Intestinal Absorption of Vitamin E in Experimental Renal Failure (43012)

M. V. PAHL, N. D. VAZIRI, I. BENAVIDES, G. KHAMISEH, AND F. OVEISI
Division of Nephrology, Department of Medicine, University of California, Irvine Medical Center, Irvine, California 92668

Abstract. **We studied intestinal absorption of vitamin E in rats with experimental renal failure (RF) and in sham-operated normal and pair-fed controls using *in vivo* perfusion and *in vitro* everted sacs. The *in vivo* absorption rates per unit of intestine length were significantly reduced in RF and pair-fed groups. Expression of data per unit of intestine weight gave normal values in the pair-fed but depressed values in the RF animals. Vitamin E uptake *in vitro* was significantly increased in RF animals, suggesting enhanced permeability. We conclude: (i) vitamin E absorption *in vivo* is impaired in experimental RF; (ii) this is in part due to reduced nutrient intake; and (iii) disparity between *in vivo* and *in vitro* results suggests the presence of some inhibitory influence(s) in intact animals with RF.** [P.S.E.B.M. 1990, Vol 193]

Vitamin E (D-α-tocopherol) is an important lipid-soluble vitamin with potent antioxidant properties (1, 2). Its deficiency has been shown to cause reproductive dysfunction, muscular dystrophy, impaired nucleoprotein synthesis, hemolytic anemia, and peripheral neuropathy (3–6).

Previous studies have shown a marked impairment of intestinal absorption of several other vitamins in experimental uremia (7–9). This study was intended to examine the effect of renal insufficiency on intestinal transport of vitamin E.

Materials and Methods

Male Sprague-Dawley rats weighing 335–400 g, fed Purina Rat Chow and tap water *ad libitum*, were randomly placed into three groups. Animals placed in the renal failure (RF) group underwent a concurrent right nephrectomy and a left two-thirds nephrectomy. The procedure was performed extraperitoneally through a single dorsal incision to avoid intra-abdominal adhesions. The mortality rate in the RF group was approximately 25%. Animals placed in the normal control group and pair-fed group (PF) were sham operated by exposing and manipulating the kidneys without performing the nephrectomy. All surgical procedures were conducted under general anesthesia employing an intraperitoneal injection of sodium pentobarbital (40 mg/kg body wt) and under aseptic conditions. Blood loss was kept to a minimum using strict hemostasis. The animals were maintained for 5 weeks prior to the absorption experiments. The PF group was included to control for uremia-induced anorexia, reduced nutrient intake, and weight loss. Pair feeding was accomplished by limiting the daily food available to each PF animal to that consumed by its RF counterpart during the preceding 24 hr. Serum creatinine concentration was determined using a kit supplied by Sigma Chemical Co. (St. Louis, MO).

Materials. Unlabeled tocopherol, glucose, and taurocholate were obtained from Sigma Chemical Co. D-[α-^{3}H]Tocopherol with the specific activity of 3.5 Ci/mmol was purchased from Amersham Laboratories (Amersham, IL) and [^{14}C]inulin with a specific activity of 260 mCi/g and [^{3}H]inulin with a specific activity of 100–500 mCi/g were purchased from New England Nuclear Research Products Inc. (Boston, MA). Inulin was used as a nonabsorbable marker (10).

Perfusates and Incubation Media. The perfusate and incubation media used consisted of micellar solutions prepared with Krebs phosphate buffer (pH 7.4) to which different concentrations of unlabeled tocopherol, taurocholate, trace amounts of [^{14}C]inulin and D-[α-^{3}H]tocopherol were added. The mixture was subjected to sonification at 70 watts of power for 5 min. The solution was protected from exposure to light at all times to prevent disintegration of vitamin E. All solutions were prepared 24 hr before the experiment, and micellar stability was verified by the presence of a clear solution at the time of all experiments.

***In Vivo* Experiments.** The *in vivo* experiments were conducted as described previously (8) with the following

Received September 20, 1988. [P.S.E.B.M. 1990, Vol 193]
Accepted October 18, 1989.

0037-9727/90/1932-0125$2.00/0

modifications. A double perfusion technique was used by isolating and cannulating two 15-cm jejunal segments. The perfusate was a micellar solution of 10 mM sodium taurocholate and Krebs phosphate buffer containing either a 400 nM or 300 μM concentration of vitamin E. The perfusions with high and low vitamin E concentrations were alternated between proximal and distal segments in different animals to cancel any effect of jejunal site on the results. The perfusion was conducted for 50 min at a flow rate of 0.5 ml/min and samples were taken at 5-min intervals. The first 10 min were considered a period of equilibration and data obtained at that time were not included in the calculations. The absorption rate was determined from disappearance of labeled vitamin E from the perfusate using the following equation:

$$A_t = A_o \frac{I_o}{E_o} \times \frac{E_t}{I_t},$$

where A_t and A_o represent the amount of vitamin E in the perfusate at time t and o and E_o and E_t equal the concentrations of labeled vitamin E at times o and t, respectively, Accordingly the data were corrected for any water shift using inulin as a nonabsorbable marker. The results were expressed in terms of dry intestine length and weight (8).

In an attempt to evaluate the general absorptive capacity of the intestine in the three study groups, the *in vivo* experiments were repeated using D-glucose as a general marker. Using a single pass technique, the intestinal segment was perfused with a 5.5 mM D-glucose solution containing [^{3}H]inulin as a nonabsorbable marker. After a 20-min equilibration period, glucose absorption was determined by its disappearance rate. Glucose concentration was measured using a colorimetric enzymatic assay purchased from Sigma Chemical Co. The radioactivity of ^{3}H and ^{14}C was determined by double isotope counting which was carried to an error of ±0.5% using a liquid scintillation counter (Beckman LS-9000) with automatic quench calibration program.

***In Vitro* Experiment.** The *in vitro* experiments were performed using everted sac technique as described previously (8). The sacs were incubated in the micellar solutions containing six different concentrations of vitamin E (400, 800 nM and 100, 200, and 400 μM) for 20 min. At the end of the incubation period, the sacs were rinsed for 1 min in a 1 mM taurocholate solution to remove any vitamin E adherent to the tissue. The *in vitro* experiments were repeated using an incubation medium containing 200 μmol/liter of vitamin E and everted sacs from normal rats filled with 0.2 ml of pooled serum from either three uremic patients or three normal individuals. Human sera were used because they were more plentiful and because the severity of uremia in these individuals is much greater than what could be achieved in the rat where dialysis is not practical.

Statistical Analysis. All data are given as mean ± SD. Data are expressed as nmol/g/min and nmol/100 cm/min. Analysis of variance (ANOVA), Student's t test, and linear regression were used in the statistical analysis of the data when indicated (11).

Results

***In Vivo* Absorption.** As expected, serum creatinine concentration in the RF group was significantly higher than those found in the normal and PF groups. The rate of intestinal absorption (expressed per length) of both 400 nM and μM concentrations of vitamin E were significantly lower in the RF and PF groups as compared with the normal control group. When the data were expressed per unit of intestine weight, the RF group continued to show depressed absorption rates while the PF group showed values comparable to those found in the normal control group (Table I).

There was no statistically significant difference in the rate of water absorption among the three groups. In the first 10 min there was net water absorption in the RF and normal (NL) control groups with slight net water secretion in the PF group. By 30 min there was mild net water secretion in most rats studied.

The rate of glucose absorption, used to evaluate the general absorptive capacity of the intestine, was similar in the three groups studies (6.6 ± 2.0 μmol/cm/min in RF animals, 5.5 ± 2.3 μmol/cm/min in NL controls, and 5.4 ± 1.3 μmol/cm/min in PF animals, P = NS for all comparisons).

***In Vitro* Uptake.** As expected, *in vitro* mucosal-to-serosal transport of vitamin E was negligible at all concentrations studied. This is because transmural transport of all fats and fat-soluble compounds requires intact lymphatic system which is lacking in the *in vitro* system. However, significant radioactivity was noted in the intestinal tissue representing vitamin E uptake by the enterocytes. The RF animals showed significantly greater vitamin E uptake when compared with the normal and PF controls at most concentrations tested. The PF group showed higher rates of vitamin E uptake when compared with normal controls (Table II). The rate of vitamin E uptake showed a linear relationship to its concentration in both the RF and control groups (r = 0.97 for RF 0.99 for NL control and 0.98 for PF, $P < 0.01$ for all correlations). Results of the *in vitro* experiment employing normal and uremic sera in the serosal compartment are summarized in Table III. The results revealed no significant difference in vitamin E uptake by sacs containing uremic sera and those containing normal sera.

Discussion

The results of the *in vivo* experiments revealed a marked reduction of intestinal absorption of vitamin E in animals with renal failure. Likewise, pair-fed animals

Table I. The *In Vivo* Absorption of Vitamin E in RF, PF, and NL Control Groups[a]

	RF	NL Control	PF	*t* test (*P*)
Creatinine (mg/dl)	2.95 ± 1.38	0.97 ± 0.32	1.07 ± 0.18	<0.001[b]
n	9	12	12	
Body weight (g)				
Initial	280.44 ± 93.64	328.83 ± 21.30	291.17 ± 84.8	NS
Final	241.33 ± 30.87	389.33 ± 43.42	193.92 ± 60.97	<0.001[c]
n	9	12	12	
Dry weight of 2-cm intestinal segments (g)	0.027 ± 0.005	0.028 ± 0.006	0.021 ± 0.004	<0.001[d]
n	54	69	65	
				ANOVA (*P*)
Absorption rate				
400 n*M*				
nmol/100 cm/min	0.607 ± 0.124	0.864 ± 0.236	0.643 ± 0.162	<0.02
n	8	9	9	
nmol/g/min	0.401 ± 0.132	0.537 ± 0.095[e]	0.541 ± 0.163	NS
n	8	9	10	
300 μ*M*				
nmol/100 cm/min	260.0 ± 72.8	405.6 ± 101.8	227.7 ± 84.8	<0.01
n	8	6	6	
nmol/g/min	155.5 ± 47.1	209.3 ± 65.6	235.5 ± 96.9	NS
n	8	7	7	

[a] Data are given as mean ± SD.
[b] RF versus NL control.
[c] RF or PF versus NL control.
[d] PF versus NL control.
[e] *t* test RF versus NL, $P < 0.05$.

Table II. The *In Vitro* Uptake of Vitamin E in RF, and PF, and NL Control Groups[a]

	RF	NL Control	PF	*t* test (*P*)
Creatinine (mg/dl)	2.83 ± 1.00	0.59 ± 0.19	0.79 ± 0.18	<0.001[b]
n	8	10	11	
				ANOVA (*P*)
Tissue (nmol/g/20 min)				
400 n*M*	0.419 ± 0.112	0.216 ± 0.436	0.342 ± 0.117	<0.01
n	7	6	7	
800 n*M*	0.810 ± 0.209	0.218 ± 0.108	0.742 ± 0.113	<0.001
n	6	4	6	
100 μ*M*	83.1 ± 16.6	45.9 ± 20.9	68.3 ± 16.2	<0.01
n	5	7	7	
200 μ*M*	96.3 ± 26.9	71.6 ± 43.2	76.3 ± 22.6	NS
n	7	6	8	
400 μ*M*	177.6 ± 69.9	124.5 ± 37.2	188.8 ± 40.3	NS
n	5	5	5	

[a] Data are given as mean ± SD.
[b] RF versus NL control.

showed reduced vitamin E absorption when compared with that of the normal control group. The abnormalities of vitamin E absorption in the renal failure and pair-fed animals were not associated with an impaired general absorptive capacity of the intestine as assessed by glucose transport experiments. Interestingly, when the absorption rates were expressed per unit of intestine weight to control for changes in intestine mass, the absorption rate in the PF animals approached that of the normal control group, whereas the RF animals continued to manifest depressed values. The observed disparity was, in part, due to the fact that despite comparable reductions in food intake and body weight the intestine mass was significantly reduced in the PF animals but was virtually unchanged in the chronic renal insufficiency model employed in the present study. This observation was recently confirmed by our group in a comparative study of intestinal morphome-

Table III. The *In Vitro* Uptake of 200 μM Vitamin E by Everted Sacs from Normal Animals Filled with Pooled Sera from Three Uremic Patients and Three Normal Individuals[a]

	Normal sera	*P*	Uremic sera
Serum creatinine (mg/dl)	1.2 ± 0.12	<0.01	12.77 ± 3.71
Uptake (nmol/g/20 min)	114.93 ± 30.14	NS	115.82 ± 37.11
	$n = 5$		$n = 6$

[a] Data are given as mean ± SD.

try in rats with chronic renal insufficiency (unpublished data) and differs from that seen in short-term (2 weeks) renal insufficiency model previously used by our group and others (7, 9) wherein intestinal mass is reduced. It thus appears that reduced nutrient intake in normal animals leads to a reduction of vitamin E absorption by reducing the absorptive tissue. In contrast reduced intestinal absorption of vitamin E in RF animals despite lack of significant change in intestine mass suggests that other factor(s) must be operative in animals with renal failure.

In contrast to the *in vivo* findings, the *in vitro* incubation studies showed greater vitamin E uptake in azotemic animals when compared with normal controls. This observation suggests increased *in vitro* permeability to vitamin E in the RF group. Since the PF animals also exhibited a similar trend toward increased uptake, it can be concluded that reduced food intake mass may be partially responsible for the observed *in vitro* abnormalities in animals with RF. However, since RF animals show greater uptake when compared with the PF group, other factors besides reduced food intake may have also contributed to the observed hyperpermeability.

The observed impairment of vitamin E absorption *in vivo* in the RF animals in the face of the demonstrated increase *in vitro* uptake is of considerable interest and points to the presence of some inhibitory influence(s) in intact animals with RF. It should be noted that the *in vitro* experiments, intended to mimic chemical environment, showed that addition of uremic serum to the serosal compartment has no effect on vitamin E uptake by normal intestinal sacs. This observation tends to exclude a rapid effect of uremic chemical environment on vitamin E absorption. It thus appears that pathophysiologic factors present only in intact azotemic animals are responsible for the observed impairment of vitamin E transport. The precise nature and mechanism of action of the inhibitory factor(s) is not known and requires further investigation.

The linear relationship of vitamin E absorption with its concentration in both RF and normal control animals suggests simple diffusion as the mode of transport in this concentration range. This confirms data previously reported by other investigators (2, 12).

1. Molenaar I, Hultaert CE, Vos J. Membrane characteristics in vitamin E deficiency and the assessment of vitamin E status. Proc Nutr Soc **32**:249–253, 1973.
2. Hollander D, Rim E, Muralidhara KS. Mechanism and site of small intestinal absorption of alpha-tocopherol in the rat. Gastroenterology **68**:1492–1499, 1975.
3. Deuei H. The importance of tocopherols (vitamin E) in various metabolic processes. The Lipids: Their Chemistry and Biochemistry. New York: Interscience, Vol **3**: p703, 1957.
4. Green J. Vitamin E and the biological anti-oxidant theory. Nutr Abstr Rev **39**:321, 1969.
5. Oski FA, Bayness LA. Vitamin E deficiency. A previously unrecognized cause of hemolytic anemia in the premature infant. J Pediatr **70**:211, 1967.
6. Traber MG, Sokol RJ, Ringel SP, Neville HE, Thellman CA, Kayden HJ. Lack of tocopherol in peripheral nerves of vitamin E deficient patients with peripheral neuropathy. N Engl J Med **317**:262–265, 1987.
7. Said H, Vaziri ND, Hollander D, Kariger R. Intestinal absorption of 5-methyltetrahydrofolate in experimental uremia. Acta Vitaminol Enzymol **6**:339–346, 1984.
8. Vaziri ND, Said H, Hollander D, Kariger D, Barbari A. Impaired intestinal absorption of riboflavin in experimental uremia. Nephron **41**:26–29, 1985.
9. Vaziri ND, Hollander D, Hung E, Vo M, Dadufalza L. Impaired intestinal absorption of vitamin D-3 in azotemic rats. Am J Clin Nutr **37**:403–406, 1983.
10. Miller DL, Schedl PS. Total recovery studies of nonabsorbable indicators in the rat small intestine. Gastroenterology **1**:40–46, 1970.
11. Draper NR, Smith H. Applied Regression Analysis. New York: John Wiley and Sons, 1966.
12. Muralidhara KS, Hollander D. Intestinal absorption of alpha-tocopherol in the unanesthetized rat. The influence of luminal constituents on the absorptive process. J Lab Clin Med **90**:85–91, 1977.