Liver α-Tocopherol Transfer Protein and Its mRNA Are Differentially Altered by Dietary Vitamin E Deficiency and Protein Insufficiency in Rats[1,2]

Huey-Mei Shaw and Ching-jang Huang[3]

Laboratory of Nutritional Biochemistry, Department of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan 10716

ABSTRACT To study how the expression of α-tocopherol transfer protein (α-TTP) and its mRNA are affected by protein and vitamin E status, Long-Evans male weanling rats were fed a vitamin E-deficient (DE), high vitamin E (HE, 5 g/kg diet of all-*rac*-α-tocopheryl acetate) or control (C) diet for 12 wk in Experiment 1; and fed a low-protein (LP) or control (C) diet for 6 wk in Experiment 2. The high and deficient vitamin E status of HE and DE groups in Experiment 1 were confirmed by changes in plasma pyruvate kinase activity as well as the concentrations of α-tocopherol in plasma and liver. As shown by the Northern and Western Blot Analysis, the expression of α-TTP in the liver of the DE group was significantly lower than, while that of the HE group was not different from, that of the controls. In contrast, the α-TTP mRNA levels did not differ among the C, DE and HE groups. α-Tocopherol in most peripheral tissues of rats fed the LP diet in Experiment 2 was significantly lower than that of the C. Both the α-TTP and its mRNA were significantly lower in the LP group than in the C. The results suggested that dietary vitamin E does not affect α-TTP gene expression except that the protein levels in the liver were lowered by vitamin E deficiency. On the other hand, protein inadequacy appeared to down-regulate the expression of the α-TTP gene. J. Nutr. 128: 2348–2354, 1998.

KEY WORDS: • *α-tocopherol transfer protein* • *vitamin E status* • *mRNA* • *protein deficiency* • *rats*

A cytosolic protein which specifically binds to *RRR*-α-tocopherol was isolated from livers of rats and humans (Behrens and Madere 1982, Catignani and Bieri, 1977, Kaplowitz et al. 1989, Kuhlenkamp et al. 1993, Sato et al. 1991, Yoshida et al. 1992). The molecular weight range was 30–36 kD. The protein was called α-tocopherol binding protein (α-TBP)[4] or α-tocopherol transfer protein (α-TTP) because it enhances the transfer of α-tocopherol between membranes (Sato et al. 1991). The full-length cDNA sequence of the rat α-TTP was reported by Sato et al. (1993); the human homolog was subsequently cloned (Arita et al. 1995).

Physiological studies indicated that liver is responsible for the preferential discrimination among the E vitamers (Burton and Traber 1990) and for the incorporation of *RRR*-α-tocopherol into nascent very low density lipoprotein (VLDL) which then distributes α-tocopherol to the peripheral tissues (Traber et al. 1990). This is in accordance with the ligand specificity (Sato et al. 1991) and tissue distribution pattern of α-TTP (Yoshida et al. 1992). Taken together with the α-tocopherol transfer activity, α-TTP is proposed to be the mediator of preferential incorporation of *RRR*-α-tocopherol into the VLDL (Kayden and Traber 1993).

FIVE (Familial Isolated Vitamin E Deficiency) patients suffer from neuropathy, including ataxia, and have very low plasma α-tocopherol which can be corrected by very large vitamin E supplementation dose (Kayden et al. 1987). The inability of these patients to discriminate between *RRR*- and *SRR*-α-tocopherol (Traber et al. 1993) led to the proposal that their liver α-TTP may be defective. Mutations in the α-TTP gene in FIVE and/or Ataxia with isolated vitamin E deficiency patients were reported and confirmed (Gotoda et al. 1995, Hentati et al. 1996, Ouahchi et al. 1995, Tamaru et al. 1997, Yokota et al. 1997).

The positive correlation between liver α-TTP mRNA and serum α-tocopherol was demonstrated in rats during development after birth (Kim et al. 1996) and during hepatocarcinogenesis (Wu et al. 1997). Arita et al. (1997) showed that α-tocopherol secretion was enhanced in cultured hepatoma cells stably transfected and expressing α-TTP. Hosomi et al. (1997) found that the biological activity of vitamin E analogs correlated with their affinity for α-TTP. These results further support the proposed physiological role of α-TTP.

We showed that dietary protein insufficiency significantly lowers α-tocopherol concentration in peripheral tissues but not in liver (Huang and Shaw 1994). Liver α-TTP might be impaired by dietary protein inadequacy. Besides, despite the regulatory role of α-TTP on vitamin E, whether the expression

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[3] To whom correspondence should be addressed.
[4] Abbreviations used: α-TTP(α-TBP), α-tocopherol transfer (binding) protein; C, control; DE, vitamin E-deficient; FIVE, familial isolated vitamin E deficiency; GTC, guanidium thiocyanate; HE, high vitamin E; IPTG, isopropyl β-D-thiogalactopyranoside; LP, low protein; MCID, microcomputer imaging device; PVDF, polyvinylidene fluoride; RT-PCR, reverse-transcriptase polymerase chain reaction; UV, ultraviolet; VLDL, very low density lipoprotein.

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TABLE 1

Composition of test diets

	Experiment 1			Experiment 2	
	C	HE	DE	C	LP
			g/kg diet		
Lactalbumin[1]	200	200	200	200	80
Corn starch[1]	570	570	570	570	690
Soybean oil[1]	150	150	150[3]	150	150
Mineral mixture[2]	35	35	35	35	35
Vitamin mixture[2]	10	10[4]	10[4]	10	10
Cellulose[1]	30	30	30	30	30
Choline[1]	3	3	3	3	3
All-rac-α-tocopheryl acetate	—	5	—	—	—

[1] Ingredient sources: lactalbumin and choline chloride, Sigma Chemical Co. (St. Louis, MO); Cellulose, J. Rettenmaier & Söhne (Ellwangen-Holzmühle, Germany); corn starch, Cerestar, GL; soybean oil, President Co. (Tainan, Taiwan).

[2] AIN-76 mineral mixture and AIN-76 vitamin mixture (AIN 1977). The AIN-76 vitamin mixture used to prepare the control (C) and the low protein (LP) diets contained 5000 mg/kg of all-rac-α-tocopheryl acetate, which contribute to 50 mg/kg diet of this form of vitamin E in the control and the LP diets. However, a vitamin E-free AIN-76 vitamin mixture was used for the preparation of DE and high vitamin E (HE) diets.

[3] For the preparation of the vitamin E-deficient (DE) diet, soybean oil was treated with active carbon to strip the vitamin E (Mohri et al. 1983).

[4] Vitamin E was excluded from the AIN-76 vitamin mixture for the preparation of DE and HE diets.

of α-TTP is affected by vitamin E intake is still unclear. Therefore, we measured the α-TTP protein and mRNA levels in the liver of rats fed a low-protein (LP) diet, a vitamin E-deficient (DE) or a high vitamin E (HE) diet in this study by Western and Northern Blot Analyses.

MATERIALS AND METHODS

Animals and diets. Male weanling Long-Evans rats, weighing 47.4 ± 8.4 g, were purchased from the Laboratory Animal Center, College of Medicine, National Taiwan University (Taipei, Taiwan). They were housed individually in stainless-steel wire cages in a room maintained at 25 ± 2°C with a controlled 12-h light/dark cycle. Rats were randomly assigned to five diet groups: three groups [control (C), HE and DE] in Experiment 1 and two groups (C and LP) in Experiment 2. Seven rats were included in each group. The composition of the test diets is shown in **Table 1.** The dietary protein was either 20% (normal protein, C, HE and DE diets) or 8% (LP diet) lactalbumin. The C and LP diets contained an adequate level of vitamin E [50 mg all-*rac*-α-tocopheryl acetate/kg diet (AIN 1977)]. The HE diet contained a high level of vitamin E (5 g all-*rac*-α-tocopheryl acetate/kg diet) while DE was a vitamin E-deficient diet. The soybean oil sample used for the formulation of the DE diet was stripped for vitamin E by active carbon adsorption (Mohri et al. 1983). The powdered diet was stored at −20°C. Tap water and test diets were freely available. Food intake was recorded every other day. Body weights were recorded weekly. In Experiment 1, the three groups of rats were respectively fed the C, the HE and the DE diets for 12 wk. In Experiment 2, the two groups of rats were fed either the C or the LP diets for 6 wk. Animal care and handling conformed to the National Institute of Health Guide for the Care and Use of Laboratory Animals (NRC 1985).

Tissue sampling and preparation. At the termination of feeding, food was withheld overnight and the rats were killed by carbon dioxide asphyxiation in the morning. Blood was collected from the abdominal vena cava into a heparinized tube. Liver was excised, weighed, quick-frozen in liquid nitrogen and stored at −70°C. A piece (2 g) of fresh liver was homogenized in SET buffer to obtain a 250 g/L homogenate which was then centrifuged with 100,000 × *g* at 4°C. The supernatant (the cytosolic fraction) was stored at −70°C for Western Blot Analysis. Aliquots of blood samples were centrifuged at 1,000 × *g* for 10 min, and the separated plasma was stored at −35°C. In Experiment 2, kidney, heart, spleen, lung, brain, adrenal gland, testis and skeletal muscle (*Vastus lateralis*) samples were also weighed and frozen-stored for the analysis of α-tocopherol.

Biochemical analysis. Plasma albumin was determined spectrophotometrically with bromcresolgreen dye (Doumas 1971). Plasma cholesterol, triglyceride and phospholipid concentrations were measured by enzymatic methods using commercial kits for cholesterol (CHOP-PAP, Randox, United Kingdom), triglyceride (GPO-PAP, Randox) and phospholipid (Boehringer Mannheim, Mannheim, Germany) determinations. Plasma and tissue α-tocopherol concentrations were determined by reversed–phase high performance liquid chromatography (Huang and Shaw, 1994). Plasma samples obtained in Experiment 1 were assayed for pyruvate kinase activity (Bucher and Pfleiderer 1955).

Preparation of cRNA probe and antiserum for the detection of α-TTP mRNA and protein. α-TTP cDNA was synthesized by reversed-transcriptase polymerase chain reaction (RT-PCR). The liver total RNA of Long-Evans rat was obtained by homogenizing and extracting the liver tissue by GTC (guanidium thiocyanate) (Chomczynski and Sacchi 1987). Liver total RNA (1–5 μg) was reverse transcribed and the resulting cDNA was used as the template for RT-PCR. The 5′ and 3′ primers used were 5′-GGGGGGGCG-CATATGGCAGAGATGC-3′ and 5′-CAAGGATGGGAAGC-*TT*TCTGGTTAC-3′, respectively. The primers corresponded to the −12 to 13(5′) and 873 to 897(3′) of the published α-TTP cDNA sequence, respectively, (Sato et al. 1993), except the GGC of the −3, −2, and −1 of the original sequence was modified to CAT to create a NedI site in this 5′ primer, and TT—A of the 884, 885 and 888 were modified to AG—T to create a Hind III site in this 3′ primer. The PCR was conducted in a thermal cycler (DNA thermal cycler 480; Perkin Elmer, Norwalk, CT) using two cycles of 94°C 5 min, 50°C 1 min and 72°C 1 min, followed by 30 cycles of 94°C 1 min, 50°C 1 min and 72°C 1 min. The PCR product was considered to be the 909 bp α-TTP cDNA, judging from the size and the restriction mapping. The PCR product was ligated into pGEM-T vector (Promega, Madison, WI) and used to transform *Escherichia coli* JM109. The transformed bacteria were screened and cultured for the large-scale preparation of α-TTP cDNA. The pGEM-T-α-TTP was isolated, purified and the α-TTP insert sequenced. The resulting sequence showed 97% homology with the reported sequence (Sato et al. 1993). Because the template used in this study was from liver of the Long-Evans strain rat and the α-TTP cDNA reported by Sato et al. (1993) was cloned from the cDNA library of the Sprague-Dawley strain rat liver, the 3% variation may be accounted for as from the strain difference. For the preparation of the α-TTP cRNA probe, 1 to 2 μg of the purified and linearized pGEM-T-α-TTP was incubated with SP6 RNA polymerase (Boehringer Mannheim), Rnasin (Rnase

TABLE 2

Body weight gain, food intake, feed efficiency, plasma albumin and lipids of rats fed a high vitamin E (HE) or deficient vitamin E (DE) or control diet for 12 wk (Expt. 1) or a low protein diet (LP) for 6 wk (Expt. 2)[1]

	Experiment 1[2]			Experiment 2[3]	
	C	HE	DE	C	LP
Initial body weight, *g*	46.8 ± 3.9[a]	47.3 ± 8.8[a]	46.7 ± 8.3[a]	47.4 ± 11.7	48.7 ± 9.7
Final body weight, *g*	402 ± 20[b]	466 ± 41[a]	407 ± 62[b]	285.2 ± 46.8	142.3 ± 30.9*
Food intake, *g/d*	14.44 ± 0.62[b]	17.96 ± 1.75[a]	16.00 ± 1.81[b]	14.2 ± 1.8	9.9 ± 2.0*
Body weight gain, *g/d*	4.23 ± 0.21[b]	4.99 ± 0.45[a]	4.29 ± 0.67[b]	5.66 ± 0.92	2.23 ± 0.53*
Feed efficiency, *g gain/g feed*	0.30 ± 0.01[a]	0.30 ± 0.01[a]	0.29 ± 0.01[b]	0.43 ± 0.02	0.24 ± 0.02*
Plasma albumin, *g/L*	43.37 ± 2.08[a]	42.38 ± 3.24[a]	40.50 ± 2.18[a]	41.12 ± 1.87	36.32 ± 2.38*
Plasma lipids, *mmol/L*					
Triglyceride	0.37 ± 0.07[a]	0.35 ± 0.08[a]	0.34 ± 0.06[a]	0.55 ± 0.18	1.60 ± 0.86*
Cholesterol	2.11 ± 0.32[a]	1.80 ± 0.31[a]	2.10 ± 0.48[a]	1.38 ± 0.17	2.33 ± 0.13*
Phospholipids	1.50 ± 0.16[a]	1.36 ± 0.23[a]	1.56 ± 0.23[a]	1.68 ± 0.32	3.31 ± 0.32*

[1] Values are means ± SD, $n = 7$.

[2] The significance of differences among the three groups in Experiment 1 was analyzed by one-way analysis of variance and Duncan's Multiple Range Test. Values not sharing a superscript letter are significantly different ($P < 0.05$).

[3] The significance of differences between the two groups in Experiment 2 was analyzed by Student's *t* test. Values with an asterisk (*) are significantly different from the control (C) group ($P < 0.05$).

inhibitor; Pharmacia, Uppsala, Sweden), transcription buffer and nucleotide mixture (Riboprobe Gemini II core system; Promega, Madison, WI) and [α-^{32}P] CTP (NEN, Boston, MA) at 37°C for 90 min The ^{32}P-labeled cRNA probe prepared by the in vitro transcription system was purified and used for hybridization on the day it was prepared.

The α-TTP cDNA produced by PCR as described above was ligated into the pET28a vector (Novagen, Madison, WI) through the Hind III and Ndel sites. *Escherichia coli* JM109 was transformed by the pET 28a-α-TTP cDNA construct, screened with Kanamycin and cultured. The pET 28a-α-TTP cDNA was isolated, purified and used to transform *E. coli* BL21. The transformant was cultured until the optical density value reached 0.6, then IPTG (isopropyl β-D-thiogalactopyranoside) was added to reach a final concentration of 0.4 mol/L and the culturing was continued for three more hours. Because a 6 His sequence upstream of the α-TTP cDNA in the construct was present, the expression of the 6 His-α-TTP protein is induced by IPTG. The protein was purified by the His.Bind Buffer kit (Novagen, Madison, WI) in which the 6 His-α-TTP was bound to the Ni^{2+} resin affinity column and eluted with imidazole according to the manufacturers instructions. The purified protein showed a single band with a molecular weight of ~34 kD. The purified 6 His-α-TTP (20–40 μg) was mixed with Freund complete adjuvant and injected directly into the spleen of the Balb/c mice under anesthesia to immunize for the production of antiserum. Mice were then boosted by i.p. injection every other week and checked for the antibody titer. Eight weeks after the first immunization when the titer was significantly increased, mice were killed and the antiserum collected. The antibody was partially purified by ammonium sulfate fractionation. To confirm the specificity of the anti-α-TTP antibody, the antibody preparation and the pre-immune serum were compared in Western Blot Analysis for immunostaining of the purified 6 His-α-TTP and cytosolic fractions of lung, liver, heart, brain, spleen and kidney from a Long-Evans rat. The anti-α-TTP antibody but not the pre-immune serum could detect the purified 6 His-α-TTP. Among the tissues, positive signal could be detected only in the cytosolic fraction of liver where one single band of ~34 kD was detected. None of the remaining rat tissue cytosolic fractions showed any positive signal.

Northern Blot Analysis. Liver total RNA was extracted by 4 mol/L of guanidium thiocyanate according to the method of Chomczynski and Sacchi (1987). Total RNA (10 μg) from each sample was separated by electrophoresis in denaturing formaldehyde agarose (1%) gel, transferred to nylon membrane (Gene Screen Plus; NEN Life Sciences, Boston, MA) and cross-linked to membrane by ultraviolet (UV) irradiation. The integrity of the extracted RNA was confirmed by observing the electrophoresized RNA samples in the agarose gel under an UV light. Blots were prehybridized at 55–60°C for 30–60 min in the hybridization solution excluding the probe. They were then hybridized at 65°C for 12–15 h with ^{32}P-labeled cRNA probe-specific for rat α-TTP. Membranes were washed and exposed to X-OMAT AR film (Kodak, Rochester, NY). Blots were deprobed and hybridized with ^{32}P-labeled cRNA probe-specific for rat L-ferritin as a control. The procedure for the preparation of L-ferritin ^{32}P-labeled cRNA probe was exactly the same as the preparation of α-TTP cRNA probe. The rat L-ferritin cDNA was generously provided by Dr. Richard Eisenstein, Department Nutritional Sciences, University of Wisconsin, Madison, WI (Brown et al. 1983). The films were analyzed by MCID image analysis system [The microcomputer imaging device (MCID); Fuji Co., Tokyo, Japan] for Density (D) and Area (A). The D × A of α-TTP mRNA and of L-ferritin mRNA for each sample were recorded and the ratio of D × A for α-TTP mRNA and for L-ferritin were calculated for each sample.

Western Blot Analysis. Aliquots of the liver cytosolic fraction containing 10 μg protein were subjected to SDS-polyacrylamide gel electrophoresis, then transferred to PVDF-plus transfer membrane. The blot was immunostained using the anti-α-TTP as the primary antibody and the rabbit antimouse IgG-alkaline phosphatase conjugate (Tropix, Bedford, MA) as the secondary antibody. The signal was visualized by reacting with CSPD (Tropix) for 5 min and exposed to a film (BioMax Light Film; Kodak, Rochester, NY) for 5 min. The film was subjected to the MCID image analysis as described above.

Statistical analysis. Data were expressed as mean ± SD of seven rats in each group. The significance of difference among the three groups for Experiment 1 were analyzed statistically by analysis of variance and Duncan's Multiple Range test using the General Linear Model of the SAS Package (SAS Institute, Cary, NC). For Experiment 2, Student's *t* test was used for the comparison between the two groups. Differences were considered significant at $P < 0.05$. Randomized Complete Block Design was employed in the analysis of data from image analysis to correct for the variation resulting from different batches or membranes.

RESULTS

Feeding the DE diet did not affect the body weight of rats but resulted in a slightly but significantly lower feed efficiency than the control (C) group (**Table 2**). Rats fed the HE group diet consumed more feed ($P < 0.05$); nevertheless, the feed efficiency was not different from that of the C. Dietary vitamin E status did not alter the plasma level of albumin, triglyceride, cholesterol and phospholipid (Table 2). However, plasma and

liver α-tocopherol concentration were affected by vitamin E intake (**Table 3**). The α-tocopherol concentration in plasma and liver of the HE group were 1.8- and 14-fold those of the C group ($P < 0.05$). The DE status of the DE group was manifested not only in the diminished α-tocopherol level in plasma and liver but also in the abnormally high activity of pyruvate kinase in plasma ($P < 0.05$). Elevated activity of this muscle enzyme in plasma was used as a biochemical indicator of vitamin E deficiency (Chow 1975, Machlin et al. 1978).

In contrast to results of Experiment 1, feeding a LP diet in Experiment 2 resulted in significantly lower body weight, feed intake, feed efficiency and plasma albumin concentration ($P < 0.05$) (Table 2). Plasma lipids were significantly higher in the LP group compared to the C group ($P < 0.05$) (Table 2), leading to a significantly lower α-tocopherol/triglyceride ratio in plasma of the LP group (**Table 4**). The α-tocopherol concentration was significantly lower in most of the peripheral tissues ($P < 0.05$), including lung, heart, kidney, muscle, brain, spleen and adrenal gland, but not in the liver of the LP group, compared to those of the C group (Table 4).

The anti-α-TTP antibody detected a single band of ~34 kD in the Western Blot Analysis of the liver cytosolic fraction (**Figs. 1***A* and **2***A*). The signals from the DE group and the LP group were relatively weaker than those from the C groups. Results of image analysis showed that DE rats had significantly lower ($P < 0.05$), but the HE group of rats had comparable, α-TTP protein compared to the control (Fig. 1C). The Density × Area of the DE group was ~70% that of the C group in the Experiment 1 ($P < 0.05$) (Fig. 1C). The D × A of the LP group was ~60% that of the C group in the Experiment 2 ($P < 0.05$) (Fig. 2C).

Figs 1B and 2B show the α-TTP and L-ferritin mRNA detected in the liver total RNA. The D × A of each signal was obtained from image analysis, and the ratio of the D × A of α-TTP and L-ferritin mRNA was calculated for each rat. The ratio was not significantly different among the C, HE and DE groups ($P > 0.05$) in Experiment 1 (Fig 1*D*). In contrast, the α-TTP/L-ferritin mRNA ratio of the LP group was 52% that of the C group ($P < 0.05$) (Fig. 2*D*).

TABLE 3

Vitamin E status indices of rats fed a high vitamin E (HE) or deficient vitamin E (DE) or control (C) diet for 12 wk (Expt. 1)[1,2]

Diet groups	C	HE	DE
Plasma			
α-Tocopherol, *μmol/L*	9.82 ± 1.91[b]	17.82 ± 2.94[a]	0.35 ± 0.16[c]
α-Tocopherol/ triglyceride, *μmol/mmol*	27.88 ± 7.98[b]	52.68 ± 11.78[a]	1.04 ± 0.35[c]
Pyruvate kinase, *unit/L*	69 ± 5[b]	68 ± 21[b]	805 ± 336[a]
Liver			
α-Tocopherol, *nmol/g*	27.38 ± 6.32[b]	394.1 ± 152.4[a]	1.47 ± 0.28[c]

[1] Values are means ± SD, $n = 7$.
[2] The significance of differences among the three groups was analyzed by one-way analysis of variance and Duncan's Multiple Range Test. Values not sharing a superscript letter are significantly different ($P < 0.05$).

TABLE 4

Plasma and tissue α-tocopherol concentrations of rats fed a low protein (LP) or control (C) diet for 6 wk (Expt. 2)[1]

Dietary group	C	LP
Plasma		
α-Tocopherol, *μmol/L*	16.39 ± 2.37[2]	17.74 ± 3.53
α-Tocopherol/ triglyceride, *μmol/mmol*	32.55 ± 11.53	14.23 ± 7.86*
Tissue α-Tocopherol, *nmol/g tissue*		
Liver	33.62 ± 7.38	45.69 ± 16.79
Lung	33.88 ± 7.36	19.80 ± 3.66*
Heart	24.56 ± 4.24	11.15 ± 2.10*
Kidney	7.93 ± 1.54	5.81 ± 1.13*
Muscle	8.72 ± 1.28	5.08 ± 0.96*
Testis	27.84 ± 2.11	26.84 ± 2.06
Brain	12.03 ± 1.97	9.33 ± 2.05*
Spleen	25.46 ± 1.79	12.14 ± 2.28*
Adrenal gland	326.0 ± 87.4	235.7 ± 48.8*

[1] Values are means ± SD, $n = 7$.
[2] The significance of differences between the two groups was analyzed by Student's *t* test. Values with an asterisk (*) are significantly different from the C group ($P < 0.05$).

DISCUSSION

The sequence of the α-TTP cDNA prepared by RT-PCR in this study showed 97% homology with that reported by Sato et al. (1993). Using this cDNA as a template, a labeled cRNA probe was synthesized by using an in vitro transcription system. The band of the mRNA detected by this probe resided between the 18S and 28S RNA bands after electrophoresis. This is in agreement with the report of Sato et al. (1993) in which they demonstrated a 2.4-kb message located between 28S and 18S RNA detected by the α-TTP cDNA probe.

Perozzi et al. (1989) found that the mRNA of albumin and transthyretin were significantly reduced in the liver of rats fed a protein-deficient diet. In contrast, the mRNA of retinol-binding protein, actin and L-ferritin was unaffected. Consequently, they used the L-ferritin mRNA as an internal control. Based on this report, we also employed the L-ferritin mRNA as an internal control. Like albumin and transthyretin, the ratio of α-TTP/L-ferritin mRNA was significantly reduced in the liver of rats fed a LP diet. In parallel, the immunoreactive α-TTP level was also lowered in the LP status. The results suggested that the expression of the α-TTP gene might be downregulated by protein malnutrition.

How protein deprivation alters gene expression remains unclear. Ogawa et al. (1997) compared liver-specific nuclear transcription factors, including C/EBPα, C/EBPβ, D-site-binding protein as well as HNF-1 (hepatocyte nuclear factor 1) of protein-deprived and control rats, but did not find significant differences between the two groups. On the other hand, carbohydrate is usually employed to replace the protein source in formulating a LP or protein-free diet. Dietary carbohydrate opposed the effect of protein or amino acids, directly or indirectly through the hormone responses it induces (Chan and Hargrove 1993).

Kayden and Traber hypothesized (1993) that α-TTP mediates the intracellular transport of α-tocopherol taken up by the liver cells into nascent VLDL for secretion. Data supporting the hypothesis were reported in recent years. For example,

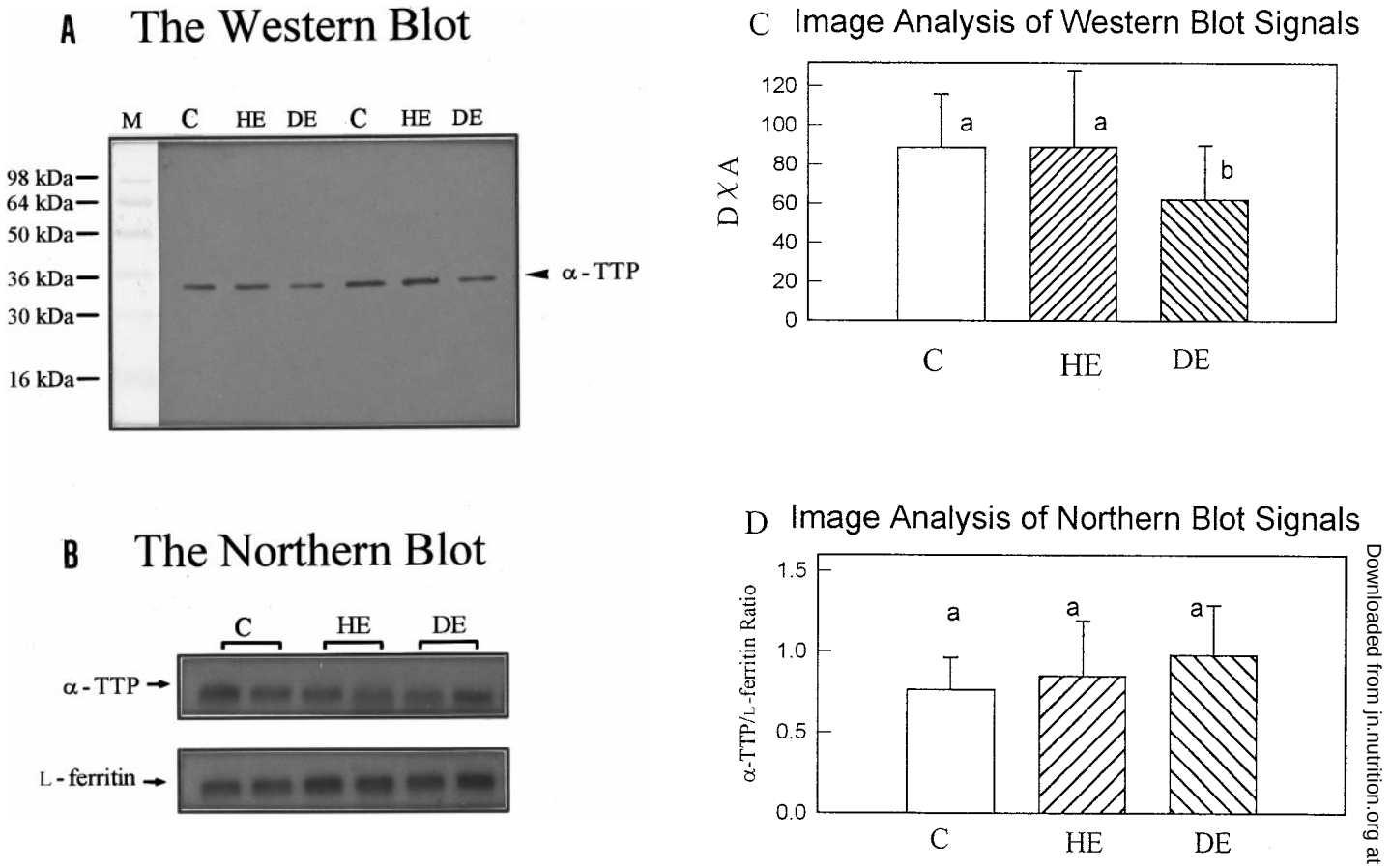

FIGURE 1 Western (A) and Northern (B) Blot Analyses of liver α-tocopherol transfer protein (α-TTP) and its mRNA of rats in Experiment 1 fed a high (HE) vitamin E diet, a vitamin E-deficient (DE) diet or a control (C) diet for 12 wk. In (A), the liver cytosolic fraction (10 μg protein) from each rat was loaded and separated by SDS-polyacrylamide gel electrophoresis, transferred to PVDF-plus transfer membrane, immunostained with anti α-TTP as the primary antibody, rabbit-antimouse IgG-alkaline phosphatase conjugate as the secondary antibody. Positive signal was visualized by reaction with CSPD (Tropix, Bedford, MA) and the membrane exposed to a film. The α-TTP was quantitated by microcomputer imaging device (MCID) (Fuji Co., Tokyo, Japan) image analysis, and data were presented as means ± SD for seven rats in each group and shown in (C). In (B), liver total RNA (10 μg) from each rat was separated by electrophoresis in denaturing formaldehyde agarose (1%) gel, transferred to nylon membrane, hybridized at 65°C for 12–15 h with ^{32}P-labeled cRNA probe specific for rat α-TTP. Membranes were washed and exposed to X-OMAT AR (Kodak, Rochester, NY) film. Blots were deprobed and hybridized with ^{32}P-labeled cRNA probe-specific for rat L-ferritin as a control. The films were analyzed by MCID image analysis system for Density and Area. The D × A (Density × Area) of α-TTP mRNA and of L-ferritin mRNA for each sample was recorded and the ratio of the D × A for α-TTP mRNA and for L-ferritin was calculated for each sample. The ratio of D × A for mRNA of α-TTP and of L-ferritin was presented as means ± SD for seven rats in each group and shown in (D). The significance of differences among C, HE and DE groups were analyzed by one-way analysis of variance and Duncan's multiple range test. Values not sharing a superscript letter are significantly different ($P < 0.05$).

a positive correlation exists between the liver α-TTP mRNA and serum α-tocopherol in rats during development after birth (Kim et al. 1996) and during hepatocarcinogenesis (Wu et al. 1997). Overexpression of α-TTP by transfection enhanced α-tocopherol secretion (Arita et al. 1997). Patients with DE neuropathy have mutations in the α-TTP gene (Gotoda et al. 1995, Hentati et al. 1996, Ouahchi et al. 1995, Tamaru et al. 1997, Yokota et al. 1997).

Using cannulated and Triton-WR1339-treated rats, we showed that the secretion rate of α-tocopherol from liver of rats fed a LP diet is significantly lower than the C rats (Shaw and Huang, unpublished data). The lower expression of the α-TTP gene observed in this study can be attributed to the decreased secretion rate of α-tocopherol from liver of protein-malnourished rats, which can also explain the lowered α-tocopherol concentration in the peripheral tissues but not in the liver (Huang and Shaw 1994). Therefore, results of this study also support the proposed physiological role of α-TTP in the liver.

Modulation of the dietary vitamin E intake did not significantly affect the liver α-TTP mRNA level. This implies that the transcription of the α-TTP gene probably is not affected by dietary vitamin E. However, the α-TTP protein level was significantly lower than control in the liver of the DE rats. When the translation process is decreased and/or the degradation of the protein is increased, a reduced protein level can result. A recent report demonstrated that ligand binding could induce conformational change and render the receptor protein more resistant to protease digestion (Dowell et al. 1997). We speculate that the lack of ligand (α-tocopherol) in DE rats may render α-TTP more susceptible to degradation.

The dietary vitamin E, liver and plasma α-tocopherol concentrations of the HE group were, respectively, 100-, 14.4- and 1.8-fold those of the C group. The smaller difference in the plasma α-tocopherol level between the two groups is in accordance with the α-TTP expression. The available α-TTP seems to be a limiting factor for the export of α-tocopherol from the liver to blood, which further supports the proposed physiological function of α-TTP.

In conclusion, the results of this study suggest that the

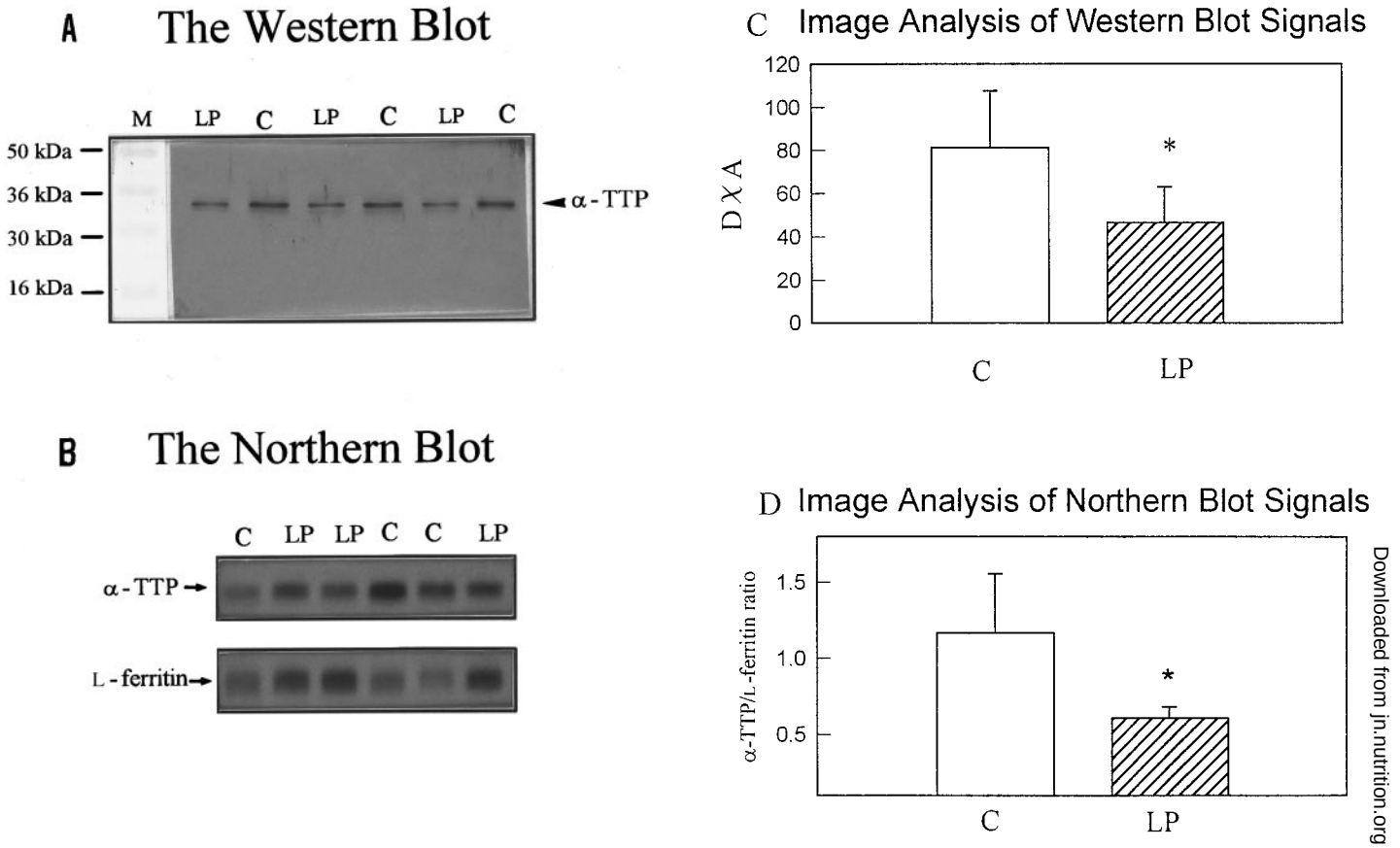

FIGURE 2 Western (*A*) and Northern (*B*) Blot Analyses of liver α-tocopherol transfer protein (α-TTP) and its mRNA of rats in Experiment 2. Rats were fed a low protein diet (LP) or a control diet (C) for 6 wk. In (A), the liver cytosolic fraction (10 μg protein) from each rat was loaded and separated by SDS-PAGE, transferred to PVDF-plus transfer membrane, immunostained with anti-α-TTP as the primary antibody, rabbit-anti-mouse IgG-alkaline phosphatase conjugate as the secondary antibody. Positive signal was visualized by reaction with CSPD (Tropix; Bedford, MA) and the membrane exposed to a film. The α-TTP was quantitated by microcomputer imaging device (MCID) image analysis and data were presented as means ± SD for seven rats in each group and shown in (*C*). In (*B*), liver total RNA (10 μg) from each rat was separated by electrophoresis in denaturing formaldehyde agarose (1%) gel, transferred to nylon membrane, hybridized at 65°C for 12–15 h with ^{32}P-labeled cRNA probe-specific for rat α-TTP. Membranes were washed and exposed to X-OMAT AR film (Kodak, Rochester, NY). Blots were deprobed and hybridized with ^{32}P-labeled cRNA probe-specific for rat L-ferritin as a control. The films were analyzed by MCID image analysis system for Density and Area. The D × A (Density × Area) of α-TTP mRNA and of L-ferritin mRNA for each sample were recorded and the ratio of the D × A for α-TTP mRNA and for L-ferritin was calculated for each sample. The ratio of D × A for mRNA of α-TTP and of L-ferritin was presented as means ± SD for seven rats in each group and shown in (*D*). The significance of differences between C and LP groups were analyzed by Student's *t* test. Values with an asterisk (*) are significantly different from the control (C) group ($p < 0.05$).

expression of liver α-TTP may be down-regulated during protein malnutrition. However, the transcription seemed not to be affected by dietary vitamin E intake, but the α-TTP protein level was significantly lowered in vitamin E deficiency.

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LITERATURE CITED

AIN (1977) Response of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. J. Nutr. 107: 1340–1348.

Arita, M., Nomura, K., Arai, H. & Inoue, K. (1997) α-Tocopherol transfer protein stimulates the secretion of α-tocopherol from a cultured liver cell line through a brefeldin A-insensitive pathway. Proc. Natl. Acad. Sci. U.S.A. 94: 12437–12441.

Arita, M., Sato, Y., Miyata, A., Tanabe, T., Takahashi, E., Kayden, H. J., Arai, H. & Inoue, K. (1995) Human alpha-tocopherol transfer protein: cDNA cloning, expression and chromosomal localization. Biochem. J. 306: 437–443.

Behrens, W. A. & Madere, R. (1982) Transfer of α-tocopherol to microsomes mediated by a partially purified liver α-tocopherol binding protein. Nutr. Res. 2: 611–618.

Brown, A. J., Leibold, E. A. & Munro, H. N. (1983) Isolation of cDNA clones for the light subunit of rat liver ferritin: evidence that the light subunit is encoded by a multigene family. Proc. Natl. Acad. Sci. U.S.A. 80: 1265–1269.

Bucher, T. & Pfleiderer, G. (1955) Pyruvate kinase from muscle. Methods Enzymol. 1: 435–440.

Burton, G. W. & Traber, M. G. (1990) Vitamin E: antioxiant activity, biokinetics, and bioavailability. Annu. Rev. Nutr. 10: 357–382.

Catignani, G. L. & Bieri, J. G. (1977) Rat liver α-tocopherol binding protein. Biochim. Biophys. Acta 497: 349–357.

Chan, D.K.C. & Hargrove, J. L. (1993) Effects of dietary protein on gene expression. In: Nutrition and Gene Expression. (Berdanier, C. D. & Hargrove, J. L., eds.) pp. 353–375, CRC Press, London.

Chomczynski, P. & Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162: 156–159.

Chow, C. K. (1975) Increased activity of pyruvate kinase in plasma of vitamin E-deficient rats. J. Nutr. 105: 1221–1224.

Doumas, B. T., Watson, W. A. & Biggs, H. G. (1971) Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chim. Acta. 31: 87–96.

Dowell, P., Peterson, V. J., Zabriskie, T. M. & Leid, M. (1997) Ligand-induced

peroxisome proliferator-activated receptor α conformational change. J. Biol. Chem. 272: 2013–2020.
Gotoda, T., Arita, M., Arai, H., Inoue, K., Yokota, T., Fukuo, Y., Yazaki, Y. & Yamada, N. (1995) Adult-onset spinocerebellar dysfunction caused by a mutation in the gene for the α-tocopherol transfer protein. N. Engl. J. Med. 333: 1313–1318.
Hentati, A., Deng, H., Hung, W., Nayer, M., Ahmed, M. S., He, X., Tim, R. & Stumpf, D. A. (1996) Human α-tocopherol transfer protein: gene structure and mutations in familial vitamin E deficiency. Ann. Neurol. 39: 295–300.
Hosomi, A., Arita, M., Sato, Y., Kiyose, C., Ueda, T., Igarashi, O., Arai, H. & Inoue, K. (1997) Affinity for α-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. FEBS Lett. 409: 105–108.
Huang, C.-J. & Shaw, H. M. (1994) Tissue vitamin E status is compromised by dietary protein insufficiency in young growing rats. J. Nutr. 124: 571–579.
Kaplowitz, N., Yoshida, N., Kuhlenkamp, J., Slitsky, B., Ren, I. & Stolz, A. (1989) Tocopherol-binding proteins of hepatic cytosol. Ann. N.Y. Acad. Sci. 570: 85–94.
Kayden, H. J., Sokol, R. J. & Traber, M. G. (1987) Familial vitamin E deficiency with neurologic abnormalities in the absence of fat malabsorption. In: Clinical and Nutritional Aspects of Vitamin E. Hayaishi, O. & Mino, M., eds. pp. 193–197, Elsevier, Amsterdam.
Kayden, H. J. & Traber, M. G. (1993) Absorption, lipoprotein transport, and regulation of plasma concentration of vitamin E in human. J. Lipids Res. 34: 343–358.
Kim, H. S., Arai, H., Arita, M., Sato, Y., Ogihara, T., Tamai, H., Inoue, K. & Mine, M. (1996) Age-related changes of α-tocopherol transfer protein expression in rat liver. J. Nutr. Sci. Vitaminol. 42: 11–18.
Kuhlenkamp, J., Ronk, M., Yusin, M., Stolz, A. & Kaplowitz, N. (1993) Identification and purification of a human liver cytosolic tocopherol binding protein. Protein Expression Purification, 4: 382–389.
Machlin, L. J., Gabriel, E., Spiegel, H. E., Horu, L. R., Brin, M. & Nelson, J. (1978) Plasma activity of pyruvate kinase as indices of myopathy in the vitamin E-deficient rats. J. Nutr. 108: 1963–1968.
Mohri, K., Dohmoto, C., Ikesu, H. & Igarasshi, O. (1983) A simple elimination method of vitamin E from vegetable and fish oils for the preparation of vitamin E deficient diet. J. Jpn. Soc. Nutr. Food Sci. 36: 122–124 (In Japanese).
National Research Council (1985) Guide for the care and use of laboratory animals, Publication No. 85-23 (rev.), National Institute of Health, Bethesda, MD.
Ogawa, A., Yano, M., Tsujinaka, T., Ebisui, C., Morimoto, T., Kishibuchi, M., Fujita, J., Morita, S., Shiozaki, H. & Monden, M. (1997) Gene expression of albumin and liver-specific nuclear transcription factors in liver of protein-deprived rats. J. Nutr. 127: 1328–1332.
Ouahchi, K., Arita, M., Kayden, H., Hentati, F., Hamida, M. B., Sokol, R., Arai, H., Inoue, K., Mandel, J. and Koenig, M. (1995) Ataxia with isolated vitamin E deficiency is caused by mutations in the α-tocopherol transfer protein. Nature Genetics. 9: 141–145.
Perozzi, G., Mengheri, E., Faraonio, R. & Gaetani, S. (1989) Expression of liver-specific genes coding for plasma proteins in protein deficiency. FEBS Lett. 257: 215–218.
Sato, Y., Arai, H., Miyata, A., Tokita, S., Yamamoto, K., Tanabe, T. & Inoue, K. (1993) Primary structure of a α-tocopherol transfer protein from rat liver, homology with cellular retinaldehyde-binding protein. J. Biol. Chem. 268: 17705–17710.
Sato, Y., Hagiwara, K., Arai, H. & Inoue, K. (1991) Purification and characterization of the α-tocopherol transfer protein from rat liver. FEBS Lett. 288: 41–45.
Tamaru, Y., Hirano, M., Kusaka, H., Ito, H., Imai, T. & Ueno, S. (1997) α-Tocopherol transfer protein gene: exon skipping of all transcripts causes ataxia. Neurology 49: 584–588.
Traber, M. G., Burton, G. W., Ingold, K. U. & Kayden, H. J. (1990) RRR- and SRR-α-tocopherols are secreted without discrimination in human chylomicrons, but RRR-α-tocopherol is preferentially secreted in very low density lipoproteins. J. Lipid. Res. 31: 675–685.
Traber, M. G., Rudel, L. L., Burton, G. W., Hughes, L., Ingold, K. U. & Kayden, H. J. (1990) Nascent VLDL from liver perfusions of cynomolgus monkeys are preferentially enriched in RRR- compared with SRR-α-tocopherol: studies using deuterated tocopherols. J. Lipid. Res. 31: 687–694.
Traber, M. G., Sokol, R. J., Kohlschutter, A., Yokota, T., Muller, D. P., Dufour, R. & Kayden, H. J. (1993) Impaired discrimination between stereoisomers of alpha-tocopherol in patients with familial isolated vitamin E deficiency. J. Lipid. Res. 34: 201–210.
Wu, C. G., Hoek, F. J., Groenink, M., Reitsma, P. H., Deventer, S.J.H. & Chamuleau, R.A.F.M. (1997) Correlation of repressed transcription of α-tocopherol transfer protein with serum α-tocopherol during hepatocarcinogenesis. Int. J. Cancer 71: 686–690.
Yokota, T., Shiojiri, T., Gotoda, T., Arita, M., Arai, H., Ohga, T., Kanda, T., Suzuki, J., Imai, T., Matsumoto, H., Harino, S., Kiyosawa, M., Mizusawa, H. & Inoue, K. (1997) Friedreich-like ataxia with retinitis pigmentosa caused by the His^{101} Gln mutation of the α-tocopherol transfer protein gene. Ann. Neurol. 41: 826–832.
Yoshida, H., Yusin, M., Ren, I., Kuhlenkamp, J., Hirano, T., Stolz, A. and Kaplowitz, N. (1992) Identification, purification, and immunochemical characterization of α-tocopherol-binding protein in rat liver cytosol. J. Lipid Res. 33: 343–350.