Limitations of Plasma or Serum Analysis in Assessing Vitamin E Status of Domestic and Wild Animals

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Venipuncture is a relatively noninvasive means of sampling tissues for determination of deviations from physiologic norms. To the extent that norms can be defined and deviations from norms can be associated with abnormality, blood analyses may be medically useful. However, studies of vitamin E metabolism have shown that neither the magnitude of body stores nor the availability of dietary supplies are consistently reflected in alpha-tocopherol concentrations within single samples of plasma or serum. Vitamin E is the major antioxidant within cell membrane lipids and protects them against oxidative damage from free radical attack. If vitamin E intake exceeds need, stores accumulate, particularly in liver , muscle, and adipose tissue. However, the kinetics of absorption, transport, and retention vary with the amount and form of vitamin E, and different tissues have different rates of vitamin E turnover. Studies with swine, horses, elephants, and white-tailed deer that were repeatedly sampled showed that individual animals had plasma or serum alpha- tocopherol concentrations that differed up to two to three-fold from those of other individuals even though age, diet, or work history were the same. Plasma or serum alpha-tocopherol concentrations in young animals with limited vitamin E stores are more likely to reflect the balance between intake, stores, and need, but that reflection is frequently distorted by individually characteristic factors. Thus, a single plasma or serum analysis is unlikely to accurately portray vitamin E status of an animal group, and it may be necessary to use repeated plasma or serum assays or functional tests that relate directly to protection against oxidative cell damage.

Key words: alpha-tocopherol, elephants, horses, swine, white-tailed deer

INTRODUCTION

Subsequent to William Harvey's discovery over 300 years ago that the blood circulates, physicians have used this tissue in attempts to solve the mysteries of the body's metabolic machinery. Venipuncture is relatively noninvasive, and the emotional cost of giving a blood sample is minimal and short-lived. A common assumption is that this blood sample can reveal useful information about the body's state of health. With the explosive growth of technology during the latter half of the 20th century, small volumes of blood can be quickly analyzed for dozens of components. A relevant question is, "Do the numbers generated have interpretable meaning?"

Components of the blood are organic or inorganic, bound or unbound. Concentrations of some are tightly regulated, while concentrations of others are quite variable. In the case of most nutrients, the levels present represent a balance between the amounts absorbed and excreted,

modulated by the amounts moving into and out of sites of storage and use. The kinetics of these processes may differ with tissue, species, and nutrient form .

HUMANS

It has been commonly stated (Hoppe et al., 1993; Machlin, 1991; Ullrey, 1981) that the concentration of alpha-tocopherol in plasma or serum can be used to assess vitamin E status. In fact, guidelines have been proposed that categorize humans as vitamin E deficient when plasma or serum alpha-tocopherol concentrations are below 5 µg/ml, low between 5 and 7 µg/ml, and adequate above 7 µg/ml (Machlin, 1991).

In human populations with widely varying plasma lipid levels, there is a positive correlation between plasma lipid and alpha-tocopherol concentrations (Horwitt et al., 1972). Thus, hyperlipidemic subjects may have falsely high plasma alpha-tocopherol levels, while hypolipidemic subjects exhibit the converse. As a consequence, it has been proposed that vitamin E status may be more accurately categorized by relating plasma alpha-tocopherol to plasma lipids, such that adults with < 0.8 mg/g lipid are classed as deficient whereas those with > 0.8 mg/g lipid are classed as adequate. Comparable values for children are < 0.6 and > 0.6 mg alpha-tocopherol/g lipid, respectively. Because plasma cholesterol may be more conveniently determined than plasma lipid, adult humans have been classified as deficient when alpha-tocopherol values are < 0.6 mg/g cholesterol and adequate when values are > 0.6 mg/g.

The correlation is poor between concentrations of plasma alpha-tocopherol and lipid or cholesterol in humans with normal lipid concentrations, and an improvement in the accuracy of categorizing vitamin E status by assaying plasma lipid or cholesterol in normolipidemic subjects is difficult to demonstrate. The same is true for many other animals, particularly herbivores, that tend to have low plasma lipid and cholesterol concentrations (Njeru et al., 1994).

SWINE

In groups of pigs, Hoppe et al. (1993) has shown that plasma or serum alpha- tocopherol concentrations can be related to dietary vitamin E concentrations. When pigs were fed from 7 to 100 kg live weight a basal diet containing 5 IU vitamin E/kg supplemented with 0, 20,40, 80, or 160 IU all-rac-alpha-tocopheryl acetate/kg, mean plasma alpha-tocopherol concentrations (from week 2 to week 20, excluding week 13) were 0.4, 1.6, 2.2, 3.0, and 3.4 µg/ml, respectively. However, these values were not only appreciably lower than those considered adequate in humans, but were highly variable as well. The coefficients of variation of plasma alpha-tocopherol concentration within treatment and sampling times ranged from 9.8% to 44.9% and were negatively related to the absolute concentration. Thus, at low vitamin E intakes, where there was the greatest need to accurately identify pigs with incipient vitamin E deficiency, there was the greatest variability in plasma alpha-tocopherol values.

A linear relationship was found between alpha-tocopherol concentrations in plasma and tissues, and while this relationship was significant for each tissue studied, correlation coefficients were not particularly high. The regression equation for alpha- tocopherol in plasma and liver was $y = 0.68 + 4.4x$, where y = ug alpha- tocopherol/g fresh liver and x = µg alpha-tocopherol/ml plasma.

The correlation coefficient (r2) was 0.54 *(P* < 0.001). No evidence of vitamin E deficiency was seen in pigs on any treatment.

Recognizing the role of vitamin E in protecting cell membranes against peroxidation and concerned that serum alpha-tocopherol concentrations may not accurately reflect the supply of vitamin E available, Krauss et al. (1995) conducted a study with pigs to measure vitamin E concentrations in subcellular (microsomal) membranes and to quantitatively evaluate the resistance of those membranes to peroxidation. They used a parenteral preparation (Vital ETM; Schering-Plough Animal Health, Kenilworth, NJ) containing 300 mg RRR-alpha-tocopherol/ml in a water- miscible base. Forty baby pigs, born to adequately nourished sows, were divided into two treatments, with 20 pigs in Group 1 injected i.m. with 0.67 ml of Vital ETM (200 mg RRR-alpha-tocopherol) at 3 and 28 days of age. The 20 pigs in Group 2 were injected i.m. with equivalent volumes of the Vital E™ vehicle without alpha- tocopherol. Blood serum was collected at 2, 14, 28, and 56 days of age. Cardiac muscle, skeletal muscle (diaphragm), and liver samples were collected at 56 days of age.

Vitamin E concentrations were measured in serum and isolated microsomal membranes using normal-phase HPLC. Microsomal membranes were also subjected to oxidative challenge in a closed system with a Clark oxygen electrode. The rate of disappearance of molecular 02 was measured as it was consumed in free-radical peroxidative reactions. Increased rates of oxygen consumption would be related to increased rates of free-radical production and would be associated with a lessened resistance of cell membrane lipids to peroxidation.

Mean serum alpha-tocopherol concentrations are shown in Table 1 and were higher *(P* < 0.01) in Group 1 than in Group 2 at each sampling interval. Significant *(P* < 0.01) effects of time and a time x treatment interaction also were noted.

TABLE 1. Mean serum alpha-tocopherol concentrations (ug/ml) of baby pigs (Krauss et al., 1995).

Group	Treatment	Day of age 2	14	28	56
1	Vit E i.m.[a]	5.4[c]	7.5[c]	2.5[c]	1.7[c]
2	Vehicle[b]	6.2[d]	5.3[d]	1.8[d]	1.3[d]

[a]Vital E™ (300 mg *RRR*-alpha-tocopherol/ml in a water-miscible base) injected i.m. (0.67 ml; 200 mg *RRR*-alpha-tocopherol) at 3 and 28 days of age.
[b]Vital E™ vehicle (without vitamin E) injected as above.
[c,d]Means in same column with different superscripts are different (*P* < 0.01).

Mean microsomal alpha-tocopherol concentrations and oxygen consumption during peroxidative challenge are shown in Table 2. Microsomal alpha-tocopherol concentrations in cardiac and skeletal muscle were unaffected by treatment but were higher *(P* < 0.01) in liver of vitamin E-

injected pigs. This difference was associated with a significant inhibition of oxygen consumption in liver microsomes of treated pigs and an enhanced resistance to peroxidation .

TABLE 2. Mean microsomal alpha-tocopherol concentrations (ug/mg protein) and oxygen consumption (mmol O_2/min/mg protein) in cardiac muscle, skeletal muscle (diaphragm), and liver.

		Vitamin E concentration			Oxygen consumption		
Group	Treatment	Card. m	Skel. m	Liver	Card. m	Skel. m	Liver
1	Vit E i.m.[a]	6.76[c]	5.69[c]	4.16[c]	1.74[c]	16.6[c]	384.6[c]
2	Vehicle[b]	6.77[c]	3.22[c]	2.88[d]	1.76[c]	11.6[c]	751.7[d]

[a]Vital E™ (300 mg *RRR*-alpha-tocopherol/ml in a water-miscible base) injected i.m. (0.67 ml; 200 mg *RRR*-alpha-tocopherol) at 3 and 28 days of age.
[b]Vital E™ vehicle (without vitamin E) injected as above.
[c,d]Means in same column with different superscripts are different ($P < 0.01$).

Although serum alpha-tocopherol concentrations seemed to be useful for assessing the alpha-tocopherol present in hepatic subcellular membranes of a group of pigs, individual serum concentrations were poorly correlated with individual liver concentrations. Thus, single serum alpha-tocopherol values were not a reliable indicator of the hepatic vitamin E status of a single pig. Serum alpha-tocopherol concentrations were not reflective of the cardiac vitamin E status either of individual pigs or of a group.

HORSES

Numerous researchers (Dill et al., 1989; Lindholm and Asheim, 1973; Maenpaa et al., 1987, 1988; Maylin et al., 1980; Roneus et al., 1986; Saastamoinen and Juusela, 1993) have explored the effects of season, age, fresh vs. dry forages, and supplemental dietary vitamin E intakes upon mean plasma or serum alpha-tocopherol concentrations in groups of horses. However, Craig et al. (1989) monitored serum alpha-tocopherol concentrations in 12 horses at 3-hour intervals for 72 hours and found that assay of a single serum sample was an unsatisfactory indicator of vitamin E status. This was due to the wide variation in serum alpha-tocopherol values within each animal, and this variance could not be eliminated by relating these values to serum total lipids or cholesterol.

In a study by Moore-Doumit (1995) of factors affecting serum alpha-tocopherol concentrations in yearling and adult mares, samples were taken from six individuals at 1- or 2-week intervals for 279 days. It was found that, as a group, serum alpha- tocopherol concentrations were lower in yearlings than in adult mares, and these levels were affected to a greater degree by vitamin E depletion or repletion in yearlings than in mares. However, mean serum alpha-tocopherol concentrations among horses within an age class varied 1.5- to 2-fold and were individually characteristic. That is, some horses consistently tended to have lower or higher levels over time than other horses. The range of values found at all sampling times was 1.6 to 7.5 ug alpha-tocopherol/ ml of serum.

ELEPHANTS

Comparable differences in plasma alpha-tocopherol concentrations were found in 28 Asian elephants *(Elephas maximus)* by Shrestha (1995). These elephants were kept at four camps maintained by the Department of National Parks and Wildlife Conservation of Nepal. They were located in the lowland jungle zone at an *elevation* of about 400 M. A seasonal monsoon climate with heavy rains was experienced from mid-June through late September (hot-wet). The monsoon was followed by a cool-dry season from *early* October to mid-February and a *hot-dry* season from mid-February to mid-June. Highest temperatures occurred from the late hot-dry season through mid- monsoon (May-July), while December and January were the coldest months.

The elephants were tethered on bare ground except when grazing or working. Grazing time in adjoining native vegetation, under the control of mahouts, was about 4 to 6 hours per day. Average daily dry matter intake during grazing was measured at one camp and was about 20 kg. Mahouts also cut fresh forage for the evening meal, and about 25 kg of dry matter from this source was consumed. The principal forages were four species of grass *(Saccharum spontanuem,* S. *bengalensis,* S. *arundinaceum,* and *Narenga porphorycoma)* and one tree species *(Bombax ceiba)*.In addition, a daily supplement of 12 to 1 5 kg unhusked rice, 1 .5 kg cane molasses, and 100 g sodium chloride was provided to each elephant.

Blood was collected monthly for 1 year from an ear vein. Mean plasma alpha- tocopherol concentrations (µg/ml) were different between ages (subadult, 0.60; adult and mature, 0.78; $p = 0.015$), camps (0.62 to 1.00; $p < 0.001$), and months (0.69 to 0.83; $p = 0.033$). However, values for all samples ranged from 0.22 to 1.57 µg/ml. Individual elephants tended to have characteristic values, as was noted for the horse, with 1 .5- to 2-fold differences in mean plasma alpha-tocopherol concentration among them.

WHITE- TAILED DEER

The effects of feeding a low-selenium (0.04 mg/kg), low-vitamin E (5 IU/kg) diet alone or with supplements of 0.15 mg selenium/kg and/or 45 IU vitamin E/kg were studied with 32 adult female white-tailed deer *(Odocoileus virginianus)* and 96 fawns born to those does over 2 years (Brady et al., 1978). Plasma selenium and alpha- tocopherol concentrations are shown in Table 3.

TABLE 3. Plasma selenium and alpha-tocopherol concentrations in white-tailed does (after 12 months) and fawns (at weaning) fed a low-selenium (0.04 mg/kg), low-vitamin E (5 IU/kg) diet alone or with supplements of 0.15 mg selenium/kg and/or 45 IU vitamin E/kg.

Deer/measure	Diet -Se -E	+Se -E	-Se +E	+Se +E	SEM[a]	Signif. effect[b]
Doe plasma						
Selenium, ug/ml	0.07	0.10	0.07	0.11	0.005	Se
Vitamin E, ug/ml	1.03	0.90	2.10	2.20	0.13	E
Fawn plasma						
Selenium, ug/ml	0.04	0.08	0.05	0.07	0.006	Se
Vitamin E, ug/ml	0.34	0.34	0.88	0.83	0.12	E

[a]Standard error of the mean.
[b]Significant effect of indicated nutrient ($P < 0.05$).

Plasma concentrations of selenium and vitamin E responded positively to supplements of these two nutrients, and both vitamin E-supplemented does and fawns had mean plasma alpha-tocopherol concentrations that were over twice as great as their respective controls. However, as noted previously in the pig, the variation (SEM) in plasma alpha-tocopherol was a relatively greater proportion of the mean when absolute concentrations were low, as seen in the fawns. Thus, it was not always possible to predict from individual plasma alpha-tocopherol concentrations which fawns were born to vitamin E-supplemented does or would show signs of white- muscle disease. Although the dietary vitamin E supplement (45 IU/kg) markedly decreased time-dependent and hydrogen peroxide-dependent hemolysis of erythrocytes of adult does, some supplemented fawns still exhibited histologic signs commonly associated with vitamin E deficiency.

CONCLUSION

Even though venipuncture allows for blood collection with minimum pain and suffering, the variation among individuals and species in "normal" plasma or serum alpha-tocopherol concentration renders a diagnosis of vitamin E deficiency or sufficiency from a single assay very uncertain. This uncertainty may not be relieved unless multiple assays are run or until specific functional tests are used that relate directly to protection against oxidative cell damage.

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