Plasma Tocopherol in Sheep and Cattle After Ingesting Free or Acetylated Tocopherol[1,2]

N. HIDIROGLOU, L. R. McDOWELL and O. BALBUENA[3]
Animal Science Department
University of Florida
Gainesville 32611-0691

ABSTRACT

Two trials were carried out to evaluate the bioavailability of dl-α-tocopherol and dl-α-tocopherol acetate administered to sheep and cattle in a single oral dose. In the first trial, two groups of five sheep were used. They received 100 mg/kg body weight of either dl-α-tocopherol or its acetylated form. The blood plasma α-tocopherol tolerance curve area was higher in the dl-α-tocopherol group than in its ester form. The time to reach maximum plasma α-tocopherol concentration was less in the dl-α-tocopherol group than in its ester form. In a second trial, four heifers received the two forms (50 mg/kg body weight) in rotation after an appropriate washing period between the two dosings. Again, plasma tolerance curve area was greater in the cattle following administration of dl-α-tocopherol than its acetylated form.

INTRODUCTION

Administration of a massive dose of vitamin E is one of the usual methods in the prophylaxes of vitamin E deficiencies (11). Overman et al. (12) reported in humans that a single massive oral dose of vitamin E is effective in increasing the free tocopherol plasma significantly after 6 h. This temporary rise with ensuing drop has been designed as a tolerance curve. The tolerance curve mirrors the intestinal absorption of vitamin E. In ruminant species, with their unique digestive system and physiologic differences in metabolic mechanisms, only few data are available on the bioavailability of the various forms of vitamin E. The objective of the experiments was to measure blood plasma response to α-tocopherol in sheep or cattle provided with different chemical forms of tocopherols but with identical weights.

MATERIALS AND METHODS

Trial 1

Ten yearling crossbred wethers weighing 40 to 50 kg were used. All sheep originated from a flock fed a commercial diet of corn meal, 56.45%; soybean meal (44% CP), 16.5%; cottonseed hulls, 25%; trace mineral salt, 1%; monocalcium phosphate, 1%; and .037% of vitamins A and D_3. They were also fed this diet throughout the experiment. Sheep were placed in individual metabolism cages 10 d before administration of vitamin E as an adjustment period. Then they were randomly allocated to two groups of five sheep each. Each group was administered intraruminally with 100 mg/kg body weight of either dl-α-tocopherol (1 mg = 1.10 IU) or dl-α-tocopherol acetate (1 mg = 1.0 IU).

Trial 2

Four crossbred dairy heifers (averaging 250 kg) were used in a crossover design with two 20-d periods. They were given ad libitum access to hay and water during the study. In the first period, two heifers were administered orally with dl-α-tocopherol and two with dl-α-tocopherol acetate. The dose given for each component was 50 mg/kg body weight. The

Received October 3, 1988.
Accepted January 30, 1989.
[1]Florida Agricultural Experiment Station Journal Series Number 9323.
[2]This research was supported. in part, by the US Department of Agriculture under CSRS special grant Number 86-CRSR-2-2843 managed by the Caribbean Advisory Group (CBAG), and Hoffmann-La Roche Inc., Nutley, NJ.
[3]Present address: Estación Experimental Agropecuaria – Colonia Benítez, Casilla de Correo 114, 3500 Resistencia, Chaco, Argentina.

end of the first period was followed by a 3-wk washing period. Then the treatment was reversed and the procedures were repeated in period 2.

Blood Samples

Blood samples were withdrawn from the jugular vein in heparinized vacutainers and immediately centrifuged. They were collected before vitamin E administration and at precise intervals (Figures 1 and 2). Plasma was separated, frozen, and stored at –20°C until analyzed for dl-α-tocopherol.

Analytical Method

Plasma samples were prepared for HPLC according to the method of McMurray and Blanchflower (10). The chromatographic apparatus consisted of a model 6000 pump and WK septumless injector (Waters Associates, Milford, MA). A Perkin-Elmer 650-150 fluorescence spectrophotometer (Perkin-Elmer Corp., Norwalk, CT), equipped with a microflow cell unit, was used for quantification. Wavelength settings were 295 and 330 nm for excitation and emission, respectively. The column was a μ Bondapak C_{18} (3.9 mm × 30 cm) of 10-μm particle size purchased from Waters Associates. The mobile phase, a solvent system (HPLC grade), consisted of methanol and water in a 97:3 ratio with a flow rate of 3 ml/min.

Statistical Analysis

Indices of bioavailability were calculated for each individual sheep and heifer. These indices were: 1) the maximum α-tocopherol in plasma (C_{max}) concentration (peak of plasma α-tocopherol curves); 2) the time of maximum (T_{max}) α-tocopherol concentration; and 3) the area under the α-tocopherol plasma concentration time curve (AUC) (4, 7). Statistical analysis was carried out by analysis of variance (sheep) or covariance (cattle) by the General Linear Model procedure (14). The following model was used for the sheep.

$$Y_{ij} = \mu + t_i + e_{ij}$$

where:

μ = mean;
t_i = i^{th} type (i = 1,2); and
E_{ij} = random error.

For cattle the model used was:

$$Y_{ij} = \mu + \alpha C_o + t_i + E_{ij}$$

where

C_o = value at time 0.

RESULTS

Trial 1

Following the oral administration of the two vitamin E preparations to sheep, plasma α-tocopherol increased (Figure 1). The peak of this increase was reached faster ($P<.05$) in the dl-α-tocopherol form than in its ester form (Table 1). The shorter T_{max} observed in the dl-α-tocopherol-dosed group may have contributed to a faster onset of biological action. The C_{max} had a tendency ($P>.05$) to be higher in the dl-α-tocopherol than in the ester-dosed sheep. The AUC was higher ($P<.05$) in sheep treated with dl-α-tocopherol than in the sheep administered with dl-α-tocopherol acetate (Table 1).

The T_{max} and the tolerance curve are dependent chiefly on factors affecting the absorption of the vitamin from the intestines. The effectiveness of the absorption indicated by the AUC was higher in the sheep dosed with free alcohol form than in the acetylated group. At the end of the 2-wk experimental period, the terminal plasma tocopherol concentrations in both groups were higher ($P<.05$) than the original values.

Trial 2

A few hours after oral dosing, plasma α-tocopherol increased in both groups of cattle (Figure 2). The free form was absorbed more effectively than its ester form. This is shown by its greater AUC and its tendency to higher C_{max} and earlier t_{max} appearance (Table 2).

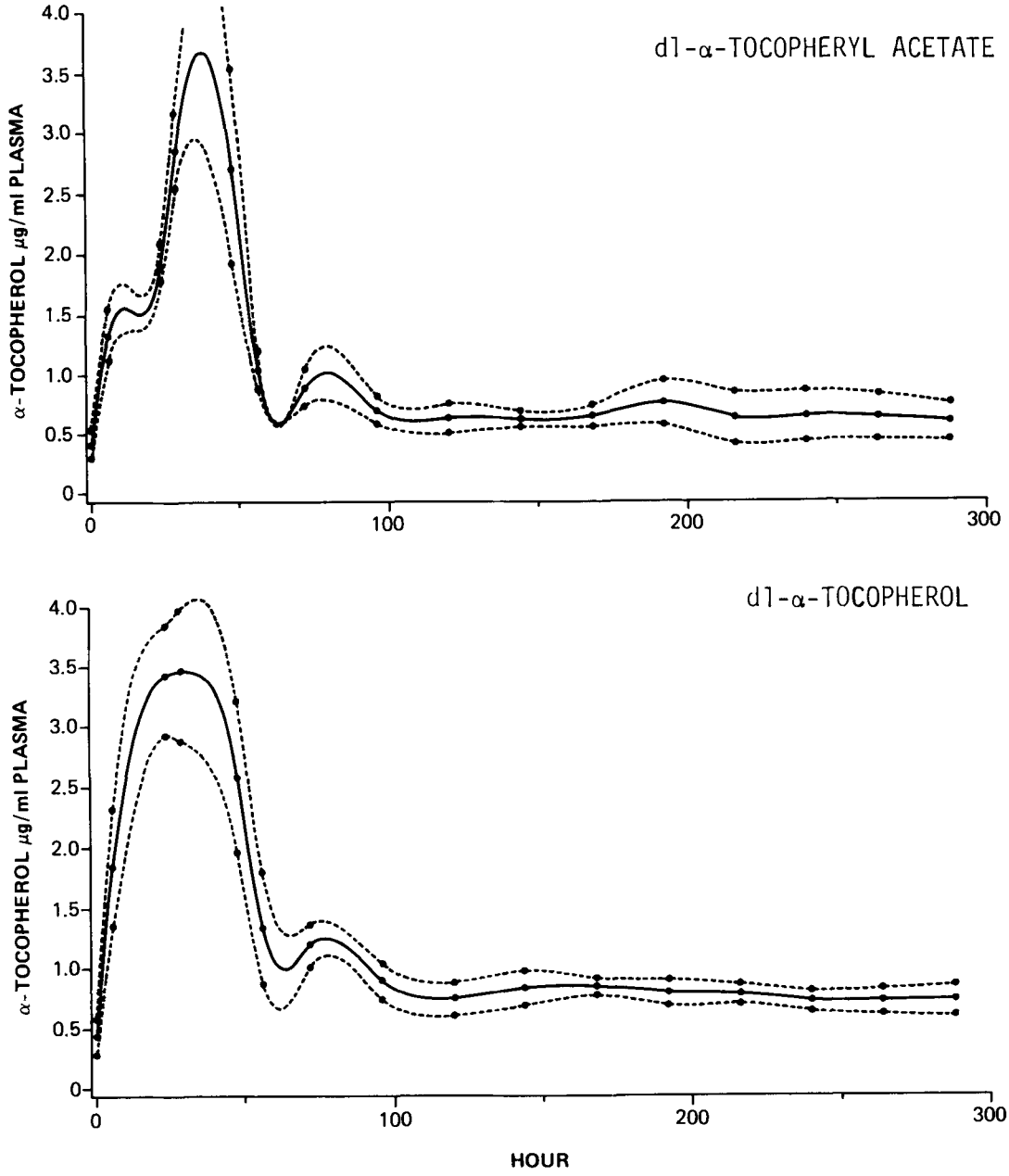

Figure 1. Plasma tocopherol concentration (μg/ml) in sheep following administration of a single megadose (100 mg/kg body weight) of dl-α-tocopherol acetate or dl-α-tocopherol. Arithmetic mean (—) and standard error (– –).

DISCUSSION

The plasma persistence curve was mainly used to provide a criterion of availability of vitamin E for physiological function following oral dosage to ruminants with two isomeric forms. The present results suggest that in the

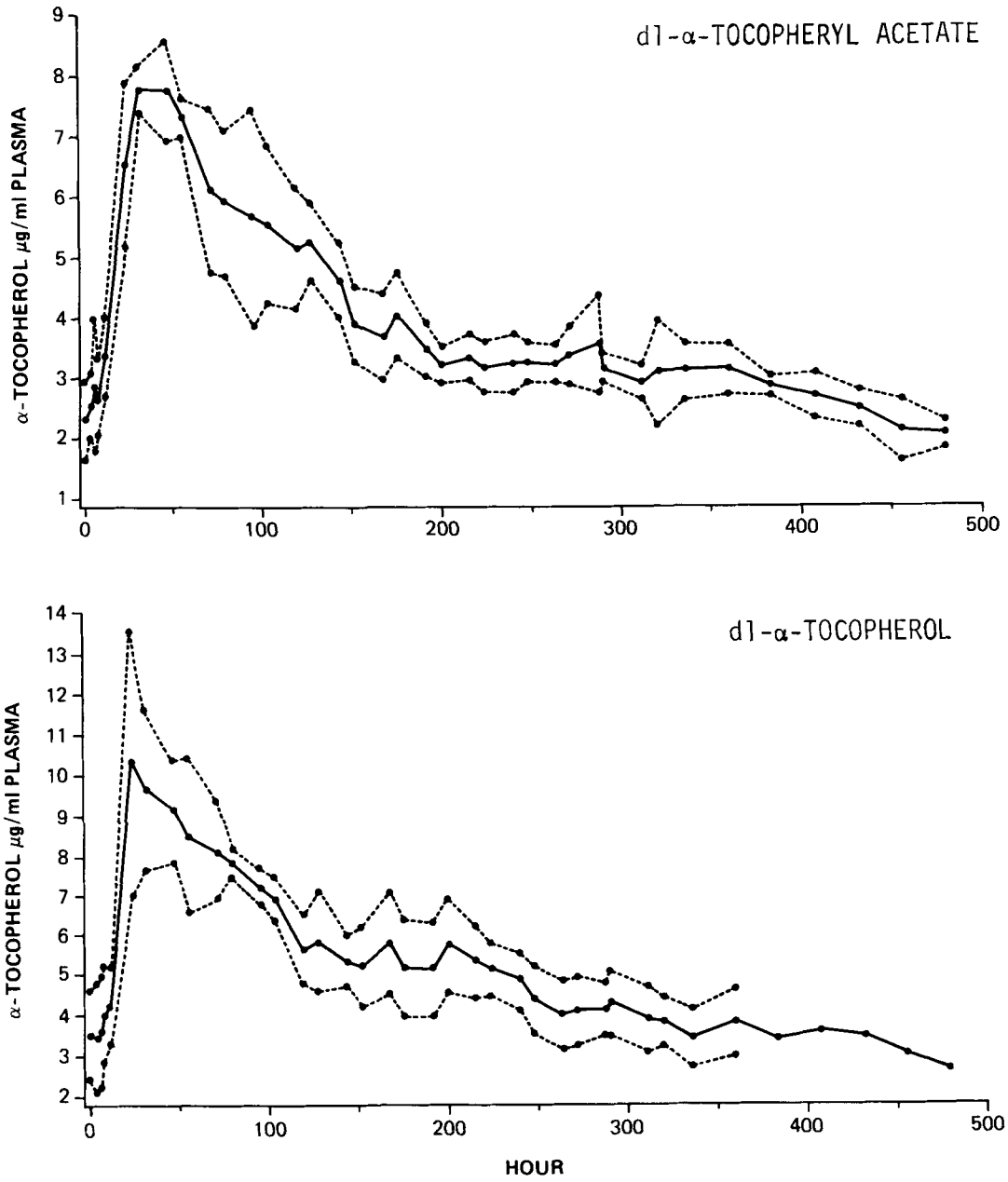

Figure 2. Plasma tocopherol concentration (μg/ml) in cattle following administration of a single dose (50 mg/kg body weight) of dl-α-tocopherol acetate or dl-α-tocopherol. Arithmetric mean (—) and standard error (– –).

environment of the rumen, despite a strong reducing system, dl-α-tocopherol was preserved sufficiently to induce an AUC persistence superior to that of the identical weight of the esterified form. It is possible that the degree of hydrolysis could be important in the shape of

TABLE 1. Mean Dl-α-tocopherol pharmacokinetic plasma values in sheep after a single oral administration of 100 mg/kg body weight vitamin E (Trial 1).

Vitamin E preparations	C_{max}[1]		T_{max}[2]		C_o[3]		C_{max}/C_o		CT[4]		CT/C_o		$\frac{C_{max}}{CT}$		AUC[5]	
	(μg/ml)		(h^{-1})		(μg/ml)				(μg/ml)						(μg/ml per h^{-1})	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
Dl-α-tocopherol	3.58	.22	26.5	4.04	.42	.06	8.99	1.13	.73	.07	1.80	.25	4.95	.54	332.18	20.27
Dl-α-tocopherol acetate	3.05	.20	40.4	3.61	.41	.05	7.67	1.01	.59	.06	1.50	.22	5.36	.48	269.63	18.13
p[6]			<.05												<.05	

[1]Maximum plasma concentration.

[2]Maximum time.

[3]Initial plasma concentration.

[4]Terminal plasma concentration.

[5]Area under plasma concentration time curve.

[6]Significance tested following analysis of variance.

TABLE 2. Mean D-α-tocopherol pharmacokinetic plasma values in cattle after a single oral administration of 50 mg/kg body weight vitamin E (Trial 2).

Vitamin E preparations	C_{max}[1]	T_{max}[2]	$\frac{C_{max}}{C_o}$	CT[3]	$\frac{CT}{C_o}$	C_{max}/CT	AUC[4]
	(μg/ml)	(h^{-1})	(μg/ml)				(μg/ml per h^{-1})
Dl-α-tocopherol	10.84	35.38	3.88	3.71	1.36	3.04	1995
Dl-α-tocopherol acetate	9.23	48.61	3.43	3.21	1.23	2.84	1589
SE	.61	14.89	.37	.40	.43	.39	88
P							<.05

[1]Maximum plasma concentration.

[2]Maximum time.

[3]Terminal plasma concentration.

[4]Area under plasma concentration time curve.

the tolerance curve. Because the stability of free tocopherol is inferior to that of tocopherol esters, it may be hypothesized that the relatively less favorable response to tocopherol ester is due to factors influencing the hydrolysis of these esters.

It seems probable that in cattle, like other mammals, dl-α-tocopherol acetate following hydrolysis is split in the intestinal mucosa and is absorbed as free tocopherol from the gut and passed to the systemic circulation via the lymphatic system. According to Tikriti (16), in cattle the acetylated form of vitamin E is hydrolyzed in the digestive system, beginning in the rumen, and appears to be utilized somewhat less efficiently than the free form. One cannot rule out, as it was suggested by Gallo-Torres (3), the possibility of a rate-limiting hydrolytic reaction occurring in the lumen of the intestine prior to entrance of the vitamin E into the intestinal wall of the ruminant animal.

Our results showed that T_{max} is much longer in ruminants than in humans following oral loading with vitamin E. Hashim and Schuttinger (6) observed that in normal human subjects, plasma tocopherol began to rise between 2 and 4 h and peaked at 7.5 h following oral administration of vitamin E. They also observed that at 24 h plasma tocopherol had declined and that at 48 h, postabsorptive concentrations were reached. Marusich et al. (8) reported that in chickens, following the administration of a single oral dose (50 IU of dl-α-tocopherol acetate), the peak plasma values obtained at 6 h were followed by a steady decline. However, in rats, Marusich et al. (9) observed that plasma tocopherol peaked at 24 h, following administration of a single oral dose of dl-α-tocopherol acetate.

The fact that the biological response to dl-α-tocopherol was greater than with its acetylated form agrees with the results reported in humans (1). These workers observed that following administration of a single oral dose (800 mg) of dl-α-tocopherol acetate, plasma d-α-tocopherol peaked (50% from the initial value) 8 h after ingestion. The same dose of free tocopherol induced a greater rise (60% from the original value) in 5 h.

It seems that dl-α-tocopherol is more pharmacological than its esterified form when ingested in amounts many times higher than the generally recognized nutritional requirements. The results demonstrated that T_{max} was faster following oral dosing of the free form than the acetylated form. This suggests that the hydrolysis rate of esterified tocopherol to the physiologically active free tocopherol is quite slow.

The AUC was influenced by the amount of vitamin E activity given. The higher vitamin E potency of dl-α-tocopherol (1.1 IU/mg) compared with that for the acetate form (1.0 IU/mg) probably contributed to the tendency of a higher plasma vitamin E peak as well as the greater AUC.

In the present experiment, initial plasma tocopherol concentrations (2 to 3 μg/ml) from hay-fed heifers were in the range of values observed by Pehrson and Hakkarainen (13) and Hakkarainen et al. (5). The initial value of α-tocopherol in the plasma of sheep fed a commercial diet was very low. In grain-fed sheep, Caravaggi (2) reported plasma concentrations of .89 ± .16 SD μg/ml. Storer (15) observed higher plasma tocopherol concentrations (1.28 ± .07 μg/ml) for sheep fed dry feed. It appears that in the ruminant animal, following a single oral dose of dl-α-tocopherol or its acetylated form, the terminal values were higher in sheep and cattle than the initial values. This may be related to a slow saturation of tissues with both forms of tocopherol. However, no difference was observed in the elimination rates (C_{max}/CT). In conclusion, the data in ruminants confirm the higher biological potency of the dl-α-tocopherol than dl-α-tocopherol acetate.

REFERENCES

1 Baker, H., O. Frank, B. Deangelis, and S. Feingold. 1980. Plasmas tocopherol in man at various times after ingesting free of acetylated tocopherol. Nutr. Rep. Int. 21:531.
2 Caravaggi, C. 1969. Vitamin E concentrations in the serum of various experimental animals. Comp. Biochem. Physiol. 30:585.
3 Gallo-Torres, H. E., F. Weber, and O. Wiss. 1971. The effect of different dietary lipids on the lymphatic appearance of vitamin E. Int. J. Vitam. Nutr. Res. 41:504.
4 Gibaldi, M., and O. Perrier. 1982. Pharmacokinetics. 2nd ed. Marcel Dekker, Inc., New York, NY.
5 Hakkarainen, J., b. Pehrson, and J. Tyopponen. 1987. Blood vitamin E, selenium and glutathione peroxidase concentrations in heifers fed either on grass or on winter feed. J. Vet. Med. A 34:508.
6 Hashim, S. A., and G. R. Schuttinger. 1966. Rapid determinations of tocopherol in macro and microquanti-

ties of plasma. Am. J. Clin. Nutr. 18:137.
7 Koch-Weser, J.A.N. 1974. Bioavailability of drugs. New England J. med. 291:233.
8 Marusich, W. L., G. Ackerman, and J. C. Bauernfeind. 1967. Biological efficiency of d-α or dl-α-tocopherol acetate in chickens. Poult. Sci. 46:541.
9 Marusich, W. L., G. Ackerman, W. C. Reese, and J. C. Bauernfeind. 1968. Relative activity on plasma levels. J. Anim. Sci. 27:58.
10 McMurray, C. H., and W. J. Blanchflower. 1979. Application of a high-performance liquid chromatographic fluorescence method for the rapid determination of α-tocopherol in the plasma of cattle and pigs and its comparison with direct fluorescence and high performance liquid chromatography ultraviolet detection methods. J. Chromatogr. 178:525.
11 Ogihara, T., Y. Nishida, M. Miks, and M. Mino. 1985. Comparative changes in plasma and RBC α-tocopherol after administrations of dl-α-tocopherol acetate and dl-α-tocopherol. J. Nutr. Sci. Vitam. 31:164.
12 Overman, R. S., J. McNeely, M. Todd, and I. S. Wright. 1954. Effects of vitamin E preparations on plasma tocopherol levels. J. Clin. Nutr. 2:168.
13 Pehrson, B., and J. Hakkarainen. 1986. Vitamin E status of healthy Swedish cattle. Acta Vet. Scand. 27:351.
14 SAS. Institute, Inc. 1980. A user's guide to SAS. SAS Inst. Inc., Raleigh, NC.
15 Storer, G. B. 1974. Fluorometric determination of tocopherol in sheep plasma. Biochem. Med. 11:71.
16 Tikriti, H. H. 1969. The metabolism of vitamin E in the lactating dairy cow in relation to oxidized flavour in milk. Ph.D. thesis, Univ. Maryland, College Park.