ARTICLES

Regulation of the α-Tocopherol Transfer Protein in Mice: Lack of Response to Dietary Vitamin E or Oxidative Stress

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ABSTRACT: The α-tocopherol transfer protein (TTP) plays an important role in the regulation of plasma α-tocopherol concentrations. We hypothesized that hepatic TTP levels would be modulated by dietary vitamin E supplementation and/or by oxidative stress. Mice were fed either a High E (1150 mg *RRR*-α-tocopheryl acetate/kg diet) or a Low E (11.5 mg/kg diet) diet for 2 wk. High E increased plasma and liver α-tocopherol concentrations approximately 8- and 40-fold, respectively, compared with Low E-fed mice, whereas hepatic TTP increased approximately 20%. Hepatic TTP concentrations were unaffected by fasting (24 h) in mice fed either diet. To induce oxidative stress, chow-fed mice were exposed for 3 d to environmental tobacco smoke (ETS) for 6 h/d (total suspended particulate, 57.4 ± 1.8 mg/m3). ETS exposure, while resulting in pulmonary and systemic oxidative stress, had no effect on hepatic α-tocopherol concentrations or hepatic TTP. Overall, changes in hepatic TTP concentrations were minimal in response to dietary vitamin E levels or ETS-related oxidative stress. Thus, hepatic TTP concentrations may be at sufficient levels such that they are unaffected by either modulations of dietary vitamin E or by the conditions of environmentally related oxidative stress used in the present studies.

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The α-tocopherol transfer protein (TTP) is a 33-kDa protein that is predominantly expressed in the mammalian liver (1) but which has also been detected in the rat brain, spleen, lung, and kidney (2); in the human brain (3); and in mouse liver, brain (4,5); and uteri (6). Based on sequence analysis, TTP is classified as a member of the SEC14-like protein family, with a characteristic CRAL_TRIO lipid-binding domain (7,8). In the closed TTP crystal structure, a mobile helical surface segment seals the hydrophobic binding pocket (9,10) and is responsible for the TTP selectivity for α-tocopherol (11).

Mutations in the TTP gene in humans [ataxia with vitamin E deficiency; (12)] are associated with extraordinarily low plasma E concentrations (13). In addition, in TTP-knockout and heterozygote mice, plasma α-tocopherol concentrations in plasma are 5 and 50% those of the wild-type mice, respectively (14,15). Clearly, TTP plays an important role in the regulation of plasma α-tocopherol concentrations. However, studies conducted on the regulation of TTP in response to dietary vitamin E intake have yielded conflicting results.

Kim *et al.* (16) showed that levels of rat hepatic TTP mRNA and protein were lower in response to high levels of dietary vitamin E (600 mg DL-α-tocopherol/kg diet) compared with rats fed the control diet (50 mg DL-α-tocopherol/kg diet). In rats that were fed vitamin E-deficient diets, TTP mRNA was higher, but protein levels were unchanged compared with controls. In contrast, Shaw and Huang (17) showed that in vitamin E-deficient rats, hepatic TTP was lower compared with rats fed control diets (50 mg all-*rac*-α-tocopheryl acetate/kg diet) but control and vitamin E-supplemented (5,000 mg all-*rac*-α-tocopheryl acetate/kg diet) rats had similar TTP concentrations. No changes in TTP mRNA were observed. Last, Fechner *et al.* (18) showed that rat hepatic TTP mRNA levels were unchanged by dietary vitamin E depletion and fasting (24 h). However, refeeding α-tocopherol to vitamin E-depleted rats followed by an additional 24-h fast increased the expression of TTP mRNA about sevenfold; however, TTP protein levels were not measured. Since α-tocopherol is secreted from the liver and associated with plasma VLDL (19), fasting, which is known to be associated with low levels of VLDL, might be expected to be accompanied by reduced liver cell release of α-tocopherol to plasma and modulations of TTP levels. Overall, no consistent changes in TTP protein in response to different dietary levels have been reported. However, oxidative stress has been suggested to increase hepatic TPP (20).

Environmental tobacco smoke (ETS) contains highly reactive components, many of which cause oxidative stress and activate host inflammatory-immune processes (21,22). Exposure of plasma to gas-phase cigarette smoke *in vitro* causes depletion of antioxidants such as vitamins C and E (23). Since smokers have a higher rate of vitamin E disappearance (24,25) despite maintaining normal or somewhat depleted plasma α-tocopherol concentrations (26–30), we hypothesized that the oxidative stress of cigarette smoke exposure might increase hepatic TTP concentrations. The purpose of the present study was to evaluate whether hepatic TTP in mice is regulated in response to variations in dietary vitamin E, fasting, or ETS exposure.

The first and second investigators contributed equally to this work.

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Abbreviations: α-CEHC, α-carboxyethyl hydroxychroman; DNPH, dinitrophenylhydrazine; ETS, environmental tobacco smoke; 4-HNE, 4-hydroxy-2-nonenal; TSP, total suspended particulates; TTP, α-tocopherol transfer protein.

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EXPERIMENTAL PROCEDURES

TTP regulation by dietary vitamin E: Animals and dietary treatment. The protocols for the care and use of animals were approved by the Institutional Care and Use Committee at Oregon State University or at the University of California at Davis. For the dietary experiments, male C57BL/6 mice that weighed ~20–22 g (~7 wk of age) were purchased from Simonsen Laboratories, Inc. (Gilroy, CA). Mice were housed individually in stainless-steel mesh cages in a room maintained at 22°C and 40–60% humidity. Animals were weighed and then randomly assigned to the High E (n = 6) or Low E (n = 7) diet, respectively (diet study 1). Mice had free access to diet and water throughout the experiments. Animal weight and diet consumption was recorded twice per week. On day 15, mice were anesthetized by CO_2 inhalation. Blood was drawn from the heart into a 2-mL purple-topped vacutainer tube (1 mg/mL EDTA), mixed, separated by centrifugation, and the plasma was stored at −80°C. The liver was rinsed with ice-cold saline, blotted, weighed, immediately frozen in liquid nitrogen, and stored at −80°C. α-Tocopherol was extracted from the plasma and liver and analyzed as described by Podda *et al.* (31). The same protocol was followed for a second group of animals (High E: n = 6, and Low E: n = 6) except that half (n = 3) of the animals in each diet group were fasted for 24 h prior to sacrifice (diet study 2).

The diet composition for the two diet studies is given in Table 1. Diets were based on AIN 93M (32) and were prepared to contain *RRR*-α-tocopheryl acetate (ADM Nutraceuticals, Decatur, IL), either 11.5 mg *RRR*-α-tocopheryl acetate/kg diet (Low E) or 1150 mg *RRR*-α-tocopheryl acetate/kg diet (High E). The diet composition was analyzed in our laboratory to confirm the dietary vitamin E contents. The Low E diet contained half of the recommended vitamin E for mice [22 mg α-tocopheryl acetate per kg diet (33). The Low E diet provided the animals with approximately 2.3 mg of *RRR*-α-tocopheryl acetate/d. This diet was designed to provide the equivalent of approximately 100 mg *RRR*-α-tocopherol per day for a 70-kg human (based on an average mouse intake of 4 g diet per day and an average weight of 20 g).

TABLE 1
Composition of Low and High E Diets[a]

Ingredients	Experimental diets (g per kg diet) Low E	High E
Vitamin-free casein	140	140
L-Cystine	1.8	1.8
Cornstarch	455.9	454.7
Maltodextrin	155	155
Sucrose	109.8	109.8
Cellulose	50	50
Tocopherol-stripped corn oil	40	40
RRR-α-tocopheryl acetate in tocopherol-stripped corn oil	0.012	1.2
Mineral mix (AIN-93M-MX)	35	35
Vitamin mix[b] (AIN-93M-VX)	10	10
Choline bitartrate	2.5	2.5

[a]Diets were prepared by Harlan Teklad (Madison, WI).
[b]Vitamin E was excluded from the vitamin mix and replaced with sucrose.

TABLE 2
Composition of Diet[a] Fed to Environmental Tobacco Exposed (ETS) Mice

Ingredients	g per kg diet
Protein	180
L-Cystine	25
Fat (ether extract)	60
Fibre (crude)	50
Mineral (Ash)	80
Vitamin E (IU/kg)	45

[a]Commercially available through Purina Lab Diet, diet #5K52 (www.labdiet.com)

TTP regulation by oxidative stress. (i) Animals and experimental treatment. C57BL6 mice (~12 wk of age) were exposed to ETS (ETS: n = 8; filtered air: n = 8, n indicated in the figure legends; insufficient sample was available for use in all assays) for 3 consecutive days. Mice exposed to ETS were purchased from Jackson Laboratory (Bar Harbor, ME). They were fed the vendor's diet (Purina 5K52; LabDiet, Richmond, IN) containing 45 IU/kg α-tocopherol (Table 2). At the end of the experimental treatment (day 3), animals were anesthetized with an intraperitoneal injection of 120 mg sodium pentobarbital/kg body weight. Sample collection, storage, and analysis were conducted as described for the diet studies.

(ii) Exposure of mice to cigarette smoke. Exposures of mice to ETS were conducted at the Institute of Toxicology and Environmental Health, University of California at Davis. The exposure system and methods used to generate ETS have been described elsewhere (34,35). Briefly, mice were exposed to a mixture of mainstream and sidestream cigarette smoke (aged and diluted) from 1R4F reference research cigarettes (1.2 mg nicotine/cigarette) obtained from the Tobacco and Health Research Institute of the University of Kentucky. An automatic cigarette-smoking machine generated cigarette smoke in a staggered manner at the rate of a 35-mL puff over a 2-s duration each min. During this period of "passive" smoking, mice were exposed to total suspended particulates (TSP, 57.4 ± 1.8 mg/m^3), carbon monoxide (262 ± 6 ppm), and nicotine (10.5 ± 1.0 mg/m^3) for 6 h/d × 3 d.

Western blot analysis for TTP and 4-hydroxy-2-nonenal (4-HNE). To obtain the soluble liver fraction for Western blot analysis, a portion of liver (frozen in liquid nitrogen on the day of sacrifice) from each animal was homogenized in PBS (0.1 M phosphate buffer, 0.0027 M KCl, and 0.137 M NaCl, pH 7.4) (20%, wt/vol). The homogenate was centrifuged at 20,000 × g for 30 min at 4°C and the resulting low-speed supernatant was centrifuged at 100,000 × g for 60 min at 4°C to obtain the high-speed supernatant. The high-speed supernatant was stored at −80°C until Western blot analyses were performed. The protein concentration in the liver supernatant samples was determined by a commercially available kit (Bio-Rad Protein Assay, Bio-Rad Laboratories, Hercules, CA).

Rabbit antirat TTP serum was a gift from Dr. Robert Farese (Gladstone Institute of Cardiovascular Disease and the

Cardiovascular Research Institute, Department of Medicine, University of California at San Francisco, San Francisco, CA). The polyclonal antibody was raised against a peptide of the C-terminus 1–21 amino acids of rat TTP and cross-reacted with mouse liver TTP (14). Proteins (40 mg per lane for ETS or 100 mg per lane for diet studies) were then subjected to electrophoretic separation under denaturing and reducing conditions and transferred onto a nitrocellulose membrane. Immunoreactive protein was detected by chemiluminescence by using goat antirabbit IgG conjugated to horseradish peroxidase (Bio-Rad, Hercules, CA) with exposure to Kodak X-OMAT XRP film (Rochester, NY). Quantitative densitometric analysis of the image was performed using an AlphaImager™ 2000 Documentation and Analysis System (Alpha Innotech Corporation, San Leandro, CA). The O.D. value represents the sum of all pixels minus the background within the specified area. For all experiments, a single band was detected for TTP corresponding to 32 kDa. This M.W. is consistent with the published apparent M.W. for mouse TTP (14).

Lipid oxidation and environmental pollutants result in the production of unsaturated aldehydes such as 4-HNE. Lung and liver homogenates from ETS and air-exposed animals were separated by electrophoresis (10% SDS-gel, reducing conditions) and after transfer onto polyvinylidene difluoride membrane probed with rabbit anti4-HNE antibody (Oxis International Inc., Portland, OR, 1:1000). Immunoreactive protein was visualized as described above.

Protein carbonyls in bronchoalveolar lavage fluid. Oxidative modification of proteins in mouse lung lavage was determined using an ELISA method described previously (36). Briefly, after derivatization of carbonyl groups with dinitrophenylhydrazine (DNPH), proteins were adsorbed onto 96-well ELISA plates, captured with a commercially available anti-DNPH antibody, and detected with a horseradish peroxidase /hydrogen peroxide, phenylenediamine system (37).

Statistical analysis. Data are expressed as mean ± SD and were analyzed for statistical significance by Student's *t*-test, or ANOVA and Fisher's LSD using StatView 5.0 (SAS Institute, Inc., Cary, NC). Liver α-tocopherol concentrations were log transformed to normalize the variances between groups for the two diet studies. Differences were considered significant at $P < 0.05$.

RESULTS

Growth and food intake. In the diet studies, final body weights, weight gains (g/d), and diet consumed (g/d) were similar for all animals, except for the weight gain in diet study 1 (Table 3). Mice fed the High E diet gained more weight than those fed the Low E diet ($P = 0.02$) because of an initial low weight gain in one animal in the Low E group. Weight gain for this animal was similar to that of the other mice at the time of sample collection. In mice fasted for 24 h, livers weighed more in nonfasted than in fasted mice, irrespective of whether the mice were fed the High E ($P = 0.0013$) or Low E diet ($P = 0.0004$).

Plasma and hepatic vitamin E and TTP levels. (i) Responses to dietary vitamin E. Mice fed the High E diet had hepatic α-tocopherol concentrations ~40 times higher ($P < 0.0001$) and plasma α-tocopherol concentrations ~8 times higher ($P < 0.0001$) than those of mice fed the Low E diet (Figs. 1, 2). In the animals fed the Low E diet that were fasted for 24 h prior to sacrifice, no effect of fasting was observed on either plasma or hepatic α-tocopherol concentrations (Fig. 2). However, in mice fed the High E diet, fasting significantly decreased hepatic ($P = 0.003$) and plasma α-tocopherol concentrations ($P = 0.0003$) (Fig. 2).

In contrast to the 40-fold increase in hepatic α-tocopherol, mice fed the High E diets had hepatic TTP levels that were only 20 ($P = 0.02$) and 25% ($P = 0.002$) higher compared with those fed the Low E diet in studies 1 and 2, respectively (Figs. 1, 2). Hepatic TTP levels were unaffected by fasting in mice fed either the High E or Low E diet (Fig. 2).

(ii) Responses to oxidative stress. Exposure of mice to ETS for 3 d caused increased oxidative stress to proteins and lipids. Figure 3A shows increased formation of protein carbonyls in the lung lavage of animals exposed to ETS ($P = 0.007$). These results and the greater amount of 4-HNE adducts in lung tissue with ETS exposure vs. air ($P < 0.01$) (Fig. 3B) demonstrate pulmonary oxidative stress. The increase in lung 4-HNE in ETS-exposed mice seems to be transient, as adduct formation returned to control levels on day 3. Exposure to ETS also caused a significant increase in 4-HNE in liver ($P = 0.005$), indicative of systemic oxidative stress. Furthermore, exposure to ETS was associated with a 40% decrease in the lung α-tocopherol concentration ($P = 0.001$; Fig. 4A). However, hepatic α-tocopherol concentrations (Fig. 4B) and TTP levels were unaffected by ETS exposure (Fig. 4C).

TABLE 3
Growth and Dietary Intake Parameters of Mice Fed Low and High E Diets[a]

	Experiment 1		Experiment 2			
			Low E		High E	
Diet	Low E	High E	Fasted	Nonfasted	Fasted	Nonfasted
Final weight (g)	23.1 ± 1.1[#]	24.8 ± 1.2*	24.4 ± 1.3	23.4 ± 1.3	25.5 ± 0.8	25.9 ± 2.2
Liver weight (g)	1.5 ± 0.1	1.6 ± 0.2	0.9 ± 0.2[§]	1.4 ± 0.0[†]	0.9 ± 0.1[§]	1.5 ± 0.1[†]
Gain/d (g)	0.2 ± 0.03	0.2 ± 0.03	0.1 ± 0.02	0.1 ± 0.01	0.2 ± 0.05	0.2 ± 0.10
Diet/d (g)	3.7 ± 0.3	3.9 ± 0.2	3.8 ± 0.2	3.8 ± 0.2	3.9 ± 0.2	3.7 ± 0.2

[a]Values are means ± SD for High E ($n = 6$) and Low E ($n = 7$). For experiment 1, the final weight was significantly greater in mice fed the High E (*) vs. the Low E (#) diet ($P = 0.02$). For experiment 2 ($n = 3$ for each treatment), in mice fasted for 24 h, liver weights were significantly higher for nonfasted (†) compared with fasted mice [High E: $P = 0.0013$ (§); Low E: $P = 0.0004$ (§)] by ANOVA and Fisher's LSD test.

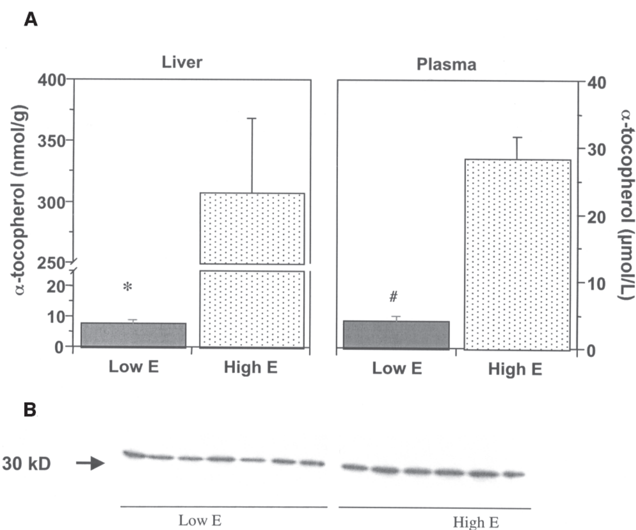

FIG. 1. Plasma and hepatic vitamin E levels and α-tocopherol transfer protein (TTP) concentrations in response to dietary vitamin E (A) Mice fed High E ($n = 6$) compared with Low E ($n = 7$) diets showed higher hepatic (*) and plasma (#) α-tocopherol concentrations, respectively (means ± SD, $P \leq 0.0001$). (B) Western blots of hepatic TTP from the livers of mice described in (A). Hepatic TTP levels were 20% higher ($P = 0.02$) in mice fed the High E diets (30,864 ± 5,199 pixels/mm^2) compared with those fed the Low E diet (24,570 ± 3,239 pixels/mm^2).

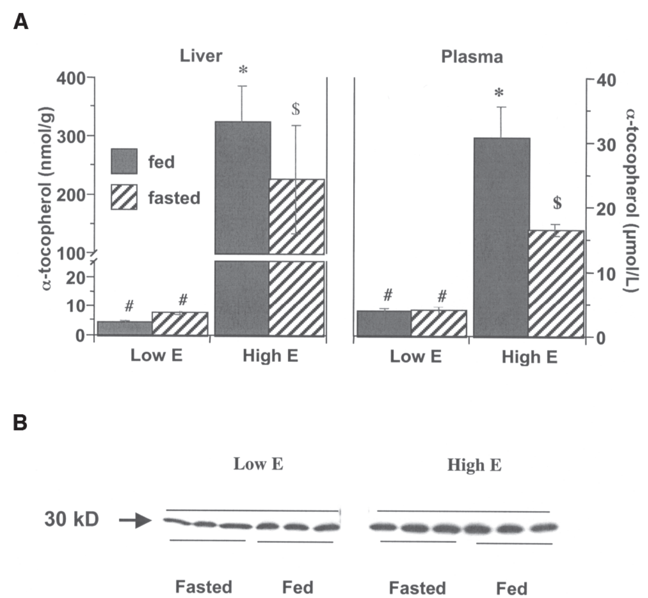

FIG. 2. Effect of fasting on plasma and hepatic vitamin E levels and TTP protein concentrations. (A) Hepatic and plasma α-tocopherol concentrations in mice (means ± SD, $n = 3$ for each of the four treatments) fed Low or High E diets followed by a 24-h fast or nonfast. Hepatic and plasma α-tocopherol concentrations were significantly lower in the Low E (#) compared with the High E diet-fed animals ($P < 0.0001$, diet effect) (*,$). Mice fed the High E diet that were fasted had significantly lower hepatic and plasma α-tocopherol concentrations than nonfasted mice [$P = 0.003$ for hepatic (* vs. $), and $P = 0.0003$ for plasma α-tocopherol (* vs. $), ANOVA and Fisher's LSD test]. (B) Western blots of hepatic TTP from the livers of mice described in (A). Hepatic TTP levels were 25% higher ($P = 0.002$) in mice fed the High E diets (40,404 ± 3,920) compared with those fed the Low E diet (29,545 ± 4,431). Hepatic TTP levels were unaffected by fasting. For abbreviation see Figure 1.

DISCUSSION

Hepatic TTP levels in mice were approximately 20% higher in response to the 100-fold greater dietary α-tocopherol levels in the High E compared with the Low E diet. This relatively small increase in TTP occurred despite a 40-fold increase in hepatic α-tocopherol in mice fed the High E diet. Plasma α-tocopherol levels increased only eightfold in the nonfasted mice fed the High E compared with the Low E diet (Figs. 1, 2). Clearly, plasma α-tocopherol concentrations do not increase to the extent they increase in the liver in response to dietary vitamin E. These data suggest that although liver α-tocopherol increased, plasma α-tocopherol levels were limited by the marginal increase in hepatic TTP and/or by the capabilities of liver-secreted lipoproteins to accept additional amounts of α-tocopherol (38).

Hepatic TTP levels were 20 and 25% higher in diet studies 1 and 2, respectively, in mice fed the High E diet compared with those fed the Low E diet. Although the levels of dietary vitamin E in our study were not identical to those used by Shaw and Huang (17), the direction and magnitude of changes in hepatic TTP levels were similar between the two studies. The authors (17) observed that TTP levels were *ca.* 25% higher in mice fed vitamin E-containing diets (for both their control and High E diets) compared with those fed a vitamin E-deficient diet, whereas no changes were observed in TTP mRNA levels (17). Using gene chip microarrays, Barella *et al.* (39) have also found a lack of significant difference in hepatic TTP mRNA expression in rats fed vitamin E-sufficient vs. vitamin E-deficient diets. Although Kim *et al.* (16) used diets and an animal species (rat) similar to those used by Shaw and Huang (17), the responses to vitamin E deficiency and supplementation did not agree for either hepatic TTP or TTP mRNA levels between the two studies. Reasons for these discrepancies have not been determined and warrant further investigation.

Fechner *et al.* (18) saw a significant increase in TTP mRNA levels in rats that consumed a vitamin E-deficient diet for 5 wk and were then fasted, refed vitamin E, and fasted again. However, this effect of fasting was evaluated only in animals fed a vitamin E-deficient diet and not in animals receiving dietary vitamin E. In contrast, we found that fasting for 24 h prior to sacrifice had no effect on the regulation of hepatic TTP levels in mice fed either low or high levels of dietary vitamin E. The significant changes in TTP mRNA levels observed by Fechner *et al.* (18) may be due to changes in vitamin E status or to changes in the animals' feeding vs. fasting state. Hormonal signals or aspects of the diet other than vitamin E levels may also regulate hepatic TTP levels (17).

Although no changes in hepatic TTP levels occurred with fasting, we observed lower hepatic and plasma α-tocopherol concentrations in fasted compared with nonfasted animals fed the High E diet, but not the Low E diet. Fasted animals that are receiving a High E diet will have a dramatic decrease in food

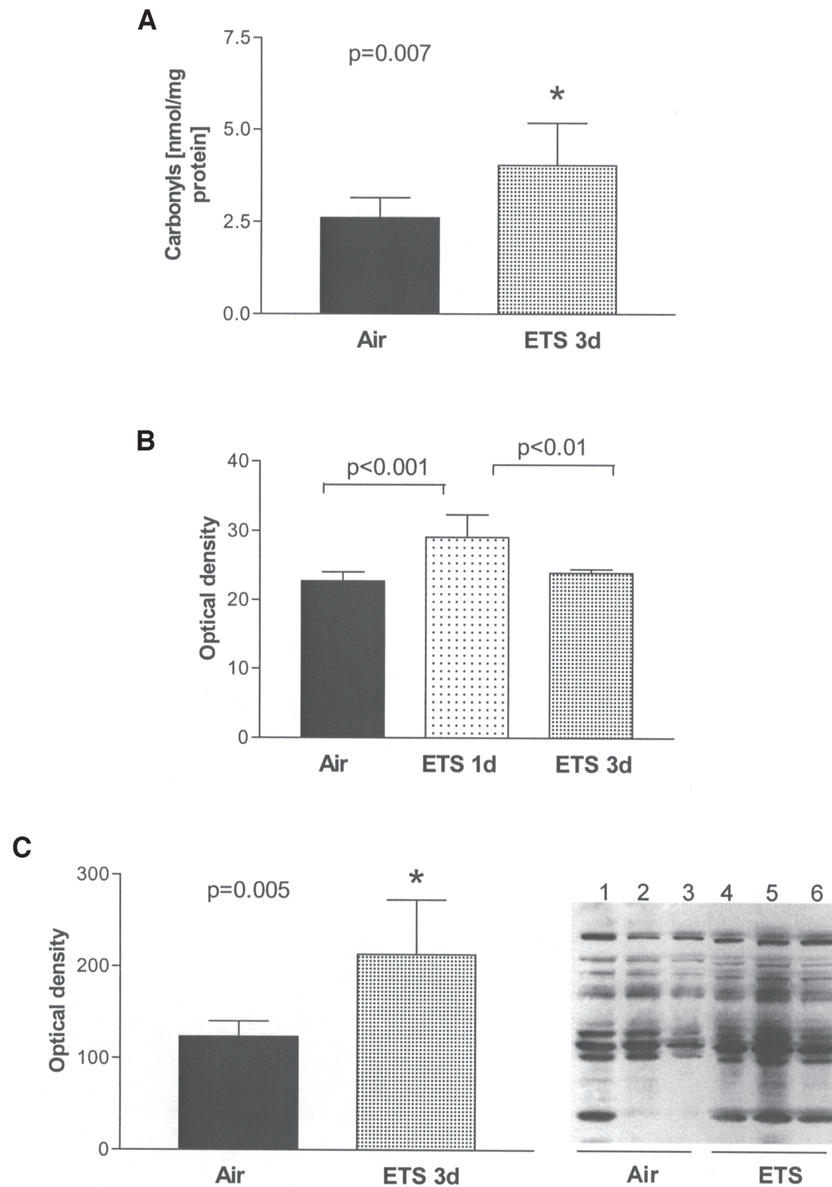

FIG. 3. Pulmonary and systemic oxidative stress in environmental tobacco smoke (ETS)-exposed mice. (A) Protein carbonyls in the lung lavage of mice exposed to ETS (3 d) vs. air (mean ± SD, n = 8). The protein carbonyl concentration was significantly higher in ETS-exposed animals (P = 0.007, Student's t-test). (B) 4-Hydroxy-2-nonenal (4-HNE) adduct formation in lung tissue of mice exposed to ETS (1 d and 3 d) vs. air (mean ± SD, n = 8). 4-HNE formation significantly increased after one day of ETS exposure but returned to control values by day 3. (O.D. 22.8 ± 1.2, 29.1 ± 3.2, and 23.9 ± 0.5 for air, ETS 1 d, and ETS 3 d, respectively, P < 0.01, ANOVA). (C) 4-HNE adduct formation in the liver tissue of mice exposed to ETS (3 d) vs. air (mean ± SD, n = 6). 4-HNE formation significantly increased after 3 d of ETS exposure (O.D. 123.6 ± 16.4 vs. 213.2 ± 59.4, P = 0.005, Student's t-test). A representative blot of liver homogenates from animals exposed to ETS and filtered air is shown (air: lanes 1–3; ETS: lanes 4–6).

intake and decreased delivery of vitamin E to both the liver and plasma. Increased metabolism of α-tocopherol may also play a role in the decreased hepatic and plasma α-tocopherol concentrations in these animals. Indeed, serum concentrations of α-carboxyethyl hydroxychroman (α-CEHC), a metabolite of α-tocopherol, have been shown to increase ~19-fold in individuals supplemented with 500 IU *RRR*-α-tocopherol per day for 29 d compared with unsupplemented individuals (40). Urinary α-CEHC is detectable at "low" vitamin E intakes (~5–10 mg/d) (41,42) in unsupplemented individuals and increases ~20-fold with supplementation of 800 mg/d (43). Hence, hepatic metabolism of α-tocopherol to α-CEHC in the mice may have been up-regulated with the high dietary levels of vitamin E.

We also considered that increased oxidative stress might up-regulate TTP. ETS exposure causes oxidative stress both because of the radicals in the smoke and through induction of airway- and systemic inflammatory-immune processes (22). Using deuterium-labeled vitamin E, Traber *et al*. (24) and

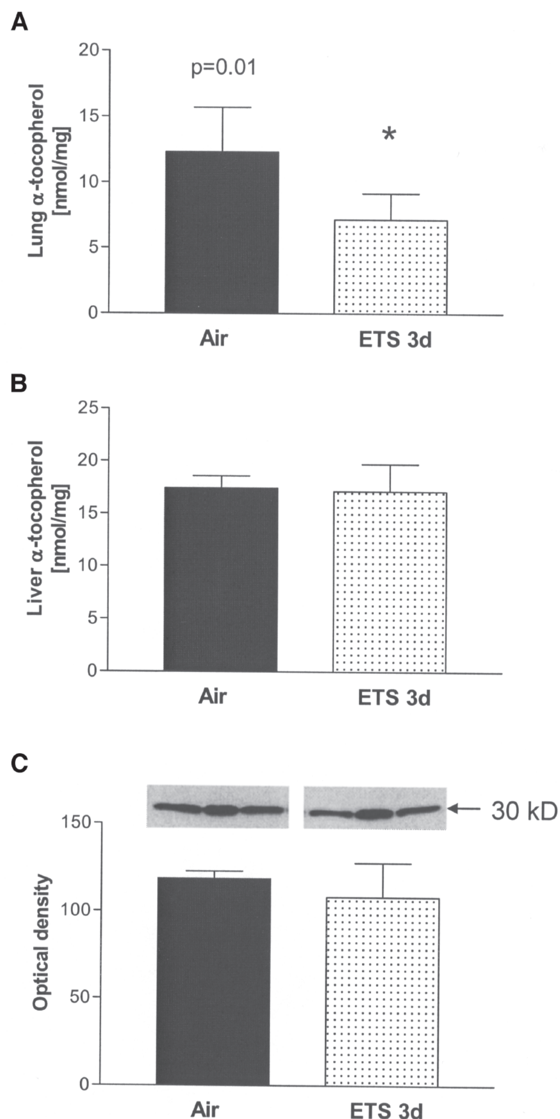

FIG. 4. Effect of ETS on pulmonary and hepatic vitamin E levels and TTP protein concentrations. (A) Pulmonary α-tocopherol concentrations in mice exposed to ETS (3 d) vs. air (mean ± SD, $n = 6$). Lung α-tocopherol concentrations decreased after 3 d of ETS exposure ($P = 0.001$, Student's *t*-test). (B) Hepatic α-tocopherol concentrations in mice exposed to ETS vs. air (mean ± SD). For hepatic tissue, ETS: $n = 5$, and air: $n = 4$. Hepatic α-tocopherol concentrations were similar by Student's *t*-test. (C) Western blots of hepatic TTP in mice exposed to ETS vs. air. No significant changes in TTP protein expression were observed ($P = 0.4$). Blots are of three representative liver homogenates from animals exposed to either ETS or air for 3 d. For abbreviations see Figures 1 and 3.

Bruno *et al.* (25) showed that cigarette smokers had a higher rate of vitamin E disappearance, yet total plasma α-tocopherol concentrations were similar in smokers and nonsmokers. We therefore hypothesized that the oxidative stress of cigarette smoking might increase hepatic TTP. Higher TTP levels could then potentially maintain plasma α-tocopherol. ETS-exposed mice exhibited increases in the oxidative modification of proteins (carbonyl formation) and lipids (HNE adducts) and a decrease in lung α-tocopherol. However, ETS exposure did not change hepatic α-tocopherol or up-regulate hepatic TTP (Fig. 4). Our exposure protocol mimics an acute ETS exposure. Although we showed increased formation of HNE adducts in the liver, it is possible that the ETS exposure regime [3 d (6 h/d) at 60 mg^3 TSP] used in this study did not cause a sufficient amount of systemic oxidative stress to deplete α-tocopherol stores and increase hepatic TPP expression. We did show, however, that exposure to ETS caused increased oxidative stress in the lung.

Thus, TTP plays an important role in the regulation of plasma levels of vitamin E. Together with Shaw and Huang (17) and Barella *et al.* (39), our study shows that the changes in hepatic TTP levels are minimal compared with changes in dietary or hepatic vitamin E levels. TTP appears to be normally expressed at sufficient levels to aid trafficking of newly absorbed dietary vitamin E. In our study, mice consuming the low vitamin E diet received half of the recommended vitamin E for mice [11.5 instead of 22 mg *RRR*-α-tocopheryl acetate/kg chow; (33)], similar to the low vitamin E diets used by others (44). TTP expression may therefore be optimized for sufficient vitamin E transport even under dietary vitamin E restrictions.

Under the conditions of oxidative stress used in this study, TTP was not up-regulated to compensate for the higher turnover or oxidative loss of vitamin E. Again, this may be due to optimal levels of TTP in the liver. However, we cannot exclude the possibility that higher levels of oxidative stress result in significant modulations in liver TTP levels. Therefore, additional studies at lower dietary and hepatic α-tocopherol concentrations are warranted.

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