrations with the disease. Unfortunately, there is strong evidence that vitamin E supplementation of affected cases does not lead to neurologic improvement.[132,143] Although the neurologic abnormalities appear to stabilize by 2–3 years of age, these horses are neurologically abnormal and often unfit for any performance activity. Prevention of the disease has been reported in genetically susceptible herds by supplementing susceptible animals with 1,000–2,000 IU/450 kg/day vitamin E.[133,140] Recent evidence, however, supports that supplementation is not necessarily entirely effective in preventing NAD/EDM, in that vitamin E supplementation appeared to decrease the severity of disease in foals born in a susceptible herd with previously diagnosed NAD/EDM cases, but it did not completely prevent new cases.[132]

Vitamin E-Deficient Myopathy. Some horses with clinical signs of EMND and a deficiency in vitamin E are not diagnosed antemortem with EMND because they lack evidence of neurogenic atrophy in the sacrocaudalis dorsalis (SC) muscle. A recent study suggests that many such undiagnosed cases are the result of a specific myogenic presentation of vitamin E deficiency.[144] Sacrocaudalis muscle from horses with a clinical presentation of EMND lacked evidence of neurogenic atrophy, but did, however, contain characteristic abnormal moth-eaten staining pattern of mitochondria. Muscle α-tocopherol concentrations from affected horses were all low, but serum α-tocopherol concentrations were inconsistently low. This vitamin E-deficient myopathy has likely been missed previously because formalin-fixed biopsy specimens are most often evaluated for a diagnosis of EMND and mitochondrial staining is not possible with this fixative. The observed generalized weakness in the horses with abnormal mitochondrial stains was suggested to be due to a reversible manifestation of skeletal muscle/mitochondrial oxidative stress associated with vitamin E deficiency. All horses recovered completely after vitamin E therapy. Vitamin E-deficient myopathy could be an entity unto itself or a predecessor to development of EMND, but this distinction was not evaluated in the study as all horses successfully responded to vitamin E therapy, precluding a postmortem examination.

Supplementation with Vitamin E

Types of Supplement. Depending on the availability of grass and fresh hay, a horse can consume less than the daily recommended amount of vitamin E. In such cases, a variety of formulations of vitamin E supplements are available for horses.

Synthetic. There are 2 types of synthetic vitamin E, all-rac-α-tocopherol and dl-α-tocopherol (Table 3).

All-rac-α-tocopherol acetate is accepted as the International Standard (1 mg = 1 IU). Forms of synthetic vitamin E are available as powdered or pelleted supplements. The all-rac-α-tocopherol acetate form is highly dependent on adequate amount of bile salts to generate hydrolysis and subsequent absorption of α-tocopherol into the plasma.[145]

Natural. RRR-α-tocopherol is available as a micellized liquid form and as an esterified form (acetate). For α-tocopherol acetates to be utilized in the body, the ester has to be removed and the α-tocopherol made water-soluble by the action of bile salts (micellization). These additional steps could limit α-tocopherol absorption in the horse.[146]

The difference between RRR-α-tocopherol and synthetic vitamin E (all-rac-α-tocopherol acetate) in the horse was recently demonstrated, where CSF concentrations of α-tocopherol were significantly elevated above baseline values after supplementation with the natural vitamin E, but not after supplementation of a synthetic all-rac-α-tocopherol acetate at equivalent high dosages (10,000 IU/500 kg horse/day).[62] In addition, although serum values of α-tocopherol increased significantly from baseline values with both the natural and synthetic forms of vitamin E, serum α-tocopherol concentrations were significantly higher in the group supplemented with the RRR-α-tocopherol compared with the all-rac-α-tocopherol acetate. These values were approximately 2× that of concentrations obtained by using all-rac-α-tocopherol acetate, which is in agreement with other studies showing that the activity of RRR-α-tocopherol is between 1.36 and 2 IU/mg higher than all-rac-α-tocopherol.[147–149]

In a recent review, it was suggested that RRR-α-tocopherol (only RRR stereoisomer) and all-rac-α-tocopherol (all stereroisomers, including RRR and SRR) are not equivalent at any dosage ratio and should be considered separate drugs.[90] Tissue concentrations of each stereoisomer are even more variable than plasma concentrations. After 154 days of supplementation with a synthetic dl-α-tocopherol acetate (which contains equal concentrations of RRR and SRR stereoisomers, Table 3), instead of having RRR : SRR ratios of 2 : 1, the ratio was 5.3 in the brain, 3.6 in red blood cells, 2.4 in plasma, 1.9 in the heart, and 1.2 in the liver.[108] Therefore, the overall ratio of 2 : 1 may only apply to plasma concentrations. In addition, RRR-α-tocopherol, in its micellized nonesterified form, was shown to be superior to both all-rac-α-tocopherol and RRR-α-tocopherol acetate at elevating serum α-tocopherol concentrations during short-term (56 days) administration to 9 Thoroughbred geldings.[146]

It is currently recommended by the authors that natural RRR-α-tocopherol (nonacetate) form of vitamin E be used to supplement deficient horses with EMND, NAD/EDM, or vitamin E-deficient myopathy because this form is the most biologically available, most readily absorbed, and has the most potent antioxidant activity.[4]

Supplementation of Healthy Horses. Research-based evidence for the need for additional vitamin E supple-

mentation above 500 IU/day is lacking in healthy young and middle-aged horses receiving adequate dietary vitamin E intake. Vitamin E, unlike other fat-soluble vitamins, does not accumulate in the body to a toxic level because of protective mechanisms.[150] Additionally, inter- and intraplasma α-tocopherol concentrations are remarkably constant despite supplementation, suggesting that genetically encoded factors determine vitamin E concentrations in vivo.[151] Excessive supplementation of α-tocopherol could increase plasma/serum and tissue concentrations; however, there is strong evidence that tissues will become saturated and additional α-tocopherol will be metabolized and/or excreted.[150]

High levels of supplementation in humans do not enhance plasma concentrations more than 3–4-fold.[4] An increase from 400 to 800 mg/day in humans was accompanied by only a minor (<10%) increase in plasma α-tocopherol.[11] Additionally, although supplementation will increase hepatic α-tocopherol concentrations in a linear manner, extrahepatic tissue concentrations plateau at high supplemental dosages in rats.[150] Excess α-tocopherol is sequestered in the liver, rapidly metabolized, excreted, or both.[150] Similarly in the horse, concentrations of α-tocopherol in the liver and muscle plateau in dosages exceeding 4.4 IU/kg, suggesting tissue saturation.[60] There appear to be considerable differences in the extent of tissue loading with α-tocopherol, with concentrations in the brain of rats increased 40% after supplementation with all-rac-α-tocopherol acetate for 4 months compared with an increase of 4.6-fold in peripheral tissue, including the liver.[109] The regulatory mechanisms that exist to control tissue α-tocopherol concentrations could play an especially prominent role in limiting the amount of α-tocopherol within the CNS.

Complications and potential toxicity resulting from high dose-supplementation of vitamin E are considered minimal in horses. The NRC set the upper safe diet concentration at 20 IU/kg BW (10,000 IU/500 kg horse) based on 2% dietary intake. Above this level, coagulopathy and impaired bone mineralization have been reported.[23]

In 2000, high-dose supplementation with vitamin E in human medicine was considered very low risk.[152] More recently, however, high-dose supplementation has been associated with increased mortality in humans.[153,154] Therefore, in humans, indiscriminate supplementation of high-dose vitamin E is not recommended to the general public.[155] Supplementation with vitamin E can alter drug metabolism and disposition because the same CYP isoforms that metabolize vitamin E metabolize >50% of therapeutic drugs.[156] In healthy exercising horses, high dosage of vitamin E supplementation (10× NRC requirements) was shown to be potentially detrimental to beta-carotene absorption and thus not recommended.[12] Therefore, it is our recommendation that healthy horses receive the 2007 NRC dietary recommendation of vitamin E (Table 2). Additional supplementation above this requirement does not appear necessary in healthy horses.

Supplementation of Horses with Neurologic Disease. Many publications in the veterinary literature recommend high levels of vitamin E supplementation for horses with neurologic disease, ranging from 1,500 IU to 12,000 IU/500 kg horse/day.[157] In horses with equine protozoal myeloencephalitis, supplementation with 5,000 IU/adult/day is recommended for "nonspecific antioxidant properties".[158] In cases of cranial trauma, 20,000 IU/adult/day is recommended.[158] In horses with cervical stenotic myelopathy, vitamin E is recommended at 3× the NRC dose.[159] The use of vitamin E for these conditions is empirical based on a belief that it could be neuroprotective in disorders not related to a deficiency of vitamin E. It is important to consider that many of these dosage recommendations exceed the NRC upper safety recommendation of 20 IU/kg (10,000 IU/500 kg horse). Furthermore, many of these studies were performed before the development of natural forms of vitamin E in horses and these amounts could well be excessive if natural vitamin E is used therapeutically. At this time, there is no scientific evidence that supplementation with doses of α-tocopherol above the 2007 NRC-recommended dose will have a therapeutic effect in horses suffering from neurologic diseases other than those associated with a vitamin E deficiency.[143] In humans, double-blinded placebo-controlled studies in patients with Parkinson[160] and Huntington[161] disease have demonstrated no beneficial effect of high dose α-tocopherol supplementation. Another major point to consider is that supplementation with high doses of α-tocopherol depletes plasma, CSF, and tissue γ-tocopherol, in contrast to supplementation with γ-tocopherol, which increases both.[6] As discussed previously, γ-tocopherol could play an important role in supporting normal brain function and depletion could be detrimental. More work is necessary to evaluate the role of γ-tocopherol in horses.

The goal of α-tocopherol supplementation in horses predisposed to or affected by EMND, NAD/EDM, and vitamin E-deficient myopathy is to increase the concentration of α-tocopherol in the CNS or muscle tissue. In horses, only short-term studies of CSF have been performed. Horses supplemented with RRR-α-tocopherol at 1,000 IU/500 kg horse/day (within NRC requirement) for 10 days found a significantly increased serum α-tocopherol concentrations compared with baseline values, but did not find a significant increase in CSF concentrations. Even with a 10-fold higher dose for the same time period, there was no significant increase in CSF α-tocopherol concentrations although a 1.3–3.4-fold increase was noted in 9/10 horses.[61] When 10,000 IU/500 kg horse/day (upper limit of NRC recommendation) was provided as RRR-α-tocopherol for additional 14 days, a significant increase in CSF α-tocopherol was apparent.[62]

For EMND-affected cases, dosages of 5,000–7,000 IU α-tocopherol/day/450 kg horse are recommended. With this treatment, approximately 40% of cases demonstrate clinical improvement within 6 weeks and some can appear normal within 3 months.[95] To

the authors' knowledge, CSF α-tocopherol concentrations in supplemented horses with EMND have not yet been evaluated. A dosage of 6,000 IU/450 kg horse/day has been recommended to supplement NAD/EDM-affected cases and clinical improvement has been noted in some cases[162]; however, none of these reported cases returned to normal and more recent evidence has found no effect of vitamin E supplementation on neurologic improvement.[132,143] Concentrations of α-tocopherol in the CSF of NAD/EDM cases after supplementation are comparable to age-matched controls on similar supplementation protocols (Finno, unpublished results).

Conclusions

In conclusion, vitamin E is a complex nutrient with many important functions. It is recommended that wherever possible the specific form of vitamin E be used in publications as the terms "vitamin E" and "α-tocopherol" are not synonymous. Plasma/serum or tissue concentrations should have the particular isoform (α-tocopherol versus γ-tocopherol) identified. In addition, the type of vitamin E supplement (RRR versus all-rac and acetate versus alcohol) and amount (IU/kg) should be clearly indicated.

Insights into equine diseases associated with vitamin E deficiencies, such as EMND, NAD/EDM, and vitamin E-deficient myopathy, could be gained by defining underlying genetic factors affecting an individual's ability to transport and metabolize α-tocopherol. Additional research is necessary to determine the effects of this potent antioxidant in exercising horses and its specific role in a variety of neuromuscular diseases. At this time, there is no strong evidence to support supplementing of α-tocopherol above the 2007 NRC dietary recommendations unless the horse has been diagnosed with EMND, vitamin E-deficient myopathy, or is part of a NAD/EDM genetically susceptible-herd.

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