Tissue α-Tocopherol Retention in Male Rats Is Compromised by Feeding Diets Containing Oxidized Frying Oil[1,2]

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ABSTRACT To investigate the effect of dietary oxidized frying oil (OFO) on tissue retention of α-tocopherol (α-T), Long-Evans male weanling rats were divided to four groups based on a 2 × 2 factorial design. Two groups were fed 15% OFO diets, and the remaining two groups were fed control diets in which OFO was replaced by vitamin E-stripped fresh soybean oil. Vitamin E as all-rac-α-tocopheryl acetate was added at the concentration of either 50 (normal E) or 500 (high E) mg/kg diet. The OFO sample was prepared by deep-frying potato sticks in fresh soybean oil at 205 ± 5°C for four 6-h periods. After 6 wks of feeding, α-T concentrations in most tissues were significantly lower in rats fed OFO diets ($P < 0.05$) than in the control groups. For rats fed the OFO diet with the normal vitamin E concentration, the α-T concentration in epididymal fat pad, plasma, liver, kidney, muscle, brain and lung were 29–64% those of the corresponding control group ($P < 0.05$). The interaction between the two dietary factors on tissue α-T was significant in liver, spleen and adrenal gland. In these three tissues, the differences between the normal and high dietary vitamin E groups were less in rats fed the OFO diets than in rats fed the control diets. The tissue α-T concentrations of the high vitamin E OFO group were comparable with or higher ($P < 0.05$) than those of the normal vitamin E control group, indicating that the negative effect of OFO on tissue α-T concentration can be alleviated by dietary supplementation of vitamin E. Compared with the controls, rats fed OFO diets had significantly higher tissue thiobarbituric acid reactive substances ($P < 0.05$). Because the amount of α-T directly added into the test oil samples was not significantly decreased through an incubation (at 37°C) period of up to 10 d, the inefficient absorption and/or enhanced catabolism or turnover of vitamin E may be involved in the inferior tissue α-T retention of OFO fed rats. J. Nutr. 125: 3071–3080, 1995.

INDEXING KEY WORDS:

- *oxidized frying oil* • *vitamin E* • *rats*
- *lipid peroxidation*

High intake of fat is a prevalent diet pattern in most developed countries. It is associated with high incidence of some chronic diseases, especially cardiovascular disease and certain types of cancer (Committee on Diet and Health 1989). Fried foods are important contributors to a high fat diet, although the relative contribution is difficult to evaluate. The possible role of lipid peroxidation products in causing the deleterious changes in lipoprotein and platelet metabolism as well as in the development of atherosclerosis has been reviewed recently (Kubow 1992, Kubow 1993).

Thermally oxidized fats were generally regarded as containing potentially toxic lipid oxidation products. This point of view originated from the early studies in which toxic fractions had been isolated from laboratory-abused oil, such as heated for long time or with a temperature higher than normally used (Artman 1969). For example, the median lethal dose (LD_{50})[5] of the nonurea-adductable fraction (NUAF) administered orally to rats weighing 40–50 g for 2 d has been shown to be 0.6 mL/100 g body wt per day (Shue et al. 1968). The NUAF used in this study was isolated from cottonseed oil that was heated at 225°C under continuous stirring at 100 rpm for ~190 h. However, long-term feeding studies using fat samples oxidized under more realistic cooking practices as part of a nutritionally balanced diet resulted in no more than mild symptoms

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[5] Abbreviations used: α-T, α-tocopherol; BHT, butylated hydroxytoluene; KOH, potassium hydroxide; LD_{50}, median lethal dose; MDA, malondialdehyde; NUAF, nonurea-adductable fraction; OFO, oxidized frying oil; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances.

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such as depression in growth and feed intake and enlargement of liver and kidney (Nolen et al. 1967, Poling et al. 1970). Liver microsomal drug-metabolizing enzymes, including phase I (the cytochrome P-450 monoxygenase system) and phase II (conjugation) enzymes, were significantly greater in rats fed diets containing 15% oxidized frying oil (OFO) for 2 d up to 8 wk, compared with rats fed the same concentration of unfried fresh soybean oil (Huang et al. 1988 and 1989). Therefore, the moderate toxicity of the OFO in rats fed a nutritionally balanced diet may be due to an effective detoxifying capability by the microsomal enzymes.

A significantly elevated RBC in vitro hemolysis was also noticed in our previous study (Huang et al. 1988). The RBC in vitro hemolysis test has long been used as a criterion for the assessment of vitamin E status. The higher RBC hemolysis implied that the vitamin E status might be compromised by the ingestion of OFO. Several studies indicated that ingestion of thermally oxidized oil resulted in increased tissue lipid peroxidation (Izaki et al. 1984, Kok et al. 1988). Moreover, Izaki et al. (1984) also found that the extent of decrease in liver and serum vitamin E concentrations were related to the degree of deterioration of the thermally oxidized oil samples.

We hypothesized that the vitamin E retention in various tissues of rats could be decreased by the feeding of a diet containing OFO. To determine not only the interaction between dietary frying oil and vitamin E but also the effectiveness of dietary supplementation of a high level vitamin E in ameliorating the deleterious effects of OFO on the tissue vitamin E status, a 2×2 factorial design with two levels of dietary vitamin E was used. An in vitro incubation experiment was conducted before the animal study to confirm that α-tocopherol (α-T) was not destroyed by direct interaction with OFO.

MATERIALS AND METHODS

Preparation of the OFO and the vitamin E-stripped soybean oil. Soybean oil (President, Tainan, Taiwan) purchased from a local supermarket was subjected to the following frying process: 9 kg of soybean oil was poured into a cast iron wok (60 cm i.d., 18 cm central depth) and heated on a gas stove that was adjusted to maintain the oil temperature at 205 ± 5°C. Potatoes were peeled, cut into sticks (10 × 1.5 × 1.5 cm) and fried in the oil. Every 30 min a batch of potato sticks (~100 g) was fried for 2.5–3 min. The frying proceeded for 6 h/d and was repeated successively for 4 d. The resultant frying oil (OFO) was stored at −20°C until the test diets were prepared. Another portion of the soybean oil was treated with active carbon (Sigma, St. Louis, MO) as described by Mohri et al. (1983) to remove vitamin E. The vitamin E-stripped soybean oil was also stored at −20°C for the preparation of the fresh soybean oil control diets.

Analysis of the test oils. The OFO and vitamin E-stripped soybean oil samples were analyzed for acid value, peroxide value and UV absorbance at 233 nm as in our previous paper (Huang et al. 1988). Total polar compounds (Waltking and Wessel 1981) and the nonurea-adductable fractions (Firestone 1961) were also determined. Fatty acid composition was analyzed by gas chromatography after methylation with sodium methoxide. Vitamin E content was analyzed by HPLC as described below. To examine whether the amount of α-T is decreased by a direct reaction with the frying oil, a measured amount of α-T was incubated in vitro with the two oil samples for up to 10 d at 37°C, and the recovery was compared.

Formulation of the test diets. Four diets were formulated according to the composition shown in **Table 1.** The two OFO diets contained 15 g/100 g of oxidized frying oil, whereas the two control diets contained 15 g/100 g of vitamin E-stripped soybean oil. One of the OFO diets and one of the control diets contained a normal level of vitamin E (50 mg/kg diet of all-rac-α-tocopheryl acetate, AIN 1977). The other OFO and control diet had a high level (500 mg/kg all-rac-α-tocopheryl acetate) of vitamin E. In preparing the diets, the powdered ingredients were mixed in advance and stored at −20°C, and the oil portions were blended in each week before feeding.

Care of animals. Thirty-seven male Long-Evans weanling rats were purchased from the Laboratory Animal Center, College of Medicine, National Taiwan University. 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. The rats were fed a nonpurified diet (Laboratory Rodent Chow, Ralston Purina, St. Louis, MO) for 3 d. They were then randomly assigned to four groups and fed the four diets. All animals had free access to food and tap water. Body weight and feed intake were recorded weekly. The NIH *Guide for the Care and Use of Laboratory Animals* was adhered to in the care and handling of the animals.

Tissue sampling and preparation. During the third week of feeding, rats were transferred to metabolic cages. Feces were collected for a 1-wk period. Fecal samples were weighed, dried at 60°C, pulverized and stored at −20°C until analysis.

After 6 wks of feeding, rats were killed by carbon dioxide asphyxiation. Blood was collected from the abdominal vena cava into an EDTA-containing tube. Liver, kidney, heart, spleen, lung, brain, adrenal gland, testis, epididymal fat pad and Vastus lateralis muscle were excised, weighed, quick-frozen in liquid nitrogen and stored in a −30°C freezer. Blood samples were centrifuged at 1000 × *g* for 10 min, and the plasma was isolated and stored at −30°C.

TABLE 1

Composition of the test diets

Fat source	Control		OFO	
	Normal vitamin E level	High vitamin E level	Normal vitamin E level	High vitamin E level
	g/kg diet			
Lactalbumin	200	200	200	200
Vitamin E-stripped soybean oil[1]	150	150		
Oxidized frying oil[1]			150	150
Corn starch	572	572	572	572
Cellulose	30	30	30	30
Salt mixture[2]	35	35	35	35
Vitamin mixture[3]	10	10	10	10
all-rac-α-tocopheryl acetate	0.05	0.5	0.05	0.5
Choline	3	3	3	3

[1] Vitamin E-stripped soybean oil: fresh soybean oil was dissolved in n-hexane and treated with active carbon according to the method of Mohri et al. (1983). Oxidized frying oil: fresh soybean oil was used for frying potato sticks for four consecutive days (6 h/d) at 205 ± 5°C.
[2] AIN-76 mineral mixture (AIN 1977).
[3] Vitamin E-free AIN-76 vitamin mixture (AIN 1977).

Analysis. The dried and pulverized fecal samples were analyzed for crude fat content by ether extraction in the Soxhlet apparatus. Fat apparent absorption was calculated by subtraction of fecal fat excretion from fat intake and divided by dietary fat intake. Tissue vitamin E was analyzed by reverse HPLC as described in a previous paper (Huang and Shaw 1994). The analyses were performed with a Jasco Model 880-PU pump (Tokyo, Japan), Model 870-UV/Vis detector, Model 851-AS autosampler and a computerized data processor. A 4 × 125-mm Lichrosphere 100RP-18 column (Merck, Darmstadt, Frankfurter, Germany) containing 5-μm particles protected by a guard column was used. The eluent (pure methanol) was pumped at a rate of 1 mL/min. The α-T was detected by UV detector (292 nm wavelength), and the retention time of a typical α-T peak was 5.2 ± 0.3 min. A software Chemlab (Shiunn-Hwa, Taipei, Taiwan) and an IBM-PC compatible personal computer were used to integrate and process the data.

For the analysis of vitamin E in oil samples, 2 mL of absolute ethanol (containing 1% pyrogallol) and 1 mL of 600 g/L potassium hydroxide (KOH) were added to 0.5-g aliquots of the sample. The mixture was incubated in a 70°C water bath for 30 min. After saponification, the mixture was cooled, and then 1 mL of distilled water and 4 mL of n-hexane [containing 1.25 g/L butylated hydroxytoluene (BHT)] was added. The mixture was vortexed for 2 min to extract the α-T. The n-hexane layer was removed, and the residue was reextracted with 2 mL n-hexane. The n-hexane extracts were combined and washed with 2 mL of distilled water. The washing was repeated twice. The organic layers were combined, solvent evaporated and the residue solubilized in 100 μL methanol. The HPLC analysis was as described above.

For the analysis of fecal vitamin E excretion in the two normal vitamin E diet groups, dried and pulverized fecal samples were saponified and extracted according to the procedure of Rushing et al. (1991) with some modification. One g of sample was mixed with 5 mL of ethanol, 0.05 g ascorbic acid and 5 mL of aqueous KOH (500 g/L) and shaken at room temperature (200 rpm) for 3 hours. Five mL of the solution was extracted with n-hexane (containing 1.25 g/L BHT), and the n-hexane extracts were washed as described (Rushing et al. 1991). For aliquots of the washed n-hexane extracts, solvent was removed in a freeze drier, the residue reconstituted in 100 μL of methanol and subjected to HPLC. The HPLC procedure was essentially the same as described above. In addition to the α-T peak, a significant tocopheryl acetate (retention time 12 min) peak was also detected in these fecal extract samples, suggesting that the hydrolysis of all-rac-tocopheryl acetate was not complete. The fecal vitamin E excretion was calculated by summing both forms of vitamin E. The apparent absorption of dietary vitamin E was calculated as 100 × (vitamin E intake − fecal vitamin E excretion)/dietary vitamin E intake.

To determine the thiobarbituric acid reactive substance (TBARS) concentration in brain, kidney, liver, lung, muscle, spleen and adipose tissue, 1 mL of the homogenates (250 g/L, Huang and Shaw 1994) of these tissues was mixed with 1 mL of 100 g/L trichloroacetic acid, centrifuged at 3500 × g for 10 min. One milliliter of the supernatant was mixed with 1.0 mL of 4 g/L thiobarbituric acid (TBA) reagent in 0.2 mol/L HCl

and 0.1 mL of 2 g/L BHT in 95% ethanol. After an incubation at 50°C for 1 h, the mixtures were cooled and the TBA-malondialdehyde (MDA) adduct was extracted with 3 mL of isobutanol, and the fluorescence was measured with excitation at 515 nm and emmision at 550 nm. 1,1,3,3-tetramethoxypropane (Sigma Chemical, St. Louis, MO) was used as the standard for the determination, and the TBARS in each tissue was expressed as nanomoles of malondialdehyde. The procedure was modified from the one described by Tatum et al. (1990).

Statistical analysis. Data were expressed as means ± SD. The significance of the effect of dietary OFO, dietary vitamin E level and the interactions of the two factors were analyzed by two-way ANOVA using the General Linear Model of the SAS Package. Dietary effects were considered significant at $P < 0.05$. The least squares mean method of the same model was used for the comparison of tissue α-T concentrations and TBARS among the four groups of rats. Student t test was used to compare the fecal excretion and apparent absorption of vitamin E between the two normal vitamin E-fed groups.

RESULTS

Analysis of the test oils. The quality of the soybean oil declined considerably through the frying process (**Table 2**). The acid value and UV absorbance at 233 nm gradually increased with frying periods. The peroxide value of the vitamin E-stripped soybean oil was somewhat higher than the original fresh soybean oil but was much lower than the frying oil sample. The concentration of total polar compounds and nonurea-adductable fractions were, respectively, 38.7 and 14.2% after the 24-h frying process and were ~6-fold and 10-fold those of the vitamin E-stripped soybean oil.

Substantial fractions of linoleic and linolenic acids were lost during the frying process (**Table 3**).

The active carbon-stripping procedure removed 97% of α-T (**Table 4**). It also removed 80% γ-T and 38% δ-T. The 24-h frying process destroyed almost all of the α-T of the soybean oil. It also destroyed 94% γ-T and 62% δ-T.

The recovery of α-T after incubation in vitro with the test oils at 37°C for up to 10 d were in the range of 77 to 89% (**Table 5**). The recovery with OFO was comparable with that with the control oil.

Animal growth and food intake. The body weight gain, food intake and feed efficiency after 6 wks of feeding are shown **Table 6.** There were no significant differences in food intake among the four groups of rats. The body weight gain of the OFO diet groups tended to be slightly lower ($P = 0.06$) than that of the control groups. The feed efficiency of rats fed the OFO diets was significantly lower than rats fed the control diets. Dietary vitamin E level did not affect body weight gain, food intake or feed efficiency. Diarrhea was observed in rats fed OFO diets but not in the control groups.

Apparent absorption of dietary fat and vitamin E. The apparent absorption of dietary fat and vitamin E are shown in **Table 7.** During the week of fecal collection, both of the OFO diet groups consumed more food than the control groups. Fat intake, fecal weight and fecal fat excretion were significantly higher in rats fed diets containing OFO than in the control groups. Nevertheless, fat and vitamin E absorption were sig-

TABLE 2

The quality of the vitamin E-stripped soybean oil and oxidized frying oil used in the feeding experiment

	Acid value	Peroxide value	UV233[1]	TBA[1] value	Total polar compound	NUAF[1]
	mg KOH/g oil	*meq/kg oil*	*OD[1]/g oil*		%	%
Fresh soybean oil[2]	0.218[3]	1.30	479			
Vitamin E-stripped soybean oil[4]	0.246	6.31	514	6.72	6.27	1.34
Oxidized frying oil (after frying for)[5]						
6 h	0.555	55.39	2350			
12 h	0.928	90.86	3260			
18 h	1.082	81.64	3792			
24 h	1.248	34.24	5663	39.15	38.66	14.16

[1] UV233: Absorbance of UV light of 233 nm after proper dilution with n-hexane, expressed as absorbance (OD, optical density) per gram oil; TBA value, thiobarbituric acid value; NUAF, nonurea-adductable fraction.
[2] The oil sample originally purchased in and not subjected to the frying or stripping process.
[3] Data were means of triplicate analyses.
[4] Vitamin E-stripped soybean oil: fresh soybean oil was dissolved in n-hexane and treated with active carbon according to the method of Mohri et al. (1983).
[5] Oxidized frying oil: fresh soybean oil was used for frying potato sticks for four consecutive days (6 h/d) at 205 ± 5°C.

TABLE 3

The fatty acid composition of vitamin E-stripped soybean oil and the oxidized frying oil

Fatty acid	Vitamin E-stripped soybean oil[1]	Oxidized frying oil[2]
	%	%
16:1	0.71[3]	0.67
18:0	4.40	4.49
18:1	27.20	25.76
18:2	59.37	47.80
18:3	8.33	5.67

[1] Vitamin E-stripped soybean oil: fresh soybean oil was dissolved in n-hexane and treated with active carbon according to the method of Mohri et al. (1983).

[2] Oxidized frying oil: fresh soybean oil was used for frying potato sticks for four consecutive days (6 h/d) at 205 ± 5°C.

[3] Data are means of duplicate analyses.

nificantly lower. The apparent absorption of dietary fat of OFO groups was 92.2% compared with 96.8% in the control groups. In contrast, the apparent absorption of dietary vitamin E was 35% in the normal E-OFO groups compared with 61% in the corresponding control. The reduction in the absorption due to feeding the diet containing OFO was more severe for vitamin E than for dietary fat. Dietary vitamin E level had no effect on fat apparent absorption.

Tissue vitamin E. Both dietary factors, namely, OFO and vitamin E concentration, had significant effects ($P < 0.05$) on tissue α-T concentrations (**Table 8**). Rats fed OFO diets had lower α-T concentration in all tissues examined except the heart (Table 8). As expected, rats fed high vitamin E diets had significantly higher α-T concentrations in all tissues than rats of the normal vitamin E groups. The interaction of the two dietary factors on tissue α-T was significant in adrenal gland, spleen and liver ($P < 0.05$). In these three tissues, the differences between the α-T concentration of the high vitamin E group and normal vitamin E group were less in the OFO-fed rats than in the control rats.

The significance of difference of tissue α-T concentrations among the four groups of rats were further tested statistically by the least squares mean methods. When the two groups of rats that were fed the normal level of dietary vitamin E were compared, the α-T concentration in epididymal fat pad, plasma, liver, kidney, muscle, brain and lung of the OFO group was, respectively, 29, 44, 45, 49, 53, 60 and 64% those of the control group ($P < 0.05$). It is noteworthy that rats fed the high vitamin E-OFO diet had tissue α-T concentrations that were comparable to or even higher ($P < 0.05$) than rats of the normal vitamin E control group.

Tissue TBARS. Dietary OFO significantly elevated the TBARS concentrations in all tissues examined except in the spleen ($P < 0.05$, **Table 9**). Among the tissues affected, the extent of increase in TBARS was greatest in brain and epididymal fat pad. For rats fed the normal level of dietary vitamin E, TBARS in brain and adipose tissue of the OFO group were two- to threefold that of the control ($P < 0.05$). On the other hand, feeding the high vitamin E diet significantly lowered TBARS concentrations in adipose tissue, brain, kidney and liver ($P < 0.05$, two-way ANOVA) but not in lung, muscle and spleen (Table 9). Interaction between dietary fat source and vitamin E level on tissue TBARS was significant for brain, kidney and adipose tissue ($P < 0.05$). For these three tissues, the differences between TBARS concentration of high and normal vitamin E groups were greater in the OFO groups than in the control groups.

DISCUSSION

Soybean oil is rich in tocopherols. However, most of these tocopherols are lost in the high temperature deep-frying process (Table 4). To reduce the vitamin E content of the control oil sample, the fresh soybean oil was subjected to a stripping process that efficiently removed most of the vitamin E. Before supplementation, the residual vitamin E in the control diets and test diets were calculated to be 1.78 and 0.44 mg α-T equivalent per kg diet, respectively. After supplementation with 50 or 500 mg of all-rac-α-tocopheryl acetate per kg diet, the difference in the residual amounts of vitamin E in the four diets was not significant.

TABLE 4

The vitamin E concentration of the vitamin E-stripped soybean oil and the oxidized frying oil

	δ-Tocopherol	r-Tocopherol	α-Tocopherol
		μg/g oil	
Fresh soybean oil[1] (before stripping)	21	485.2	67
Vitamin E-stripped soybean oil[2] (% *residual ratio*)	13 (61.9)	95 (19.6)	2.3 (3)
Oxidized frying oil[3] (% *residual ratio*)	8 (38.1)	28 (5.8)	*[4] —

[1] The oil sample originally purchased in and not subjected to the frying or stripping process.

[2] Vitamin E-stripped soybean oil: fresh soybean oil was dissolved in n-hexane and treated with active carbon according to the method of Mohri et al. (1983).

[3] Oxidized frying oil: fresh soybean oil was used for frying potato sticks for four consecutive days (6 h/d) at 205 ± 5°C.

[4] * Undetectable.

TABLE 5

The recovery of α-tocopherol after incubation with oxidized frying oil or vitamin E-stripped fresh soybean oil at 37°C for 10 d[1,2]

	α-Tocopherol		
	Spiked amount	Recovered amount	Recovery
	μg/g oil	*μg/g oil*	*%*
Vitamin E-stripped soybean oil[3]	666.66	591.26 ± 157.17[3]	88.69
	1333.33	1026.92 ± 222.37	77.03
Oxidized frying oil[4]	1333.33	1160.65 ± 378.77	87.05
	1500.00	1199.02 ± 266.08	79.93

[1] The spiked amount of all-rac-α-tocopherol was dissolved in the oil samples at the indicated concentration and incubated at 37°C for 10 d.
[2] Values are means ± SD for five replicates.
[3] Vitamin E-stripped soybean oil: fresh soybean oil was dissolved in n-hexane and treated with active carbon according to the method of Mohri et al. (1983).
[4] Oxidized frying oil: fresh soybean oil was used for frying potato sticks for four consecutive days (6 h/d) at 205 ± 5°C.

The frying process led to substantial oxidation of the soybean oil. The data indicated that the 24-h frying oil used in this experiment was abundant in secondary products of lipid peroxidation and relatively scarce in the primary product, i.e., hydroperoxides. The quality of the OFO sample used in this experiment was in the range of those obtained from street vendors in the Taipei area (Hau et al. 1987).

Although a substantial fraction of polyunsaturated fatty acids was lost during the frying process, the linoleic acid concentration of the formulated OFO and control diets were calculated as 71.7 and 89.1 g/kg diet, respectively, values in excess of the required level of 6 g/kg diet (NRC 1978).

Although in our diet preparation the oil portion was blended in each week before feeding, there is still a possibility that the all-rac-α-tocopheryl acetate premixed in the diet could be lost by direct reaction with the OFO. Because there was no difference between the recovery of the α-T added to the vitamin E-stripped fresh soybean oil and OFO in the in vitro incubation experiment and because all-rac-tocopheryl acetate is more stable than the all-rac-α-T, the all-rac-α-tocopheryl acetate concentration in the diet fed was presumably similar in OFO and control diets and close to the expected value.

Fecal total vitamin E accounted for 38.7% of the vitamin E intake of the normal vitamin E control group in the present study. This is in agreement with the value (37.5%) reported by Bieri and Tolliver (1982). Because most of the fecal vitamin E detected in the HPLC was in the form of tocopheryl acetate (~90% of total vitamin E), it appears that the mild alkali treatment before extraction used in this study was relatively inefficient in the hydrolysis of the fecal tocopheryl ester. However, this also suggested that the inefficient absorption of dietary vitamin E is associated with an incomplete digestion.

The radioisotope tracer studies of Kanazawa et al. (1985) and Oarada et al. (1986) demonstrated that a substantial proportion of the secondary products of lipid autoxidation could be absorbed, but the secondary products, especially polymeric fractions, were less readily absorbable. Poor absorbability of the secondary products in the OFO may account for the lowered apparent fat absorption and diarrhea. Further investigation is needed to determine whether the secondary oxidation products present in OFO disturbed micelle formation and interfered with the digestion and absorption of vitamin E.

Tissue concentrations of α-T denote the net balance between the uptake as well as the catabolism or turnover. The uptake of α-T by a tissue depends on the plasma level and the specific uptake mechanism of that tissue. The uptake mechanism and turnover rate for α-T may be different in different tissues. For example, an HDL-specific binding mechanism in the adrenal gland may contribute to the high uptake efficiency for α-T leading to the high α-T concentration in this tissue (Gwynne and Hess 1980), because HDL possess the highest α-T concentration among the plasma lipoprotein fractions in rats (Bjorneboe et al. 1987a). Using deuterium-labeled stable isotope, Ingold et al. (1987) demonstrated that the tissue half-lives of RRR-α-T were as follows: lung, 7.6; liver, 9.8; plasma, 10.9; kidney, 11.2; heart, 13.3; muscle 15.8; spleen, 16.6; brain, 29.4; testes, 33.3 and epididymal fat, 34.8 d.

The reduced absorption of dietary vitamin E in rats fed the OFO diets may have led to the lower plasma α-T concentration; this in turn, may have contributed to the lower α-T concentration in peripheral tissues. Accordingly, the reduction of α-T concentration in liver, kidney, lung and muscle were comparable, to an extent, with the reduction in plasma. That these tissues have short half-lives of α-T may have rendered their α-T content more susceptible to a decrease in plasma

TABLE 6

Body weight gain, food intake and feed efficiency of rats fed oxidized frying oil (OFO) diets or control diets supplemented with 50 (normal) or 500 (high) mg/kg all-rac-α-tocopheryl acetate for 6 wk[1]

Fat source	Vitamin E level	Body wt gain	Food intake	Feed efficiency
		g/wk	*g/wk*	*g gain/g food*
Control[2]	Normal (n = 8)	47.82 ± 4.06	124.81 ± 7.57	0.384 ± 0.014
Control[2]	High (n = 9)	44.66 ± 6.70	118.33 ± 16.71	0.379 ± 0.019
OFO[2]	Normal (n = 10)	42.62 ± 5.99	123.11 ± 12.02	0.347 ± 0.024
OFO[2]	High (n = 10)	43.02 ± 4.15	119.91 ± 11.19	0.357 ± 0.014
Dietary factor[3]				
Fat source		0.0619	0.9872	0.0026
Vitamin E		0.4404	0.2445	0.6301
Oil × vitamin E		0.3264	0.6190	0.2815

[1] Values are means ± SD.
[2] Control, Vitamin E-stripped soybean oil: fresh soybean oil was dissolved in n-hexane and treated with active carbon according to the method of Mohri et al. (1983). OFO, oxidized frying oil: fresh soybean oil was used for frying potato sticks for four consecutive days (6 h/d) at 205 ± 5°C.
[3] Analyzed by two-way ANOVA, each value represents P value.

α-T concentration than tissues with long half-lives, such as testes.

The reduction of α-T concentration in adrenal gland and testes were the least among the tissues affected. The adrenal gland may be protected by a specific uptake mechanism for α-T (Gwynne and Hess 1980). The long half-life of α-T in testes may explain why its α-T content was minimally affected by lower plasma α-T. The half-lives for α-T turnover are also long in brain and adipose tissue. However, the α-T in these tissues were reduced markedly by the ingestion of OFO. Noticeably, among the tissues measured, brain and adipose tissue showed the highest increase in TBARS due to OFO in rats fed normal vitamin E (two- to threefold that of normal vitamin E control group, Table 9). Because these two tissues are relatively high in lipid content, it is possible that more oxidation products from the OFO diets may have entered these tissues and enhanced lipid peroxidation. This in turn may accelerate catabolism and/or turnover of α-T in these tissues.

The interaction of dietary fat source and vitamin E on tissue α-T level was significant in adrenal gland, liver and spleen. When the high and normal vitamin E control groups were compared, the greatest differences in α-T concentration were also observed in these three tissues. It therefore appears that these three tis-

TABLE 7

Fecal excretion and apparent absorption of fat and vitamin E by rats fed the oxidized frying oil (OFO) or control diets supplemented with 50 (normal) or 500 (high) mg/kg α-tocopheryl acetate for 6 wk[1]

Fat source	Vitamin E level	Food intake	Fat intake	Fecal weight	Fat excretion in feces	Fat apparent absorption	Vitamin E intake[2]	Fecal vitamin E excretion[3]	Vitamin E apparent absorption[4]
		g/wk	*g/wk*	*g/wk*	*g/wk*	%	*μg/wk*	*μg/wk*	%
Control[5]	Normal	116.4 ± 6.18	17.46 ± 0.93	6.86 ± 0.53	0.55 ± 0.19	96.83 ± 1.16	5820 ± 309	2284 ± 706	61.4 ± 10.8
Control[5]	High	116.9 ± 10.17	17.54 ± 1.53	7.54 ± 0.65	0.56 ± 0.19	96.76 ± 1.26			
OFO[5]	Normal	134.6 ± 17.75	20.12 ± 2.66	10.43 ± 1.90	1.47 ± 0.23	92.32 ± 1.71	6707 ± 888	4397 ± 1311[6]	34.6 ± 17.2[6]
OFO[5]	High	125.1 ± 15.95	18.76 ± 2.39	9.79 ± 2.01	1.53 ± 0.24	91.89 ± 1.54			
Dietary factors[7]									
Fat source		0.0107	0.0107	0.0001	0.0001	0.0001			
Vitamin E[7]		0.3323	0.3323	0.9480	0.8152	0.6961			
Fat source × vitamin E		0.3236	0.3236	0.2168	0.9722	0.6499			

[1] Values are means ± SD for 8–10 rats.
[2] All-rac-α-tocopheryl acetate.
[3] Vitamin E = α-tocopherol + α-tocopheryl acetate.
[4] Apparent absorption = 100 × (vitamin E intake − fecal vitamin E)/vitamin E intake.
[5] Control, Vitamin E-stripped soybean oil: fresh soybean oil was dissolved in n-hexane and treated with active carbon according to the method of Mohri et al. (1983). Oxidized frying oil: fresh soybean oil was used for frying potato sticks for four consecutive days (6 h/d) at 205 ± 5°C.
[6] Significantly different from control normal E group by Student t test ($P < 0.01$).
[7] Analyzed by two-way ANOVA, each value represents P value.

TABLE 8

Tissue α-tocopherol concentrations of rats fed oxidized frying oil (OFO) diets or control diets supplemented with 50 (normal) or 500 (high) mg/kg all-rac-α-tocopheryl acetate for 6 wk[1,2]

	Fat source						
	Control[3]		OFO[3]		Two-way ANOVA[4]		
	Normal vitamin E level	High vitamin E level	Normal vitamin E level	High vitamin E level	Fat source	Vitamin E	Oil × vitamin E
	μmol/L				*P value*		
Plasma	11.17 ± 2.04[b]	19.15 ± 3.25[c]	4.88 ± 1.46[a]	9.82 ± 0.61[b]	0.0001	0.0001	0.1123
	nmol/g tissue						
Adrenal gland	235.54 ± 36.68[a]	455.40 ± 54.58[c]	193.98 ± 36.36[a]	347.24 ± 24.26[b]	0.0001	0.0001	0.0395
Brain	21.96 ± 5.13[b]	29.33 ± 4.90[b]	13.26 ± 4.76[a]	22.20 ± 6.27[b]	0.0031	0.0023	0.4001
Adipose[5]	7.94 ± 2.48[bc]	11.38 ± 4.81[c]	2.32 ± 1.61[a]	7.52 ± 3.74[b]	0.0009	0.0021	0.4929
Heart	16.30 ± 7.01[ab]	25.96 ± 6.83[c]	14.84 ± 6.94[a]	25.19 ± 6.78[bc]	0.2750	0.0017	0.8262
Kidney	12.17 ± 5.20[b]	17.39 ± 3.41[c]	5.97 ± 1.35[a]	10.17 ± 4.69[b]	0.0001	0.0023	0.7156
Liver	36.45 ± 5.66[b]	115.83 ± 19.94[d]	16.53 ± 4.57[a]	55.10 ± 5.78[c]	0.0001	0.0001	0.0003
Lung	23.03 ± 4.97[b]	42.81 ± 7.94[d]	14.63 ± 2.55[a]	29.67 ± 6.11[c]	0.0001	0.0001	0.2339
Spleen	15.74 ± 5.48[a]	44.72 ± 11.94[b]	11.75 ± 5.62[a]	21.45 ± 7.80[a]	0.0002	0.0001	0.0018
Testis	21.08 ± 5.43[a]	38.12 ± 5.13[b]	17.23 ± 2.86[a]	33.50 ± 5.32[b]	0.0155	0.0001	0.8135
Muscle[5]	13.18 ± 1.14[b]	22.36 ± 3.55[d]	6.93 ± 1.49[a]	16.83 ± 2.25[c]	0.0001	0.0001	0.6733

[1] Values are means ± SD for 8–10 rats.
[2] The significance of difference between any two groups was tested by least squares means method. Data in the same row with the same superscript letter are not significantly different.
[3] Control, Vitamin E-stripped soybean oil: fresh soybean oil was dissolved in n-hexane and treated with active carbon according to the method of Mohri et al. (1983). Oxidized frying oil: fresh soybean oil was used for frying potato stick for four consecutive days (6 h/d) at 205 ± 5°C.
[4] Analyzed by two-way ANOVA, each value represents *P* value.
[5] Adipose: epididymal fat pad; muscle: vastus lateralis.

TABLE 9

The concentrations of thiobarbituric acid reactive substance (TBARS) in tissues of rats fed oxidize frying oil (OFO) diets or control diets supplemented with 50 (normal) or 500 (high) mg/kg all-rac-α-tocopheryl acetate for 6 wk[1,2]

	Fat source						
	Control[3]		OFO[3]		Two-way ANOVA[4]		
	Normal vitamin E level	High vitamin E level	Normal vitamin E level	High vitamin E level	Fat source	Vitamin E	Oil × vitamin E
	nmol/g tissue				*P value*		
Adipose[5]	12.70 ± 5.13[ab]	8.03 ± 4.52[a]	33.30 ± 5.20[c]	14.73 ± 3.44[b]	0.0001	0.0001	0.0001
Brain	11.86 ± 6.64[a]	9.98 ± 6.43[a]	34.51 ± 8.67[b]	14.52 ± 8.58[a]	0.0001	0.0004	0.0026
Kidney	16.38 ± 3.13[b]	8.94 ± 2.49[a]	25.66 ± 4.38[c]	10.10 ± 3.67[a]	0.0003	0.0001	0.0019
Liver	4.07 ± 1.05[c]	2.24 ± 0.50[a]	5.28 ± 1.03[d]	3.17 ± 0.64[b]	0.0005	0.0001	0.6433
Lung	1.82 ± 0.89[a]	1.74 ± 0.93[a]	5.73 ± 1.04[b]	4.63 ± 1.97[b]	0.0001	0.2071	0.2761
Muscle[5]	49.37 ± 9.09[a]	47.65 ± 19.84[a]	70.26 ± 11.94[b]	52.63 ± 23.41[a]	0.0361	0.1115	0.1878
Spleen	40.41 ± 14.92	38.27 ± 16.59	51.01 ± 30.01	49.49 ± 21.11	0.2656	0.8492	0.9750

[1] Values are means ± SD for 8–10 rats.
[2] The significance of difference between any two groups was tested by least squares means method. Data in the same row with the same superscript letter are not significantly different.
[3] Control, Vitamin E-stripped soybean oil: fresh soybean oil was dissolved in n-hexane and treated with active carbon according to the method of Mohri et al. (1983). Oxidized frying oil: fresh soybean oil was used for frying potato sticks for four consecutive days (6 h/d) at 205 ± 5°C.
[4] Analyzed by two-way ANOVA, each value represents *P* value.
[5] Adipose: epididymal fat pad; muscle: vastus lateralis.

sues have relatively high α-T retention efficiencies in response to dietary vitamin E supplementation. The significant interaction observed suggests that the accumulation of tissue α-T was less efficient when OFO was present in the diet. Again, the interference of OFO on tissue retention of vitamin E is noteworthy. It is not clear why α-T concentration in heart was not affected by dietary OFO because the α-T turnover is rapid (half-life 13.3 d) in this tissue. The half-life of α-T in spleen is also short; nevertheless, the reduction of α-T in spleen of the normal vitamin E-OFO group was not as marked as that in those tissues with short α-T half-lives. Noticeably, TBARS in spleen was not affected by either dietary OFO or vitamin E.

A negative correlation (Pearson's correlation coefficient = 0.3115, $P < 0.0001$, $n = 216$) was observed between concentrations of TBARS and vitamin E of the seven tissues of all four groups. However, it is difficult to identify the cause and effect relationship between these two factors. It is generally accepted that lower vitamin E status may lead to elevated tissue TBARS. The present data also showed that supplementation of high dietary vitamin E to the control diet significantly raised α-tocopherol (Table 8) and reduced TBARS (Table 9) in adipose tissue, brain, liver and kidney. The lower tissue α-T resulting from reduced absorption may partially contribute to the high TBARS in rats fed the OFO diet with normal vitamin E.

The secondary products of lipid oxidation (Kanazawa et al. 1985, Kanazawa et al. 1986) and compounds isolated from these fractions such as 9-oxononanoic acid (Minamoto et al. 1985), 12-keto oleic acid (Fukuzawa and Sato 1975) and 4-hydroxynonenal (Esterbauer et al. 1986) have been shown to increase tissue TBARS or lipid peroxidation. Therefore, enhanced formation of TBARS due to insult of oxidation products can also be surmised. More studies are needed to support this hypothesis.

It is believed that free radicals could be generated in the microsomal cytochrome P-450 catalyzed reactions. Like Xenobiotics and ethanol administration, OFO feeding can induce hepatic microsomal cytochrome P-450. Nevertheless, the response in tissue α-T to these agents was different. Long-term (5–6 wks) ethanol feeding reduced α-T content (by 25%) in the liver but not in the peripheral tissues (Bjorneboe et al. 1987b). In contrast, administration of polychlorobiphenyls (PCB), a xenobiotic, has been shown to increase absorption and tissue concentration of α-T (Koremura et al. 1990). These comparisons did not support the possibility that the induced liver cytochrome P-450 monoxygenase reactions could directly be attributed to the decreased tissue liver α-T. On the other hand, methyl ethyl ketone peroxide was shown to decrease liver α-T (Warren and Reed 1991). Oxidative products in the OFO are more likely to play a major role in the enhancement of catabolism or turnover and the reduction of tissue retention of α-T.

Much attention has been accorded to the status of vitamin E in tissues because of its chain-breaking antioxidant activity in the protection against oxidative stress and the associated pathological conditions. The present study shows that tissue α-T concentration cannot be predicted directly from dietary vitamin E level if such an interfering dietary factor as OFO is present. The adverse biological effect of dietary OFO, i.e., to compromise tissue vitamin E status, may not be acutely detrimental but may increase the risk of the organism to various diseases and pathological conditions. Nonetheless, data from the present study also showed that the decreased retention of vitamin E in tissues resulting from the ingestion of OFO can be ameliorated by dietary supplementation of vitamin E.

In conclusion, the retention of dietary vitamin E in rat tissues was unfavorably affected by dietary OFO. The significantly reduced α-T concentration in most tissues of rats fed the diet containing OFO was presumably related to a lower plasma level associated with a lower absorption and a faster catabolism or turnover as a result of enhanced lipid peroxidation. In addition, the reduced α-T and increased TBARS in tissues of rats fed the OFO diet can be alleviated by supplementation with high concentration of dietary vitamin E.

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