Dietary Oxidized Frying Oil Enhances Tissue α-Tocopherol Depletion and Radioisotope Tracer Excretion in Vitamin E–Deficient Rats[1,2]

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ABSTRACT Rats fed a diet containing 15% oxidized frying soybean oil (OFO) have been shown to have significantly lower tissue α-tocopherol (α-T) concentration than rats fed a 15% fresh soybean oil diet. To examine the turnover of α-tocopherol, a depletion-repletion experiment and a radioisotope tracer study were conducted. Two groups of male weanling Long-Evans rats were fed vitamin E–deficient diets containing either 15% OFO or 15% vitamin E–stripped fresh soybean oil (control). After 9 wk of depletion, rats fed the OFO diet had significantly higher plasma pyruvate kinase (PK) activity and lower concentrations of α-T in RBC, adrenal gland, heart, kidney, liver, spleen, testis and muscle compared with controls ($P < 0.05$), indicating that the vitamin E–deficient status was aggravated by feeding the OFO diet. After 12 wk, the depleted rats were intraperitoneally injected with a dose of all-*rac*-α-T (2.5 mg/rat, dissolved in Vitamin E–stripped corn oil) every other day. Three doses were administered to each rat during the 1-wk repletion period. Plasma PK activity decreased in both groups ($P < 0.05$) after repletion but that of the OFO rats was still significantly higher than that of the control group. The repleted OFO group also had significantly lower α-T concentration in adrenal gland, epididymal fat, liver and spleen than the repleted control group. Two rats from each group that had been vitamin E–depleted for 16 wk were injected intraperitoneally with a single dose of 5-methyl-^{14}C-*RRR*-α-T (740 kBq/kg body weight). During the week after dosing, the radioactivity excreted in urine and feces of the OFO group was 1.3- and 1.7-fold, respectively, that of the control group. Tissue retention of radioactivity was also lower in the OFO rats than in the control rats. The results suggest that more of the α-T in the body was catabolized or turned over in rats fed the OFO-containing diet. J. Nutr. 126: 2227–2235, 1996.**

INDEXING KEY WORDS:

- *oxidized frying oil* • *vitamin E* • *rats*
- *depletion* • *radioisotope tracer*

Rats fed a diet containing 15% oxidized frying oil (OFO)[5] had significantly lower α-tocopherol (α-T) concentrations in plasma, liver, kidney, muscle, brain, epididymal fat and lung than rats fed a diet containing a similar level of fresh soybean oil (Liu and Huang 1995). The elevated fecal vitamin E excretion indicated a lower absorption of dietary vitamin E in these OFO-fed rats. This partially explained their compromised tissue vitamin E status. Because a significant increase in tissue thiobarbituric acid reactive substances (TBARS) concentration was also observed (Liu and Huang 1995), the reduced tissue α-T level may also have arisen from an enhanced catabolism and/or turnover of α-T because tissues were exposed to lipid peroxidation products in OFO.

To test this hypothesis, a vitamin E depletion-repletion experiment and a radioisotope tracer study were conducted. The vitamin E status was assessed by plasma pyruvate kinase activity (PK) (Chow 1975 and 1990) and tissue α-T concentration. In the depletion experiment, these indices of vitamin E status were compared between the OFO and control groups after they were fed the vitamin E–deficient diets for 9 wk. Because no exogenous α-T was available, a more severe vitamin E–deficient status would indicate an elevated turnover of this vitamin in the body. It may also suggest an enhanced catabolism and/or turnover in some tissues. The vitamin E status of the two groups was further compared after administration of unlabeled and labeled α-T through intraperitoneal injection. To avoid

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[5] Abbreviations used: OFO, oxidized frying oil; PK, pyruvate kinase (EC 2.7.1.40); α-T, α-tocopherol; TBARS, thiobarbituric acid reactive substances.

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the interference of vitamin E malabsorption, the administration of α-T in the repletion and the tracer experiments was achieved by intraperitoneal injection.

MATERIALS AND METHODS

Test oil samples and diets. The OFO and the vitamin E–stripped soybean oil samples used in this experiment were essentially from the same batch as those used in the experiment reported previously (Liu and Huang 1995). The OFO sample was prepared by deep frying potato sticks in fresh soybean oil in a cast iron wok at 205 ± 5°C for four 6-h periods. Because most of the vitamin E contained in the fresh soybean oil was destroyed during frying, the fresh soybean oil used for preparing the control diet was stripped of vitamin E. A portion of the fresh soybean oil was treated with active carbon (Sigma Chemical, St. Louis, MO) as described by Mohri et al. (1983) to strip vitamin E. The quality index and residual vitamin E content of the two prepared oil samples were described in detail in our previous paper (Liu and Huang 1995). Briefly, the non-urea adductable fraction, total polar compound and peroxide value of the vitamin E–stripped soybean oil were as follows: 1.34 g/100 g oil, 6.27 g/100 g oil, and 6.31 meq/kg oil, respectively. In the OFO, these values were 14.16 g/100 g oil, 38.66 g/100 g oil and 34.24 meq/kg oil.

The two fat sources were incorporated into the two test diets at 15 g/100 g diet. The remaining ingredients were as follows: lactalbumin, 20%; cornstarch, 57.2%; cellulose, 3%; AIN-76 mineral mixture, 3.5%; vitamin E–free AIN-76 vitamin mixture, 1%; choline chloride, 0.3%. The composition was similar to that reported in our previous paper (Liu and Huang 1995) except that the vitamin E was excluded. Because the vitamin E concentration of the vitamin E–stripped fresh soybean oil was slightly higher than that of the OFO, to adjust to an equal amount of residual vitamin E level in the two diets, nine portions of the unstripped fresh soybean oil were mixed into 91 portions of OFO sample before blending into the test diets. The final residual vitamin E concentrations (mg/kg diet) of the OFO and control diets were as follows: α-tocopherol, 0.90 and 0.35; γ-tocopherol, 10.37 and 14.30; δ-tocopherol, 1.38 and 1.95, respectively. 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.

Animals. Twenty-three male Long-Evans weanling rats were purchased from the Laboratory Animal Center, College of Medicine, National Taiwan University. Their initial body weight was 50.5 ± 7.5 g. Three rats were killed at the beginning of the experiment to determine tissue α-T concentration before depletion. The remaining 20 rats were housed individually in stainless steel wire cages in a room maintained at 25 ± 2°C with a controlled 12-h light:dark cycle. They were randomly assigned to the two diet groups with 10 rats in each of the OFO and the control groups. The two diets as well as tap water were given freely. Body weight and food intake were recorded weekly. The *Guide for the Care and Use of Laboratory Animals* (NRC 1985) was adhered to in the care and handling of the animals.

To obtain the plasma PK activity of rats fed normal vitamin E diets, 10 male Long-Evans weanling rats from the same source were randomly assigned to two groups with five rats in each group. They were fed the control and OFO diets, respectively, supplemented with 50 mg/kg diet of all-*rac*-α-tocopheryl acetate. After 9 wk, blood samples were collected, plasma isolated and assayed for plasma PK activity as described in the following.

Depletion, repletion and radioisotope tracer experiments. For the depletion of tissue vitamin E, the two groups with 10 rats in each group were fed the two vitamin E–deficient diets. After 9 wk, five rats from each group were killed. These were the depleted rats. Three rats from each group were depleted for a total of 12 wk and then repleted by intraperitoneal injection of all-*rac*-α-tocopherol [2.5 mg/(rat · dose)] (Merck, Pointe Claire, Canada), dissolved in vitamin E–stripped corn oil (U.S. Biochemical, Cleveland, OH) (10 g/L), one dose on every other day. After 1 wk of repletion during which each rat received three doses of α-T injections, the two groups of repleted rats were killed. The remaining two rats in each group were depleted for a total of 16 wk and subjected to the radioisotope tracer experiment. The labeled tracer, 5-methyl-^{14}C-*RRR*-α-T (generously provided by Eisai Pharmaceutical, Tokyo, Japan as a gift) was purified by TLC. Two batches were purified, and the specific radioactivities were 598.7 and 346.0 kBq/mg, respectively. Each batch was used for a rat from each group. The purified tracer was dissolved in vitamin E–stripped corn oil (2.69 and 2.46 g/L for the two batches, respectively). Two vitamin E–depleted rats from each group were injected intraperitoneally with the purified tracer (740 kBq/kg body weight) and transferred to metabolic cages. Feces and urine were collected each morning and evening for1 wk. The expired CO_2 during this period was collected by introducing the expired air into a saturated KOH solution. At the end of the week, these four rats were also killed.

Tissue sampling and preparation. The night before killing, food cups were removed. Blood was drawn from the abdominal vena cava into a EDTA-containing syringe. Upon killing, the weanling rats, depleted rats, repleted rats and the radioisotope-injected rats were asphyxiated by CO_2. Liver, kidney, heart, spleen, lung, brain, adrenal gland, testis, epididymal fat pad and vastus lateralis muscle were excised, weighed and stored at −30°C. Aliquots of blood were centrifuged at 1000 × *g* for 10 min and the separated plasma and RBC were stored at −30°C. For rats administered the radioisotope,

the gastrointestinal tract was also excised. Stomach, small intestine, cecum and colon were collected. The contents in stomach and small intestine were separated from the wall. The carcass, along with the tissues, was stored at −30°C. The two groups of rats that were fed the normal vitamin E diets were killed in a similar manner, but only plasma samples were collected.

Analysis. The detailed procedure for the determination of α-T concentration was described (Huang and Shaw 1994). Tissue samples were homogenized, saponified, extracted by *n*-hexane and analyzed by reverse-phase HPLC. The assay for plasma pyruvate kinase activity was according to the method of Bucher and Pfleiderer (1955).

The radioactivity of the feces, urine, expired CO_2, tissues and carcass of the isotope-traced rats was measured by liquid scintillation counting (LS 5000CE, Beckman, Buckinghamshire, UK). Solid samples (tissues, feces) were minced with scissors, mixed thoroughly and aliquots were mixed with the appropriate volume of Solvable reagent™ (NEN, Dupont, Boston, MA). Aliquots of liquid samples (urine, blood, plasma and RBC) were also mixed with the appropriate volume of Solvable reagent™. The mixtures were incubated at 50°C until clear. If the solubilized solution was colored, appropriate amounts of 30% hydrogen peroxide were added and incubated at room temperature until the color was bleached. For RBC, small amounts of an EDTA solution were added prior to the addition of hydrogen peroxide to minimize the bubbling. Ten milliliters of the scintillation fluid (Formula-989, NEN, Dupont) was added to each of the solubilized samples, mixed and counted. The carcasses were immersed in 500 mL of alcoholic KOH (4.46 mol/L) for 4–5 d until solubilized. Aliquots of these KOH solutions were mixed with the scintillation fluid and counted.

Statistical analysis. Data are expressed as mean ± SD except those of the radioisotope tracer experiment in which data of individual rats are presented. For body weight gain, food intake and feed efficiency, the significance of differences between control and OFO groups were analyzed statistically by one-way ANOVA. To test the significance of the effects of fat source, vitamin E repletion and their interaction on blood vitamin E status indicators and tissue α-T, data from the four groups (depleted control, depleted OFO, repleted control and repleted OFO) were analyzed by two-way ANOVA. In addition, to compare the tissue α-T after depletion and after repletion with that before depletion, the data were further analyzed by one-way ANOVA and Duncan's multiple range test. Because the variance of the tissue α-T data was not homogeneous, data were transformed to log values and then subjected to the statistical analysis. The General Linear Model of the SAS system (SAS Institute, Cary, NC) was employed and differences were considered significant at $P < 0.05$.

RESULTS

Growth, food intake and feed efficiency. Rats fed the vitamin E–devoid OFO diet had significantly lower body weight gain and food intake than rats fed the vitamin E–deficient control diet ($P < 0.05$)(**Table 1**). However, the two groups of repleted rats had comparable body weight gains and food intakes ($P > 0.05$). The feed efficiency was not significantly different between the two depleted groups or between the repleted groups.

Vitamin E status indicators in blood. The plasma PK activity of rats fed control and OFO diets supplemented with 50 mg/kg diet of all-*rac*-α-tocopheryl acetate for 9 wk were 94.9 ± 5.4 and 123.6 ± 16.3 μmol/(min · L) for the control and OFO groups, respectively. The plasma PK activities of both the depleted groups were four- to fivefold this value (**Table 2**). When the two depleted groups were compared, the plasma PK activity was significantly higher in the OFO group ($P < 0.05$). In addition, the RBC α-T concentration of the OFO group was about one third that of the control group ($P < 0.05$).

For the repleted control rats, the RBC α-T was 2.3-fold and plasma PK activity was 44% of that of depleted controls ($P < 0.05$), indicating the amelioration of the vitamin E–deficient status. Compared with the depleted OFO group, the repleted OFO group also had greater RBC α-T and lower plasma PK after the week of repletion ($P < 0.05$). However, the plasma PK of the repleted OFO group was still 1.8-fold that of the repleted control group ($P < 0.05$).

Tissue α-T concentration. Compared with the initial values (before depletion), the tissue α-T concentrations of both depleted groups were drastically lower after 9 wk of vitamin E depletion except in brain and adrenal gland (**Table 3**). The difference generally was greater in the OFO group than in the control group. When the two groups of depleted rats were compared, the OFO group had significantly lower α-T concentration in adrenal gland, heart, kidney, liver, spleen, testis and muscle ($P < 0.05$). After 1 wk of repletion, tissue α-T was markedly greater in both groups. For the control group, the epididymal fat pad had the most striking accumulation of α-T. Liver, spleen, adrenal gland, testis and lung also accumulated significant amounts of α-T ($P < 0.05$, vs. depleted controls). However, the muscle α-T of the repleted controls was not significantly higher than in depleted controls. Repletion elevated the α-T concentration in most tissues of the control rats to a level either comparable to or higher than that of rats before depletion. For the OFO group, repletion significantly increased α-T in all tissues except for brain ($P < 0.05$, vs. depleted OFO group). Compared with the repleted control group, the repleted OFO group had significantly lower α-T in the adrenal gland, epididymal fat, liver and spleen ($P < 0.05$). The α-T concentration in epididymal fat, kidney and liver of the repleted OFO rats was still significantly lower than

TABLE 1

The body weight gain, food intake and feed efficiency of rats fed the vitamin E–deficient diets containing 15% oxidized frying soybean oil (OFO) or control soybean oil for 9 wk (depleted) and injected intraperitoneally with α-tocopherol for 1 wk (repleted)[1,2]

	Body weight gain		Food intake		Feed efficiency	
Group	Depleted	Repleted	Depleted	Repleted	Depleted	Repleted
	g/wk				*body wt gain(g)/food intake(g)*	
Control	34.65 ± 1.72[b]	29.40 ± 0.13	104.21 ± 5.88[b]	104.63 ± 1.51	0.33 ± 0.01	0.28 ± 0.06
OFO	28.74 ± 2.66[a]	28.17 ± 0.04	89.16 ± 6.60[a]	104.47 ± 2.65	0.32 ± 0.01	0.27 ± 0.02

[1] Each value represents the mean ± SD for five rats in the depleted groups, and for three rats in the repleted groups. Depleted groups, weanling rats were fed the vitamin E–deficient diets containing 15% oxidized frying oil (OFO) or vitamin E–stripped fresh soybean oil (control) for 9 wk. Repleted groups, rats fed the two depleted diets for 12 wk were injected intraperitoneally with 2.5 mg all-*rac*-α-tocopherol on every other day for 1 wk.

[2] Values not sharing the same superscript letter in a column are significantly different by ANOVA ($P < 0.05$).

those of rats before depletion. Analysis of data from the four groups using two-way ANOVA showed that fat source had a significant effect on α-T in all tissues except in brain and lung (Table 3). As expected, vitamin E repletion had a significant effect on α-T concentration in all tissues. A significant interaction of these two factors was found for α-T concentrations in epididymal fat and muscle ($P < 0.05$).

Excretions and distribution of the radioactivity. The urinary and fecal excretions of the radioactivity within the week after the administration of ^{14}C-α-tocopherol are shown in **Figure 1**. The rats of the OFO group excreted 170% more radioactivity in urine than the control rats on the first day (Fig. 1*A*). The accumulated percentage dose in urine of the two OFO-fed rats for the week was 1.3-fold greater than that of the two control rats (**Table 4**). Within the first day after dosing, the radioactivity excreted in feces of the two groups was equally low (Fig. 1*B*). However, from the second day on and through the rest of the week, the OFO rats excreted more radioactivity in feces than the control rats. The accumulated percentage dose from feces of the OFO rats for the week was 1.7-fold that of the control rats (Table 4).

Table 5 shows the distribution of radioactivity in the organs and tissues. The liver, epididymal fat pad and gastrointestinal tract accumulated more radioactivity than other tissues. The OFO rats apparently had

TABLE 2

The RBC α-tocopherol concentration and plasma pyruvate kinase activity of rats fed the vitamin E–deficient diets containing 15% oxidized frying soybean oil (OFO) or control soybean oil for 9 wk (depleted) and injected intraperitoneally with α-tocopherol for 1 wk (repleted)[1,2,3]

	RBC α-tocopherol		Plasma pyruvate kinase activity	
Group	Depleted	Repleted	Depleted	Repleted
	μmol/L packed cells		*μmol/(min · L plasma)*	
Control	0.46 ± 0.23[b]	1.09 ± 0.30[c]	423.2 ± 38.6[b]	186.6 ± 10.6[a]
OFO	0.16 ± 0.02[a]	0.65 ± 0.09[bc]	575.5 ± 50.9[c]	342.6 ± 62.2[b]
			P values	
Vitamin E repletion	0.0054		0.0001	
Fat source	0.0265		0.0004	
Vitamin E × fat source	0.6151		0.9472	

[1] Each value represents the mean ± SD for five rats in the depleted groups, and for three rats in the repleted groups. Depleted groups, weanling rats were fed the vitamin E–devoid diets containing 15% oxidized frying soybean oil (OFO) or vitamin E–stripped fresh soybean oil (control) for 9 wk. Repleted groups, rats fed the two depleted diets for 12 wk were injected intraperitoneally with 2.5 mg all-*rac*-α-tocopherol on every other day for 1 wk.

[2] The effects of vitamin E repletion, dietary fat source and their interaction were analyzed by two-way ANOVA. Data were also analyzed by Duncan's multiple range test. Values not sharing the same superscript letters among the four groups are significantly different ($P < 0.05$). Data were transformed to log values before analysis.

[3] The plasma pyruvate kinase activity of a group of rats fed the control diet supplemented with 50 mg/kg diet of all-*rac*-α-tocopheryl acetate was 101 μmol/(min · L plasma).

TABLE 3

The tissue α-tocopherol concentrations of rats fed the control soybean oil or oxidized frying soybean oil (OFO) diets before depletion, after depletion and after repletion with vitamin E[1,2]

	Before depletion	Depleted		Repleted		Treatment		
		Control	OFO	Control	OFO	Vitamin E repletion	Fat source	Vitamin E × Fat source
	nmol/g tissue					*P value*		
Adrenal gland	9.94 ± 1.95[b]	7.03 ± 4.13[b]	2.55 ± 0.05[c]	19.78 ± 3.44[a]	10.6 ± 2.60[b]	0.0001	0.0053	0.6453
Brain	2.23 ± 0.32[ab]	2.48 ± 0.67[ab]	1.83 ± 1.16[b]	4.09 ± 1.46[a]	3.92 ± 1.44[ab]	0.0370	0.3911	0.4727
Epididymal fat	8.06 ± 2.32[a]	0.19 ± 0.14[c]	0.07 ± 0.02[c]	15.30 ± 5.78[a]	1.72 ± 0.35[b]	0.0001	0.0008	0.0275
Heart	4.62 ± 2.55[a]	1.25 ± 0.39[b]	0.37 ± 0.33[c]	3.09 ± 0.81[ab]	1.07 ± 0.49[ab]	0.0097	0.0044	0.5597
Kidney	9.17 ± 1.78[a]	1.23 ± 0.56[b]	0.46 ± 0.23[c]	2.46 ± 0.49[b]	1.67 ± 1.09[b]	0.0113	0.0275	0.5660
Liver	10.89 ± 1.11[a]	0.79 ± 0.16[c]	0.42 ± 0.14[d]	12.24 ± 2.48[a]	6.43 ± 2.44[b]	0.0001	0.0016	0.9461
Lung	4.55 ± 2.46[a]	1.00 ± 0.51[b]	0.70 ± 0.23[b]	5.74 ± 2.34[a]	4.50 ± 3.09[a]	0.0004	0.3398	0.8446
Spleen	2.02 ± 0.98[c]	0.39 ± 0.14[d]	0.05 ± 0.02[e]	12.33 ± 0.54[a]	4.37 ± 2.41[b]	0.0001	0.0001	0.0679
Testis	9.31 ± 5.85[a]	1.79 ± 0.30[b]	0.72 ± 0.39[c]	10.45 ± 5.62[a]	7.69 ± 2.07[a]	0.0001	0.0315	0.1260
Muscle	2.83 ± 2.46[a]	0.54 ± 0.21[b]	0.14 ± 0.02[c]	0.81 ± 0.32[ab]	0.79 ± 0.28[ab]	0.0004	0.0101	0.0112

[1] Values represent the means ± SD for five rats (depletion groups) or three rats (before depletion group and repletion groups). Tissue α-tocopherol concentration is expressed as nmol/g tissue. Depleted groups, weanling rats were fed the vitamin E–devoid diets containing 15% oxidized frying oil (OFO) or vitamin E–stripped fresh soybean oil (control) for 9 wk. Repleted groups, rats fed the two depleted diets for 12 wk were injected intraperitoneally with 2.5 mg all-*rac*-α-tocopherol on every other day for 1 wk. Before depletion indicates the weanling rats before the feeding regimen started.

[2] The effects of vitamin E repletion, dietary fat source and their interaction were analyzed by two-way ANOVA, excluding data of the before depletion group. All five groups of data were also compared by Duncan's multiple range test. Values not sharing the same superscript letters in a row are significantly different ($P < 0.05$). Data were transformed to log values before analysis.

a lower distribution of radioactivity in adrenal gland, epididymal fat pad, kidney, liver, spleen, stomach, and small intestine than the control rats. The distribution of the dosed radioactivity in the excreta, visceral organs/tissues and the carcass is presented in Table 4. The radioactivity in expired carbon dioxide was very low (0.03% of dose) and not different among the four rats. The percentage of dosed radioactivity distributed in the carcass of the two groups of rats was comparable. However, the OFO-fed rats excreted more radioactivity in urine and feces, and retained less radioactivity in the viscera and brain (Table 4).

DISCUSSION

The requirement of normal rats for vitamin E has been estimated to be 20 mg/kg diet of all-*rac*-α-tocopheryl acetate and hence about 16 mg/kg diet of α-tocopherol equivalent (Bieri 1972). The AIN-76 diet formula recommends a vitamin E level of 50 mg/kg diet of all-*rac*-α-tocopheryl acetate (AIN 1977). The calculated α-tocopherol equivalent of the control and OFO diets used in this study was 1.79 and 1.96 mg/kg diet, respectively. The vitamin E–deficient status of rats fed these diets for 9 wk was evident from the elevated plasma PK activity and reduced tissue α-T. Elevated plasma PK activity has been shown in vitamin E–deficient rats (Chow 1975 and 1990); this is considered a result of muscular degeneration caused by vitamin E deficiency (Machlin et al. 1978).

The decreases in tissue α-T concentration after vitamin E depletion could be ascribed to tissue growth and tissue α-T turnover and/or catabolism. Ingold et al. (1987) demonstrated that the half-lives of α-T in rat tissues are as follows: lung, 7.6 d; liver, 9.8 d; plasma, 10.9 d; kidney, 11.2 d; heart, 13.3 d; muscle, 15.8 d; spleen, 16.6 d; brain, 29.4 d; testes, 33.3 d and epididymal fat, 34.8 d. When the tissue total α-T of the control group was calculated from tissue weight and the concentration of α-T, it was found that for those tissues with longer α-T half-life such as epididymal fat, brain and testis, the decreases in the α-T concentration were mainly from the increase in tissue weight. For tissues with shorter α-T half-life such as liver and kidney, the total tissue α-T also decreased markedly. However, the more severe vitamin E–deficient status of the OFO group compared with the control group could not be explained by differences in organ weight. Because there was no exogenous source of α-T for the body through the depletion period, the inferior vitamin E status indicated an enhanced α-T turnover in the rat body. Because OFO interferes with vitamin E absorption (Liu and Huang 1995), this enhanced turnover could be due in part to the effect that OFO exerted on the presumable entero-hepatic recirculation/reabsorption of α-T in the intestine, although very little is known about this recirculation of α-T, especially in vitamin E–deficient animals. Nevertheless, the enhanced depletion of

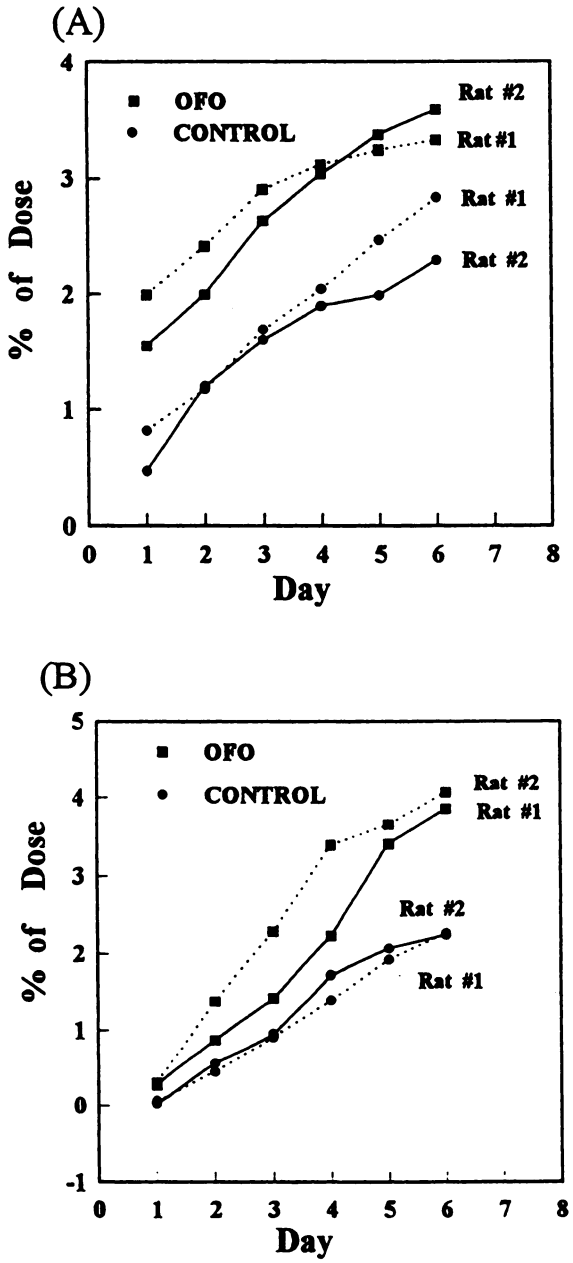

FIGURE 1 The accumulative radioactivity excreted in urine (A) and feces (B) within 1 wk after rats fed vitamin E–deficient oxidized frying oil (OFO) or control rats were injected intraperitoneally with ^{14}C-α-tocopherol. Rats were fed the vitamin E–devoid control or OFO diets for 16 wk and injected intraperitoneally with 740 kBq/kg body weight of 5-methyl-^{14}C-*RRR*-α-tocopherol (specific activity: 598.7 kBq/mg for the #2 rats, 346.0 kBq/mg for the #1 rats). Two rats per group and each curve indicate data of an individual rat.

tissue α-T in the OFO group may also arise from enhanced turnover and/or catabolism of α-T in some tissues.

The dose used for repletion in this study [2.5 mg/(rat · 2 d)] was equivalent to the dietary vitamin E intake from an AIN-76 diet by an adult rat (assuming a daily food intake of 25 g). Repletion elevated α-T in all tissues. Among these, muscle had the lowest replenishment in α-T. This may explain why the plasma PK activity did not return to normal after the week of repletion because the elevated plasma PK is believed to originate from the muscle damaged by vitamin E deficiency (Machlin et al. 1978). On the other hand, the accumulation of α-T was most striking in epididymal fat and liver in the control group. This agrees with previous reports (Bieri 1972, Machlin and Gabriel 1982).

The response of the OFO group to repletion was less than that of the controls. Not only was the plasma PK activity significantly higher (1.8-fold), but the α-T concentration in adrenal gland, epididymal fat pad, liver and spleen were also significantly lower in the repleted OFO group than in the repleted control group. In contrast to the control group, replenishment of α-T in the epididymal fat was far less in the OFO group. Because the tissue concentration is determined by the uptake and turnover during the repletion period, either the turnover/catabolism was enhanced or the tissue uptake was hampered by the feeding of OFO.

As reported (Gallo-Torres 1980), the excretion of the injected radioactive α-T was mainly via the urinary and fecal routes. The accumulated percentage dose excreted was approximately equal via these two routes. However, excretion of radioactivity in urine seems to be an earlier event than that in the feces because most of the urinary radioactivity was excreted within the first 4 d, whereas that appearing in the feces during the first 2 d was relatively low. Besides the GI tract, epididymal fat and liver retained high radioactivity among the major organs, which is in agreement with the results of the repletion experiment.

Assuming that the dose of the injected α-T was not a limiting factor, the depleted OFO rats theoretically should retain more of the administered α-T in their tissues because they originally had lower tissue α-T concentration and should have more "tissue sites" for α-T. However, despite the inferior vitamin E status after depletion, the OFO group excreted a higher percentage dose of radioactivity than the control group, via both urinary and fecal routes, and retained less radioactivity in the major organs, especially plasma, adipose tissue, adrenal gland, liver, spleen and the gastrointestinal tract, in agreement with data obtained in the repletion study. The higher excretion of radioactivity in feces could be attributed to an increased secretion of the tracer into the bile and a decreased entero-hepatic recirculation/ reabsorption. However, the elevated urinary excretion of radioactivity could result from a change in the handling of the administered α-tocopherol occurring in a part of the body other than the digestive tract. Whatever the mechanism may be, the observed high excretion and low retention of the tracer may indicate an enhanced α-T catabolism/turnover in rats fed the OFO-containing diet. Therefore, the results of the radioisotope tracer study support our hypothesis that feeding the OFO could enhance α-T catabolism

TABLE 4

Recovery (percentage of dose) of radioactivity 1 wk after vitamin E–depleted oxidized frying oil (OFO) or control rats were injected intraperitoneally with ^{14}C-α-tocopherol[1]

Diet group	Rat no.	Body weight	Total injected dose	Excretion				Tissues[2]	Carcass	Total
				Urine (U)	Feces (F)	(U + F)	CO_2			
		g	*kBq/mg*	*% of dose*						
Control	(#1)	500	425.5/1.23	2.84	2.27	5.11	0.03	5.96	75.37	86.71
	(#2)	436	351.5/0.59	2.29	2.51	4.80	0.03	7.43	55.60	68.03
OFO	(#1)	421	339.7/0.99	3.59	3.86	7.18	0.03	1.30	79.40	88.34
	(#2)	368	260.5/0.43	3.33	4.06	7.39	0.03	3.41	42.94	53.93

[1] Rats were fed the vitamin E–devoid control or OFO diets for 16 wk and injected intraperitoneally with 740 kBq/kg body weight of 5-methyl-^{14}C-*RRR*-α-tocopherol (specific activity: 598.7 kBq/mg for the #2 rats, 346.0 kBq/mg for the #1 rats). Tissues were analyzed for radioactivity 1 wk after the administration of the isotope tracer.

[2] Tissues include adrenal gland, brain, heart, kidney, lung, liver, spleen, testis, stomach (content and wall), small intestine (content and wall), cecum and colon, as shown in Table 5. Radioactivity in the epididymal fat pad and vastus lateralis muscle shown in Table 5 is not included.

TABLE 5

The distribution of radioactivity in percentage dose tissue of rats fed the vitamin–E deficient control or oxidized frying oil (OFO) diets for 16 wk and injected intraperitoneally with ^{14}C-α-tocopherol[1,2]

Rat no.	Control		OFO	
	1	2	1	2
	% dose/tissue			
RBC[3]	0.117	0.107	0.116	0.059
Plasma[3]	0.097	0.046	0.056	0.014
Epididymal fat pad	0.865	0.673	0.306	0.169
Vastus lateralis muscle	0.031	0.021	0.044	0.013
Adrenal gland	0.011	0.007	0.006	0.002
Brain	0.017	0.010	0.018	0.007
Heart	0.020	0.014	0.019	0.013
Kidney	0.054	0.027	0.046	0.016
Liver	0.760	1.789	0.312	1.318
Lung	0.035	0.033	0.031	0.030
Spleen	0.065	0.111	0.024	0.051
Testis	0.049	0.047	0.025	0.044
Stomach content	0.037	0.032	0.023	0.038
Stomach	0.267	0.361	0.009	0.020
Small intestine content	0.104	0.066	0.001	0.011
Small intestine	3.571	3.668	0.185	1.216
Cecum (content + wall)	0.607	0.346	0.452	0.268
Colon (content + wall)	0.367	0.915	0.153	0.280
Total[4]	5.964	7.426	1.304	3.073

[1] n = 2/group. Each value is a datum from an individual rat.

[2] Rats were fed the vitamin E devoid–control or OFO diets for 16 wk and injected intraperitoneally with 740 kBq/kg body weight of 5-methyl-^{14}C-*RRR*-α-tocopherol (specific activity: 598.7 kBq/mg for the #2 rats, 346.0 kBq/mg for the #1 rats). Tissues were analyzed for radioactivity 1 wk after the administration of the isotope tracer.

[3] It was assumed that the total weight (and volume) of blood is 7% of the body weight.

[4] Subtotal of the visceral organs and brain; radioactivity in blood, fat and muscle not included.

and/or turnover as well as interfere with the absorption of fat and fat-soluble substances.

The understanding of the metabolism of α-T and its regulation in the body is far from complete (Parker 1989). Early radioactive tracer studies described but did not identify conjugated forms of metabolites in feces, bile and urine (Gallo-Torres 1980, Parker 1989), such as tocopheryl quinone and tocopheronic acid/lactone (Simon et al. 1956) which were not identical to α-T. More recently, Bjørneboe et al. (1987a) and Ingold et al. (1987) demonstrated that some of the α-T in the body may be turned over and excreted without further catabolism. Because the tracer compound excreted was not further identified in the present study, it is not known if the OFO diet enhanced turnover or catabolism.

The tissue α-T that is "turned over" and "catabolized" is that lost by chemical and physical processes. The chemical processes that consume α-T, for example, include the oxidation of α-T by lipid peroxyl radicals. The α-tocopheryl radical produced will be converted to its oxidation product if it is not regenerated by ascorbate or other water-soluble reducing agents. The physical processes include, for example, cell death and exchange into plasma or lymph.

Lipid oxidation occurs during frying by free radical–mediated reactions. However, under the high temperature conditions of frying, the lipoxy radical is not stable. These radicals rapidly react with adjacent molecules and produce a wide range of secondary products such as polymers and carbonyl compounds (Paulose and Chang 1973, Chang et al. 1978). Although lipid hydroperoxides, the primary lipid oxidation products, can be converted to alkoxyl radicals in the presence of, for example, a metal catalyst, an oxidized frying oil usually has a much lower peroxide value and higher content of secondary products than an autoxidized lipid (Chang et al. 1978). The OFO used in the present study had a low peroxide value but contained substantial lev-

els of non-urea adductable fractions (Liu and Huang 1995). Furthermore, Bergan and Draper (1970) demonstrated in a radioisotope tracer study that the fatty acid hydroperoxide introduced into the gastrointestinal tract was converted to hydroxy and trienoic fatty acids during absorption. It is thus difficult to ascribe the enhanced disappearance of tissue α-T to the lipid hydroperoxide ingested from the OFO.

On the other hand, we did not observe a significant loss of α-T when it was added into the OFO and incubated at 37°C for up to 10 d in our previous study (Liu and Huang 1995). It is thus unlikely that the lipid oxidation products in the OFO would directly react and destroy α-T. A biological process elicited by the ingestion of OFO seems to be involved in the enhanced catabolism/turnover of α-T.

Although the mechanism is not understood, the secondary products of lipid oxidation (Kanazawa et al. 1985 and 1986) and compounds isolated from these fractions such as 9-oxononanoic acid (Minamoto et al. 1985) and 12-keto oleic acid (Fukuzawa and Sato 1975) were shown to increase tissue TBARS or lipid peroxidation. Increased tissue TBARS in rats fed the OFO diet was also observed in our previous study (Liu and Huang 1995) and by others (Izaki et al. 1984, Kok et al. 1988). Methyl ethyl ketone peroxide was shown to decrease liver α-T (Warren and Reed 1991). Enhanced lipid peroxidation by the insult of secondary oxidation product in OFO may result in the increased consumption and metabolism of α-T. More studies are required to confirm this speculation.

Reactive oxygen species can be generated in the microsomal cytochrome P-450 catalyzed reactions (Terelius and Ingelman-Sundberg 1988). The potential of free radical generation varies among different isoforms. The P450 2E1, the isoform specifically induced by ethanol, has a relatively high potential in free radical generation and lipid peroxidation (Ekström and Ingelman-Sundberg 1989). Long-term (5–6 wk) ethanol feeding reduced α-T content (by 25%) in the liver but not in the peripheral tissues (Bjørneboe et al. 1987b). In contrast, administration of polychlorobiphenyls, a xenobiotic, increased absorption and tissue concentration of α-T (Koremura et al. 1990). Like xenobiotics and ethanol administration, OFO feeding can induce hepatic microsomal cytochrome P-450 (Huang et al. 1988) but the isoform(s) has not been identified. The role of the induced hepatic microsomal phase I and phase II drug-metabolizing enzymes in the enhanced α-T catabolism/turnover cannot be elucidated by results obtained in this study.

Body weight gain and food intake were not affected by dietary OFO when a nutritionally balanced diet was fed (Huang et al. 1988, Liu and Huang 1995). In this study, however, these indicators of gross toxicity in the OFO group were significant in the vitamin E–deficient animals, but not after repletion. This suggests the importance of vitamin E in protection against OFO toxicity.

The data collected in the present study, i.e., exacerbated vitamin E deficient status upon depletion, less amelioration upon repletion, and most of all, more of the parenterally administered radioisotope-labeled α-T tracer excreted, all support our hypothesis that ingestion of the oxidized frying oil–containing diet accelerated α-T catabolism/turnover in the body. The observed effects of dietary oxidized frying oil may have significance in oxidative stress–related diseases and pathological conditions (Kubow 1993).

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