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Influence of Dietary Vitamin E on the Intermembrane Transfer of α-Tocopherol as Mediated by an α-Tocopherol Binding Protein[1] (42779)

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Abstract. The effect of dietary vitamin E on the intermembrane transfer of (3*R*)-α-tocopherol, a spontaneous process accelerated in the presence of an α-tocopherol binding protein (αTBP), was examined. The transfer activity of this cytosolic liver protein was assayed via *in vitro* transfer of (3*R*)-α-[^{3}H]tocopherol (α[^{3}H]T) from egg lecithin liposomes to human erythrocyte ghosts (EG). Male Fisher 344 rats (1 and 20 months old) were fed diets containing 0, 30, and 500 mg/kg vitamin E (*dl*-α-tocopheryl acetate) for 15 weeks. Liver cytosol fractions were assayed for α[^{3}H]T transfer activity (αTTA). Among young rats, those fed vitamin E-deficient diets had the highest αTTA, 5.02 ± 3.10 pmole α[^{3}H]T/min (mean ± SD), which was different ($P < 0.05$) from the spontaneous transfer rate of 2.10 pmole/min. Neither young rats fed 30 and 500 mg/kg vitamin E diets nor any of the aged rats showed αTTA which differed significantly from the spontaneous transfer rate. To examine the relationship between hepatic α-tocopherol levels and αTTA, α-tocopherol concentration per gram of wet liver was assayed by HPLC. A steep positive slope (6.39 ± 1.46 pmole min^{-1} nmole g^{-1}) and strong correlation ($r = 0.873$) between hepatic α-tocopherol and αTTA were observed ($P < 0.005$) among young vitamin E-deficient rats. The data indicates that αTTA varies directly with hepatic α-tocopherol concentration when total liver vitamin E stores are very low. Thus, αTBP-mediated transfer of α-tocopherol may be manifest only when vitamin E status is compromised.

Vitamin E is found primarily in intracellular membranes at concentrations one to two orders of magnitude greater than the soluble fraction of various tissues (1). (3*R*)-α-Tocopherol (αT) is practically insoluble in water but is detected in the soluble fraction of tissue homogenates in association with amphiphilic molecules and proteins. One of these proteins, a hepatic protein associated specifically with αT, has been identified as the α-tocopherol binding protein (αTBP) (2). This cytosolic protein has been reported to transfer αT between membranes in a manner similar to that of other cytosolic proteins which transfer or exchange phospholipids (3), cholesterol (4), and fatty acids (5). αTBP (M_r 31,000–32,000) binds αT with an affinity greater than all-*rac*-α-tocopherol, *d*-γ-tocopherol, or α-tocotrienol and is not displaced by *dl*-α-tocopheryl acetate, α-tocopheryl quinone, or *rac*-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) (6). The intestinal absorption, transport, and liver uptake of vitamin E are postulated to be highly specific for αT, supported by the observation that a small dose of αT will displace γ-tocopherol in rats (7, 8). It is also interesting to note that supplements of α-T lower circulating γ-tocopherol in humans (9, 10). Tissue preference for αT over γ-tocopherol as indicated in these *in vivo* studies is consistent with the biological potencies described elsewhere (11) and with the binding order of these vitamers to αTBP (12), suggesting that the hepatic αTBP may play a part in the systemic handling of αT.

The αTBP-mediated αT transfer activity (αTTA) has been assayed by measuring the *in vitro* transfer of α-[^{3}H]tocopherol (α[^{3}H]T) from liposomes to rat liver microsomes (13) and mitochondria (14, 15), a process that occurs spontaneously but slowly in the absence of αTBP. When the αTBP from rat liver cytosol was purified by Sephadex G-75 permeation, αTTA to rat liver microsomes (14) and mitochondria (15) was improved 9- and 12-fold, respectively, and coincided with α[^{3}H]T binding. Binding and

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transfer activity are lost with Pronase treatment and, to a lesser degree, with trypsin digestion. The binding of α[^{3}H]T to αTBP was reported to be 4-fold greater when the protein was isolated from rats fed a vitamin E-deficient diet, although αTTA was unaffected (14).

This study examines the influence of deficient, 30 and 500 mg/kg vitamin E diets on the αTTA in the presence of liver cytosol obtained from 1- and 20-month-old rats. Aged rats were included in the experimental design to ascertain any age-associated changes in αTTA. In addition, the correlation between αTTA and αT liver stores was determined. αTTA was determined using an assay that measures the rate of transfer of α[^{3}H]T from egg phosphatidylcholine liposomes to human erythrocyte ghosts (EG) in the presence of these cytosolic fractions. Under our conditions, αTTA responded in a linear fashion with respect to cytosol concentration and semipurified αTBP (16). Our purpose was to see if liver αTBP activity changes in response to dietary vitamin E or liver αT concentration.

Materials and Methods. *Materials.* (3*R*)-α-[^{3}H]tocopherol and glycerol tri[1-^{14}C]-oleate ([^{14}C]triolein) were obtained from Amersham Radiochemicals, Arlington Heights, Illinois. (3*R*)-α-tocopherol and *dl*-α-tocopheryl acetate were obtained from Kodak Chemicals, Rochester, New York, and egg phosphatidylcholine (lecithin), butylated hydroxytoluene (BHT), and bovine serum albumin (BSA) from Sigma Chemical Co., St. Louis, Missouri. Sephacryl S-200 superfine and Sepharose 4B-CL are trademarks of Pharmacia Fine Chemicals (Uppsala 1, Sweden) and Unicord is a trademark of LKB Instruments (Bromma, Sweden). Distilled, deionized water was prepared using a Barnstead Still (Sybron, Boston, MA). Spectrophotometric measurements were made in a Lambda 1 spectrophotometer (Perkin–Elmer, Norwalk, CT). The buffer (SET) used throughout the experiments consisted of 250 m*M* sucrose (Fisher Scientific, reagent grade), 1 m*M* EDTA (Baker Chemical Co., reagent grade), and 50 m*M* Tris–HCl (Sigma Chemical Co.), adjusted to pH 7.4. The buffer used for homogenization of livers contained 10 μ*M* pepstatin A (Sigma Chemical Co.). All dietary ingredients were obtained from Teklad, Madison, Wisconsin, except where noted otherwise.

Instrumentation. The high-performance liquid chromatography (HPLC) system consisted of a single reciprocating piston Beckman 112 pump, a Rheodyne injector with a 100-μl sample loop, and a 250 × 4.6-mm, 5-μm particle-size reverse-phase C-18 column (Ultrasphere ODS, Altex Instruments). Calibration was accomplished by 20- to 40-μl injections of αT standard mixed with α-tocopheryl acetate (internal standard). αT was isocratically eluted with 100% methanol at a flow rate of 2.0 ml/min and detected by a spectrofluorescence detector exciting at 292 nm, with emission set at 330 nm. The peak areas were quantified by a Waters 840 chromatography data station (Millipore Corp., Milford, MA).

Ultracentrifugations were performed using a 50.2 Ti rotor in a L8-80 ultracentrifuge (Beckman, Palo Alto, CA). ^{3}H and ^{14}C activities in liposomes and pellets were determined in a LS3000 liquid scintillation counter (Beckman, Palo Alto, CA) using Ready-Solv HP/B cocktail. Liposomes were formed in a bath-type sonicator (Laboratory Supplies, Inc., Hicksville, NY). A Beckman Microfuge 12 tabletop centrifuge was used for pelleting small samples in 1.5-ml plastic centrifuge tubes. Samples were stored at −80°C in a Revco freezer.

Animals and diets. Twenty 1-month-old and twenty 20-month-old male Fisher 344 rats were kept in a common room controlled for humidity and temperature with a 12-hr light/dark cycle. Animals were randomly assigned to individual stainless steel cages with an automatic deionized drinking water system. During a 1-week acclimation period, rats were fed a standard commercial diet (Prolab 3000, Agway, Inc., Syracuse, NY) after which the dietary treatment began. Experimental diets consisted of the basal vitamin E-deficient diet (Table I) supplemented with *dl*-α-tocopheryl acetate (500 U/g) to make 30 and 500 mg/kg concentrations, with the exception of the vitamin E-deficient diet for which sucrose was substituted. Each age group was randomly divided into three dietary groups: vitamin E deficient (n = 8), 30 mg/kg vitamin E (n = 6), and 500 mg/kg

TABLE I. BASAL DIET

Ingredient	Amount (g/kg diet)
Sucrose	610.0
Casein, vitamin free	200.0
Corn oil, tocopherol-stripped	93.0
Cellulose	50.0
Mineral mix, AIN-76	35.0
Vitamin mix, vitamin E free[a]	9.5
DL-Methionine	3.0
Choline bitartrate	0.5

[a] Vitamin mix consisted of a modified AIN-76A formula missing vitamin E.

vitamin E (n = 6). Rats were allowed to feed *ad libitum* from food jars that were filled twice weekly for a period of 15 weeks. Rats were monitored for weight gain twice weekly for the first 2 weeks, then once a week for the duration of the study. One rat from the vitamin E-deficient treatment aged group and two from the 30 mg/kg diet treatment aged group (one had testicular tumor) were not maintaining their weight and were removed from the study at Weeks 3, 14, and 15, respectively. The mean weight of young and old rats at the end of study was 330 and 400 g, respectively. There was no significant difference in mean weights between diet treatments within each age group. There was no significant difference in the amount of food consumed between groups during the 3 days before sacrifice. On the day of sacrifice, 18-hr-fasted rats were anesthetized with metophane and livers were perfused with cold isotonic phosphate-buffered saline (PBS), pH 7.4. Rinsed livers were quick-frozen in liquid nitrogen and stored at −80°C.

Isolation of cytosol. The method of isolating rat liver cytosol was adapted from the method of Hogeboom (17). Five grams of frozen liver was accurately weighed with minimal thawing just before homogenization. The liver pieces were quantitatively transferred to a 55-ml Potter–Elvehjem tissue grinding tube (A.H. Thomas & Co.) with 15.0 ml of SET–pepstatin A buffer. Liver was homogenized with three strokes, transferred to a centrifuge tube, and spun at 600g for 10 min at 4°C. The pellet was separated and rehomogenized as before in 10.0 ml of SET–pepstatin A buffer. This homogenate was combined with the first and then centrifuged again 10 min at 600g at 4°C. Twenty milliliters of the resulting supernatant was centrifuged at 100,000g for 2 hr at 4°C. Thirteen milliliters of the clear supernatant below the upper lipid layer was aspirated and 150 μl was pipetted into a microcentrifuge tube, another 100 μl was kept for total protein determination using the biuret reaction (18), and the remainder was saved in 5-ml aliquots. All aliquots were kept at −80°C until assayed.

Preparation of human erythrocyte ghosts. Preparation of EG was adapted from the method of Burton *et al.* (19). Blood was collected from human volunteers by venipuncture into 10-ml heparinized Vacutainer tubes and pooled. The blood was centrifuged at 600g for 15 min at 4°C and the supernatant aspirated and discarded. The packed red blood cells (RBC) were resuspended in 3 vol of cold 0.8% NaCl/10 mM Na-phosphate, pH 7.0, (PBS) and then centrifuged as before. This wash treatment of the RBC was repeated twice. The RBC were then hemolyzed by suspending in 5 to 7 vol of hypotonic phosphate buffer [5 mM Na-phosphate/1 mM EDTA buffer/1 mM ascorbic acid, pH 7, (HPB)] at 4°C for 30–40 min. EG were separated from the hemolysate by centrifugation at 11,000g for 30 min. The supernatant was aspirated and discarded, and the pelleted EG were resuspended with cold HPB and allowed to stand at 4°C for 10 min. The EG were repelleted at 17,000g for 12 min. The supernatant was aspirated and discarded, and the EG pellet washed three times by repeated resuspension in PBS and centrifugation at 17,000g for 12 min. After the final wash, the packed EG pellet was white in color. An equal volume of PBS containing 20% dimethyl sulfoxide was added and the EG were mixed and stored at −80°C in 1-ml aliquots. One milliliter of packed EG consisted of 2.24×10^8 ghosts, containing 2.63 nmole αT and 2.13 μmole lipid phosphorus. One batch was used for all experiments.

Preparation of liposomes containing α-[^3H]tocopherol and [1-^{14}C]triolein. α[^{3}H]T was purified by HPLC prior to use in the liposome preparation. Two separate HPLC runs, each consisting of a 86-μl injection of α[^{3}H]T (1.0 mg/ml in ethanol; 55 μCi/mg),

were made with a 100-μl Hamilton gas-tight syringe. The effluent containing α[^{3}H]T, as detected by uv absorbance at 292 nm, was collected. Fifty microliters of a 100 mg/ml solution of lecithin in $CHCl_3$, 1–2 μCi of [^{14}C]triolein in benzene (64 μCi/mg), and 0.44 mg of BHT were added to α[^{3}H]T and the solvent was evaporated under a stream of nitrogen. Two milliliters of diethyl ether (previously washed with 5% aqueous $FeSO_4$ and dried over anhydrous $MgSO_4$ to reduce peroxides) was added to dissolve the lipid residue and then evaporated under nitrogen. Nitrogen-purged SET buffer (2.5 ml) was added, and the tube was sealed under prepurified nitrogen and then vortexed to suspend the lipid residue as a milky emulsion. The emulsion was kept in the dark overnight at 4°C. The suspension was sonicated for 20–45 min at 20–37°C. The liposome preparation was diluted 5-fold with nitrogen-purged SET buffer and then filtered through a 0.1-μm pore-type polycarbonate filter (Nuclepore, Inc., CA). An aliquot was mixed with scintillation cocktail and counted to determine the ^{3}H:^{14}C ratio. This liposome preparation was kept capped under nitrogen at 4°C for a period of 2 days before use in the assay. Final concentration of α[^{3}H]T in the liposomes was 200 nmole α[^{3}H]T/mg lecithin. HPLC analysis of the liposomes on the day of assay confirmed the radioactive purity of α[^{3}H]T to be >98%.

α-Tocopherol transfer assay. αTTA was measured by adapting the phospholipid exchange protein assay of Bloj and Zilversmit (4). Liposomes pelleted with EG contribute "nontransferred" α[^{3}H]T to the total [^{3}H] and introduce error. [^{14}C]Triolein was added as a nonexchangeable marker of liposomes so that contaminating α[^{3}H]T could be accounted. One hundred microliters of liposomes and 150 μl of SET buffer were mixed with 150 μl of cytosol for a 2-min preincubation at 4°C before the addition of 100 μl of EG. Completed volume was 500 μl contained in 1.5-ml polypropylene centrifuge tubes. Final concentrations per milliliter incubation mixture were 80 μg lecithin liposomes and 0.43 μmole EG as lipid phosphorous. The average total protein concentration of the cytosol was 7.50 ± 0.95 mg/ml (±SD). Samples were incubated 20 min in a shaking 37°C water bath. Blank runs consisted of the same components with cytosol replaced by SET buffer. After incubation, tubes were chilled in an ice bath, and then EG were pelleted by centrifugation at 10,000*g*. EG were washed by 10,000*g* centrifugation with 0.50 ml SET buffer and then resuspended in 1.2-ml scintillation cocktail for determination of ^{3}H and ^{14}C. Each cytosol was assayed in triplicate.

Calculation of α-[^{3}H]tocopherol transfer. The equation used to calculate α[^{3}H]T transfer was

$$[^3H]_t = [^3H]_p - [^3H]_c, \qquad [1]$$

where

$$[^3H]_c = [^{14}C]_p\left(\frac{[^3H]_0}{[^{14}C]_0}\right). \qquad [2]$$

$[^3H]_t$ represents dpm of α[^{3}H]T transferred to EG, $[^3H]_p$ and $[^{14}C]_p$ are the total ^{3}H and ^{14}C counted, respectively, in the EG pellet, $[^3H]_c$ is the α[^{3}H]T in liposomes contaminating the EG pellet, and $[^3H]_0/[^{14}C]_0$ is the ratio of α[^{3}H]T to [^{14}C]triolein measured in the liposomes prior to start of the assay. The ratio of α[^{3}H]T to [^{14}C]triolein in incubating liposomes changed less than 5% from their initial ratio, based on measurements of ^{3}H/^{14}C in postassay supernatants. From $[^3H]_t$, moles of αT transferred was calculated from the specific radioactivity of α[^{3}H]T previously determined by HPLC. The mean αTTA for each cytosol was calculated from triplicate αTTA measurements.

Liver α-tocopherol content. The liver extraction and HPLC procedure was adapted from Zaspel and Csallany (20). Each liver (0.19–0.21 g) was weighed with minimal thawing and homogenized with 20 vol of acetone containing 0.25 mg *dl*-α-tocopheryl acetate (added as an internal standard) and 0.2% BHT added as an antioxidant (21). Acetone supernatants of liver extracts from young, vitamin E-deficient rats were concentrated by evaporation; otherwise, the αT content in 40 μl of unconcentrated acetone supernatants was determined by duplicate injections into the HPLC as described above. The αT concentration in livers from young, vitamin E-deficient rats was determined from two samples of each liver which were

TABLE II. LIVER α-TOCOPHEROL CONTENT

Age group	Diet[a] (mg/kg)	N	nmole/g Wet tissue[b]	μmole/mole of Triglycerides[b]
1 month	0	8	1.5 ± 0.4	3.3 ± 1.3
	30	6	41.7 ± 7.6	101.0 ± 38.7
	500	6	122.9 ± 63.2	286.6 ± 161.0
20 month	0	7	14.3 ± 2.7	22.3 ± 6.7
	30	4	71.1 ± 19.0	120.1 ± 29.9
	500	6	290.3 ± 76.2	512.6 ± 166.1

[a] Concentration of α-tocopheryl acetate in the diet.
[b] Values represent the mean ± standard deviation.

extracted and analyzed separately. The mean values were used having a typical 5% variation between replicates.

Liver triglyceride content. Liver (0.9–1.1 g) was used for the quantification of triglyceride content based on the method of Vandekamer (22).

Statistical calculations. Unpaired Student's *t* test and the analysis of variance (ANOVA) test were used to determine levels of significance. Simple linear regressions were determined by the least-squares method.

Results. The liver concentration of αT as determined by HPLC is shown in Table II. Livers from aged rats fed vitamin E diets contained about twice the concentration of αT found in young rats. The degree of αT depletion in aged animals was markedly less than that in young animals. Young rats fed a vitamin E-deficient diet had the lowest αT content. There were no significant differences in liver weights of animals between dietary categories with age groups.

Figure 1 illustrates the αTTA values of liver cytosol obtained from young and aged rats fed deficient, normal, and supplemented vitamin E diets. Livers from young rats fed a vitamin E-deficient diet ($n = 8$) show the highest αTTA values, 5.02 ± 3.10 pmole α[^{3}H]T/min (mean ± SD). This value is sig-

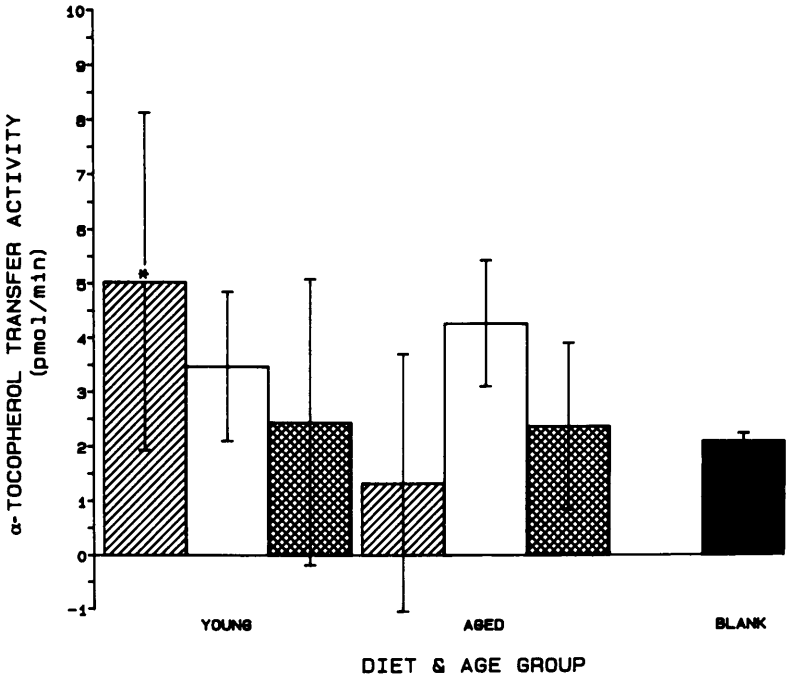

FIG. 1. Transfer rates of α-[^{3}H]tocopherol from liposomes to human erythrocyte ghosts were measured during incubation with rat liver cytosol obtained from 1- or 20-month-old rats fed a diet deficient in vitamin E (cross-hatch bars) or containing 30 (open bars) or 500 mg/kg α-tocopheryl acetate (X-hatch bars) for 15 weeks. Buffer blank incubations provided a measure of spontaneous transfer (solid bar). Error bars represent standard deviations of the mean. Bars significantly different from blank ($P < 0.05$) are marked (*).

nificantly greater ($P < 0.05$) than the spontaneous transfer of $\alpha[^3H]T$ in the absence of liver cytosol (i.e., blank sample), 2.10 ± 0.15 pmole/min. Adjustment of αTTA values for milligrams of total cytosolic protein did not improve the significance. Young rats fed 30 and 500 mg/kg vitamin E-diet ($n = 6$) exhibited αTTA of 3.48 ± 1.38 and 2.45 ± 2.63 pmole/min, values not significantly different from spontaneous transfer rates. This negative trend in αTTA with increasing dietary vitamin E is not significant by analysis of variance. Aged rats fed deficient and 30 and 500 mg/kg vitamin E diets averaged αTTA of 1.33 ± 2.37, 4.27 ± 1.16, and 2.38 ± 1.54 pmole/min, respectively, values not significantly different from spontaneous transfer rates. Neither did aged animals show any consistent trend of the relationship between dietary vitamin E and αTTA.

There was no significant correlation between individual liver αT concentrations from all pooled groups and respective αTTA values (data not shown). However, when linear regression analysis was applied to pooled young rats (Fig. 2), a negative correlation between αTTA and liver αT concentration ($r = 0.460$) was noted with a slope of −0.0200 ± 0.009 pmole min^{-1} nmol g^{-1} (±SD, $P < 0.05$). Figure 3 shows the linear correlation between the αT concentration of each liver from young vitamin E-deficient rats and their respective αTTA values. The positive slope of 6.39 ± 1.46 pmole min^{-1} nmole g^{-1} is highly significant ($P < 0.005$) with a strong correlation coefficient ($r = 0.873$). No correlation was found within any of the other groups. When the concentration of αT was expressed per mole of liver triglyceride, the significance was reduced.

Discussion. The *in vitro* intermembrane transfer of αT, a process which occurs spontaneously but is accelerated by the hepatic αTBP, was explored in young and aged rats fed diets containing 0, 30, and 500 mg/kg of vitamin E. Among groups of young rats, αTTA was observed to decrease as dietary vitamin E increased. When total liver αT concentrations were correlated with αTTA in these same animals, this association was

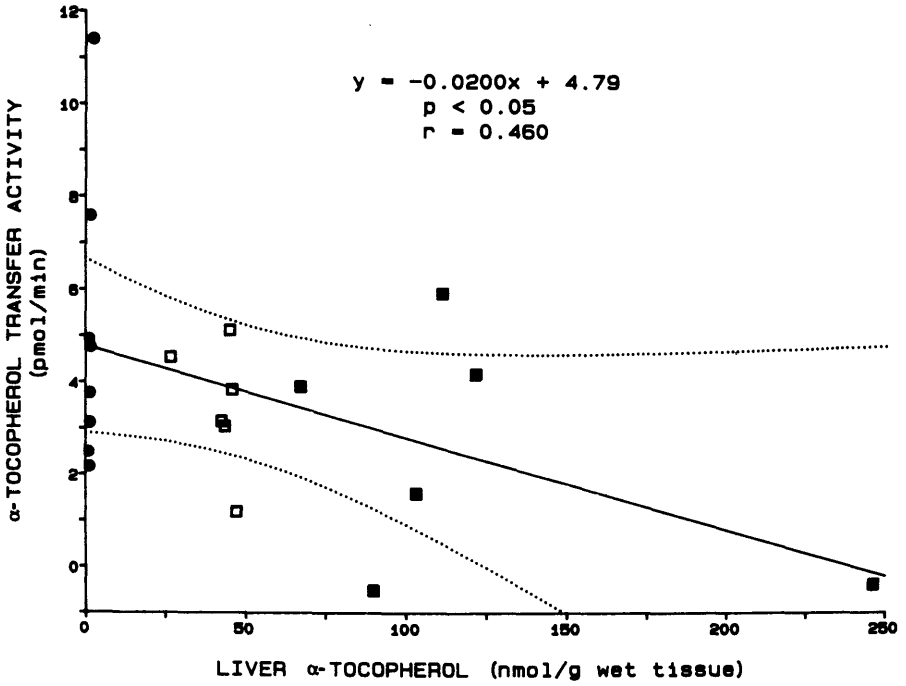

FIG. 2. Correlation between liver α-tocopherol concentration and α-tocopherol transfer activity of individual livers from young rats fed 500 mg/kg (■), 30 mg/kg (□), and vitamin E-deficient diets (●). Dotted lines represent 95% confidence limits.

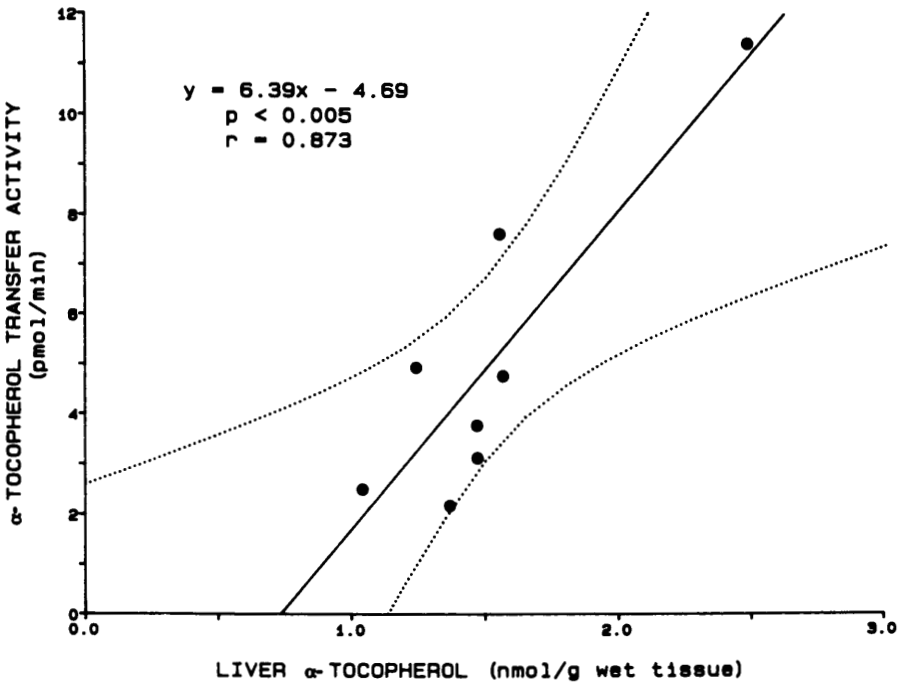

FIG. 3. Correlation between liver α-tocopherol concentration and α-tocopherol transfer activity of individual livers from young rats fed a vitamin E-deficient diet. Dotted lines represent 95% confidence limits.

again inversely related. These observations are consistent with reports that binding of α[^{3}H]T to αTBP is increased in rats fed low vitamin E diets (14, 23).

Assuming a low dissociation constant (K_d) for the binding of αT to αTBP, the amount of endogenous bound αT could be important in determining the initial transfer rate between membranes. Thus, rats fed an adequate vitamin E diet should exhibit lower αTTA since their αTBP binding sites would be already occupied and unable to transfer α[^{3}H]T within the time period of the assay. On the other hand, if the exchange between α[^{3}H]T and αT for αTBP's binding site is rapid, then an equilibrium might be quickly reached so that the amount of endogenously bound αT would not affect the measured overall rate of α[^{3}H]T transfer to EG membrane. While the K_d for αTBP has not been determined, it has been reported that exchange of α[^{3}H]T for αT on αTBP reaches equilibrium with a few minutes in the presence of α[^{3}H]T-loaded liposomes (13). In our experiments, liposomes were preincubated with cytosol for 2 min before the addition of EG membranes. If the K_d for αTBP is large, then a possible alternate hypothesis for the observed increase in αTTA with lower dietary vitamin E would be the activation of αTBP. If this were the case, the data would suggest an adaptive response to increase αTBP synthesis (or decrease degradation) in the liver of vitamin E-deficient rat.

A corollary to the "activated αTBP" hypothesis is plausible; i.e., rats that had a stronger adaptive response to the vitamin E deficient diet did so by increasing αTBP concentrations which resulted in greater liver αT concentrations. Consistent with this extension of the hypothesis are the data shown in Fig. 3. A strong positive correlation ($r^2 = 0.762$, $P < 0.005$) exists between the lowest αT stores (among the young vitamin E-deficient group) and the respective αTTA in each liver sample. This is opposed to the negative correlation that would have been expected if competition for endogenous αT occupied αTBP binding sites were ineffective; i.e., the K_d for binding was very low. Of

course, the small increase in liver αT content as shown in Fig. 3 might not be important in terms of protecting against pathological consequences of vitamin E deficiency, but might reflect of the beginning of a more involved adaptive response induced by deficiency. The "activated αTBP" hypothesis is consistent with the finding that the rat liver is a discriminating organ which preferentially stores αT over γ-tocopherol even during a challenge dose of γ-tocopherol (7). In that study, the rat liver appeared to respond to αT deficiency by increasing its αT storage in deference to the γ-, β-, or δ-vitamers. Our experiment with aged animals found there was no significant correlation between liver αT concentrations and their respective αTTA. This may be because the relatively high hepatic αT stores in aged rats were above a threshold value required for αTBP activation, even in those receiving a vitamin E-deficient diet.

These interpretations remain speculative pending the availability of additional experimental data. Problematic to this area of investigation was the large αTTA variation between animals. Triplicate αTTA measurements of individual cytosol samples resulted in a typical coefficient of variation of 15%; however, a larger variation ranging from 40 to 178% was observed in mean αTTA values between different animals within a group. A variable not directly controlled in this study was the amount of endogenous αT contributed by the liver cytosol not bound to αTBP (which conceivably could compete for binding and transfer). We were unable to determine cytosolic αT concentrations directly; however, its contribution from young, vitamin E-deficient rat livers to the total αT pool in the assay mixture approximated 25 pmole/ml while liposomes contributed 16,000 pmole αT/ml. This estimate is based on the finding that liver cytosol from the vitamin E-deficient rat contains about 28% of the total liver αT (24); the vitamin E-deficient rats had an average liver αT concentration of 1.5 nmole/g wet wt and the cytosolic fractions represented approximately 6% of the wet wt during the assay. It is unlikely that the variability in αTTA values between livers in young vitamin E-deficient rats can be explained by variations in nonliposomal αT contributions. However, it may explain the greater degree of variation in animals supplemented with vitamin E.

Future studies should attempt to increase the reproducibility in processing the cytosolic fractions, measure cytosolic αT concentration, and perhaps manipulate the levels of αTBP-bound αT to study the effect of endogenous cytosolic αT on αTTA. The purification of αTBP has been recently reported (25). Thus, new efforts may now be undertaken to quantify further αTBP and αTTA relationships in different tissues and to define the molecular processes that regulate the movement of αT between cellular membranes and tissues.

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