CHAPTER 4

DISTRIBUTION AND BIOAVAILABILITY OF TOCOTRIENOLS IN ANIMALS

4.1 INTRODUCTION

4.1.1 Tocotrienols in Palm Oil

Tocotrienols and tocopherols, better known as vitamin E, are commonly present as minor components in vegetable oils at a concentration of less than 1000 ppm (see Table 4.1) [196,444,587]. Among the common edible oils, palm oil is the richest source in providing natural tocotrienols [507,683,684] which constituted about 83% of its total vitamin E. Two types of tocotrienols (i.e. α-T_3 and γ-T_3) were reported to be present in palm oil in the 1950's [228,661]. Four major types of vitamin E components are now known to be present in palm oil, namely α-T (17%), α-T_3 (23%), γ-T_3 (49%) and δ-T_3 (11%). Vitamin E is present in crude palm oil at a concentration of 600-1000 ppm [195,622], and the vitamin E contents in other palm oil products obtained from various stages of processing are shown in Table 4.2. In palm fatty acid distillate (PFAD), a by-product from physical refining of palm oil, the vitamin E is present at the highest concentration, i.e. about 0.4% [200] but usually varies from 0.16% to 1% [492]. The vitamin E accumulated in the PFAD has become an important source of tocotrienols [199], whereas oil palm leaves with a vitamin E content of 0.5% (dry weight basis) is also a potential source of vitamin E [197,198,321].

4.1.2 Bioavailability of Vitamin E

"Bioavailability" is a study on the rate and extent of absorption of a substance from its dosage form to reach circulation. Distribution and bioavailability studies of vitamin E components are important in view of the fact that although these vitamers have similar structural skeletons, chemical properties and antioxidant activities, they may not be bioequivalent in various biological aspects and clinical functions. Since plasma vitamin E levels increase following oral administration of vitamin E [27], the relative biopotency of various forms of vitamin E could also be expected to closely relate to their bioavailability.

Table 4.1 Vitamin E composition in various vegetable oils (ppm)

Oil	Tocopherols*				Tocotrienols*				Total
	α-T	β-T	γ-T	δ-T	α-T_3	β-T_3	γ-T_3	δ-T_3	
Coconut oil	5	-	-	6	5	1	19	-	36
Corn oil	112	50	602	18	-	-	-	-	782
Cottonseed oil	389	-	387	-	-	-	-	-	776
Olive oil	51	-	-	-	-	-	-	-	51
Palm oil	152	-	-	-	205	-	439	94	890
Peanut oil	130	-	216	21	-	-	-	-	367
Rice bran oil	324	18	53	-	236	-	349	-	980
Safflower oil	387	-	174	240	-	-	-	-	801
Sesame oil	12	6	244	32	-	-	-	-	294
Soyabean oil	101	-	593	264	-	-	-	-	958
Sunflower oil	487	-	51	8	-	-	-	-	546

* Structures of the tocopherols and tocotrienols are shown in page 3.

Table 4.2 Vitamin E content in various palm oil products

Palm oil product	Total vitamin E / ppm
Crude palm oil	600-1000
Crude palm olein	800-1000
Crude palm stearin	250-530
Palm kernel oil	80-100
RBD* palm oil	356-630
RBD* palm olein	468-673
Palm fatty acid distillate	1600-10000
Oil palm leaflets (dry basis)	5000

* RBD = Refined, bleached and deodorized.

Many nutritional studies on tocopherols [28,36,37,51,104,248,308,624] have shown that the plasma content of α-T is a few times higher than γ-T despite the diets containing more γ-T than α-T [51,52]. Oral administration of either *RRR*-α-T or all-*rac*-α-T also resulted in a remarkable increase of α-T and a decrease of γ-T concentration in the plasma [128,36,242]. Various reasons have been advanced to explain such observations including discriminating absorption, secretion, excretion, transport, uptake, retention or deposition differences among various vitamin E components, and the most profound explanation is the presence of a highly specific binding protein [33,96,442]. All of these factors must be taken into consideration when investigations on the bioavailability of vitamin E components is to be related to their biological potencies.

4.1.3 Fate of Ingested Vitamin E Components in Humans and Animals

Absorption

Dietary tocopherols are absorbed in the intestine and secreted into the lymph within triglyceride-rich chylomicrons [56,391,546,636]. In the absence or presence of a 50-fold excess of α-T, similar concentration of γ-T was secreted in the chylomicrons [634]. The chylomicrons are rapidly catabolized in the circulation by lipoprotein lipase to form chylomicron remnants [534] and in the meantime transfer fatty acids [451] as well as some of the vitamin E to extrahepatic tissues [39]. The chylomicron remnants deliver the remainder of the vitamin E to liver through remnant receptors by hepatic parenchymal cells [396,571]. Lipoprotein-lipase-deficient patients have most of the circulating tocopherols in the chylomicrons [596] as well as very low density lipoproteins (VLDL) up to 24 hours in response to oral supplementation of tocopherols [638]. Previously, a higher level of plasma α-T was thought to be the result of preferential absorption of α-T over γ-T [28] but this has been suggested as due to a specific intestinal receptor which has a higher affinity for α-T than for γ-T [36,242,505]. In recent years, compelling evidence obtained demonstrated that α-T and γ-T are equally well absorbed from human intestine and secreted within chylomicrons [200,418,503]. Similar increases of both α-T and γ-T for up

to 12 hours were found in the plasma after an administration of α-T and γ-T [638]. In fact, an earlier report using radiolabelled compounds demonstrated that there is no difference in the absorption of α-T and γ-T [51].

Secretion and excretion

Vitamin E delivered to the liver is resecreted in plasma VLDL [57,109,639,640] which becomes the source of vitamin E in low density lipoprotein (LDL) and high density lipoprotein (HDL). After injection of α-T and γ-T into rats, γ-T was found excreted more rapidly than the α-T [503], and it has been suggested that γ-T is much more rapidly excreted than α-T in most tissues [38,212,503]. Compared to the γ-T, α-T is preferentially secreted by the liver within nascent lipoproteins [418,637]. The α-T:γ-T ratio was not appreciably different between the bile and plasma of patients undergoing gall bladder surgery [638]. Therefore, there is still inadequate research data to support the hypothesis that γ-T is excreted by the liver into bile [253,418].

Transport and uptake

Vitamin E is transported in blood within plasma lipoproteins [194,393]; LDL and HDL are the major carriers of vitamin E [34,59,129,327,383,409,506,548,620], and the vitamin E is transferable between the LDL and HDL. Since HDL and LDL exchange their tocopherols rapidly [58,402], simultaneous increases of the tocopherols in HDL and LDL have been observed [638]. The vitamin E is delivered to extrahepatic tissues mainly *via* a mechanism mediated by a specific LDL receptor [110,629,633]. In Watanabe heritable hyperlipidemic (WHHL) rabbits which are deficient in LDL receptor activity, elevated plasma levels of α-T and triglycerides, cholesterol and phospholipids were observed despite the fact that their liver α-T levels were similar to those in normal rabbits [110]. It has been shown that uptake of α-T and γ-T by tissues are almost equally efficient [212,503]; however, α-T is always found to be the major vitamin E in plasma and tissues

[520]. Plasma γ-T appears to come from chylomicrons through catabolism, and transfer to HDL [638].

Retention and disappearance

A lot of uncertainties still remain regarding the disappearance of γ-T. For example, discrepancies arise from some reports pertaining to a decrease in plasma γ-T after a high dose of oral supplementation of α-T [28,36,40,607]. The retention of α-T and disappearance of γ-T levels in vitamin E-deficient rats after oral administration of γ-T, have been rationalized as a sparing effect of γ-T on α-T [287]. Compared to α-T, lesser γ-T secreted in VLDL leads to a lower level of γ-T in plasma and tissues [638]; a faster disappearance of γ-T over α-T may be another factor to explain the low level of γ-T in tissues [524]. Catabolism of chylomicrons, uptake of chylomicron remnants and secretion of VLDL by the liver were suggested to be responsible for the disappearance of γ-T from the plasma [638]. It has been reported that tocopherols were more efficiently absorbed than the corresponding tocotrienols in rat's liver [505]. However, the actual mechanism for the metabolism of the vitamin E components which are not retained is not yet fully understood.

Tocopherol binding protein

Liver tocopherol binding protein isolated from rats has an affinity for α-T, a few-fold higher than for γ-T [33,96,430,442]. This protein was thought to determine the incorporation of α-T or γ-T into nascent lipoprotein particles prior to secretion from the liver [638].

4.1.4 Biokinetics and Biodiscrimination among Vitamin E Stereoisomers

The plasma tocopherol level after oral administration of *RRR*-α-tocopheryl acetate was found to be higher when compared to those supplemented with all-*rac*-α-tocopheryl acetate which consisted of eight types of stereoisomers [4,279]. Competitive biokinetic

experiments using deuterium labelled compounds indicated that *RRR*-α-tocopheryl acetate is more bioavailable than the *rac*-α-tocopheryl acetate, by a ratio of about 2:1 [5]. Results of Ingold *et al.* have demonstrated that bioavailability of *RRR*-α-T was greater than the *SRR*-α-T [290], and retention of the *RRR*-α-T is more effective than the *SSR*-α-T [88]. However, only a small preferential uptake of the *RRR*-α-T compared to other stereoisomers was observed when rats fed low levels of *RRR*-α-T or all-*rac*-α-T (i.e. 35 ppm in the diet), but no discrimination when rats were given 200 ppm of the vitamin E in the diet; the results suggest that the α-T uptake by tissues is not entirely specific for *RRR*-α-T [39]. Although α-T stereoisomers with the 2*R* configuration accumulated preferentially over the 2*S* stereoisomers, there was no significant discrimination among the 2*R* stereoisomers (i.e. *RRR*, *RRS*, *RSR*, *RSS*) [674]. The absorption rates of the 2*S* and 2*R* enantiomers of α-T were not significantly different [702]. Experiments using rats [382,667,668,672], chickens [135,136], seals [156] and sheep [268] also indicated that the retention of the stereoisomers with the natural 2*R* configuration was higher than that of the 2*S* configuration. Studies in humans showed that *RRR*-α-T and *SRR*-α-T were secreted without discrimination in chylomicrons; however, *RRR*-α-T was preferentially secreted in VLDL [639].

4.1.5 Biosynthesis of Vitamin E in Plants

The biosynthesis of vitamin E in plants is well established, two possible pathways leading to the biosynthesis of α-T are the tocotrienol route and the tocopherol route as described in Fig. 4.1. The tocotrienol pathway probably occurs in oil palm and *Hevea* latex, involves methylation of the precursor, i.e. δ-T_3, to β-T_3 or γ-T_3, and further methylation to α-T_3; methylation followed by reduction of the unsaturated side chains of the tocotrienols will form various corresponding tocopherols [148,507]. In tocopherol pathway, δ-T is the major precursor of γ-T and α-T. Tyrosine has been demonstrated to be the carbon source for the aromatic carbon atoms and the 8-methyl carbon atom of α-T is incorporated through the homogentisate pathway [680]. The 5-methyl and 7-methyl

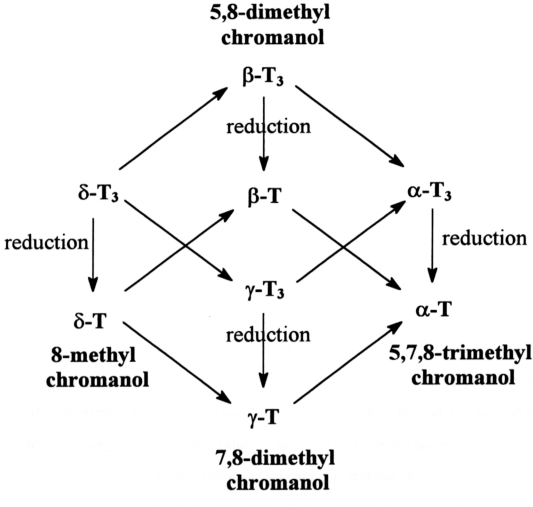


Fig. 4.1 Possible biosynthetic pathways of vitamin E in plants.

carbons in α-T and γ-T are derived from L-methionine [69,631,685] in the form of *S*-adenosylmethionine which are incorporated *via* the homogentisate pathway. The 5-methyl group of tocopherols has been demonstrated to be derived from methionine since incorporation into β-T has been noted in blue-green alga *Anabaena variabilis* [69]. This conversion also occurs in plants and has been proven by *in vitro* incorporation of ^{14}C-methyl-L-methionine using *Hevea* latex [685], *Hevea* leaves [562] and *Ficus elasticus* leaves [677]. The elevated level of incorporation of ^{14}C-label into other tocopherol forms [677] is in agreement with other workers' findings [631,681,682]. The transfer of tritium from the nicotinamide ring of stereospecifically labelled NADPH (*viz.* a coenzyme for the reduction process) to the isoprene residues of phytyl and α-T [677] has been reported and was detected in the side chain of α-T formed during biogenesis in seedlings of *Phaseolus vulgaris* and *Avena sativa* [676].

In the biosynthesis of α-T from δ-T_3, methylation appears to precede hydrogenation [677]. The unique vitamin E composition in *Hevea* latex and palm oil with tocotrienols being predominant is in contrast to many other vegetable oils, and seems to support the tocotrienol pathway in which reduction is effective only at the final stage to transform α-T_3 into α-T, thus only negligible amounts of other tocopherols (i.e. β-T, γ-T and δ-T) can be found in palm oil. However, to date it is still not clear what is the missing enzyme causing higher proportion of tocotrienols over tocopherols in palm oil and how it functions in other oils.

4.1.6 Objectives of the Present Research

The distribution and bioavailability of tocotrienols are not adequately documented in spite of the growing interest of these compounds to be used in the pharmaceutical and food industries. To explore the pharmacological uses of tocotrienols and their role in nutrition, it is of importance to understand the bioavailability of these compounds. Guinea pigs were orally fed with palm oil vitamin E mixture, subsequently the concentration and compositional changes of the various forms of vitamin E in the plasma, liver and kidney

were examined. Various purified vitamin E components were also added to semi-synthetic diets for rabbits; this was attempted to obtain more information on the overall bioavailability of ingested tocotrienols and their distribution in various organs.

4.2 EXPERIMENTAL

4.2.1 Vitamin E Feeding Experiments on Guinea Pigs

Five male guinea pigs, fed commercial pellets, were orally given palm-oil vitamin E mixture (denoted as POVE) at a daily dosage of 1000 mg per kg body weight for a period of two and a half months. The purity of the vitamin E mixture was 95.4% and its composition was 42.7% α-T, 20.1% α-T_3, 26.7% γ-T_3 and 10.5% δ-T_3. Six guinea pigs fed commercial pellets without administration of palm-oil vitamin E were used as time-matched controls. At the end of the treatment, animals were sacrificed and the vitamin E composition in their plasma, livers and kidneys were determined by HPLC.

4.2.2 Vitamin E Feeding Experiments on Rabbits

Experiment 1

New Zealand White rabbits were fed *ad libitum* with the following diets for 30 days:- (a) commercial rabbit pellets (CP), (b) semi-synthetic diet containing soyabean oil (SO), (c) semi-synthetic diet containing refined-bleached-deodorized palm olein (PO) or (d) semi-synthetic diet containing refined-bleached-deodorized palm olein and 1% (w/w) of palm-oil vitamin E (PO+E). The compositions of the vitamin E components in various diets in this experiment are given in Table 4.3. The compositions (weight %) of the vitamin E components for PO+E diet were 30.4% α-T, 13.1% α-T_3, 31.2% γ-T_3 and 25.1% δ-T_3. The composition of the semi-synthetic diets were 20% (w/w) commercial rabbit pellets and 80% (w/w) purified feed materials consisting of 15% (w/w) of dietary fat, 20% protein, 39% carbohydrate, 20% fibre, the remainder being vitamins and mineral mixtures.

Experiment 2

New Zealand White rabbits were fed palm-oil semi-synthetic diets and divided into groups based on the supplementation of vitamin E components as follows:- (a) diet enhanced with 186 mg γ-T_3 per kg diet (PO+γ-T_3), (b) diet enhanced with 863 mg δ-T

Table 4.3 Vitamin E composition in various diets (mg/kg)

Vitamin E	Diets *			
	CP	SO	PO	PO+E
α-TAc#	0	176	176	176
α-T	22	7	23	2817
α-T_3	5	0	16	1293
γ-T	0	52	< 1	0
γ-T_3	6	0	12	3068
δ-T	0	24	0	13
δ-T_3	5	0	18	2469
Total	38	259	246	9836
$T:T_3$ ‡	1.4:1	1:nil	4.3:1	0.44:1
[α]:[γ+δ] ‡	1:0.77	1:0.42	1:0.14	1:1.30

* CP = commercial pellets, SO = semi-synthetic diet contained soyabean oil, PO = semi-synthetic diet contained refined-bleached-deodorized palm olein; PO+E = semi-synthetic diet contained refined-bleached-deodorized palm olein and enriched with 1% of palm-oil vitamin E.

α-TAc is *dl*-α-tocopheryl acetate supplemented as α-T equivalent in the semi-synthetic diet.

‡ $T:T_3$ is the ratio of total tocopherols to the tocotrienols, [α]:[γ+δ] is the ratio of total α-vitamers to the sum of γ- and δ-vitamers.

per kg diet (PO+δ-T), (c) diet enhanced with *dl*-α-tocopheryl acetate, α-TAc (PO+α-T) and (d) diet depleted of α-T and without vitamin E enhancement (PO–E). Palm olein depleted of vitamin E used in this experiment was prepared by column chromatography using 0.063-0.200 mm neutral alumina (Merck) and *n*-hexane and the final α-T and α-T_3 contents contributed by the oils were reduced to less than 20 and 15 ppm, respectively. All of the semi-synthetic diets contained 5% (w/w) commercial rabbit pellets and 95% purified feed materials consisting of 15% palm olein, 20% casein, 40% dextrose monohydrate, 20% fibre and 0.5% cholesterol, the remainder being vitamins and mineral mixtures. Vitamin supplements specifically omitting vitamin E (i.e. *dl*-α-tocopheryl acetate) were added in the diets depleted of vitamin E, i.e. for dietary groups labelled PO+γ-T_3, PO+δ-T and PO–E. All of the purified materials were purchased from US Biochemical Corporation. γ-T_3 was purified from a tocotrienol mixture isolated from palm fatty acid distillate (PFAD) [218]. δ-T was purchased from Sigma Chemical Co. (St. Louis, MO), and was purified by column chromatography (40-63 μm (Merck) silica gel) prior to mixing in the semi-synthetic diet. Compositions of the vitamin E components in various diets are given in Table 4.4. All of the rabbits were fed with the respective dietary regimes for a duration of 12 weeks.

Experiment 3

New Zealand White rabbits fed *ad libitum* semi-synthetic diets enhanced with *dl*-α-tocopheryl acetate were divided into groups based on the type of dietary fats as follows:- (a) soyabean oil (SO), (b) coconut oil (87%) and corn oil (13%) mixture (CO), (c) refined-bleached-deodorized palm olein (PO) and (d) refined-bleached-deodorized palm olein without cholesterol supplement (POWC); the SO, CO and PO diets contained 0.5% of cholesterol. Rabbits fed with commercial pellets were also used for comparison. Formulation of the semi-synthetic diets was same as in Experiment 2, compositions of the vitamin E components in various diets are given in Table 4.5. All of the rabbits were treated with respective dietary regimes for a duration of 12 weeks.

Table 4.4 Vitamin E compositions in palm-oil diets enhanced with various vitamin E components (mg/kg)

Vitamin E	Palm-oil semi-synthetic diets*			
	PO+γ-T_3	PO+δ-T	PO+α-T	PO–E
α-TAc *	0	0	176	0
α-T	6	15	20	10
α-T_3	3	9	14	8
γ-T	0	34	0	0
γ-T_3	186	32	22	20
δ-T	0	863	0	0
δ-T_3	14	12	8	16
Total	209	965	240	54
% α-T	2.9	1.6	81.7	18.5
T:T_3 #	0.03:1	17.2:1	4.4:1	0.23:1
[α]:[γ+δ] #	1:22	1:39	1:0.14	1:2

* PO+γ-T_3 = diet enhanced with 186 mg γ-T_3 per kg diet, (b) PO+δ-T = diet enhanced with 863 mg δ-T per kg diet, (c) PO+α-T = diet enhanced with *dl*-α-tocopheryl acetate and (d) PO–E = palm oil diet depleted of α-T and without vitamin E supplementation. The weight of *dl*-α-tocopheryl acetate (α-TAc) is given in α-T equivalent.

T:T_3 is the ratio of the total tocopherols to the tocotrienols, [α]:[γ+δ] is the ratio of total α-vitamers to the sum of γ- and δ-vitamers. n is the number of rabbits in each group.

Table 4.5 Compositions of the vitamin E components in various diets (mg/kg)*

Vitamin E	Semi-synthetic diets enhanced with α-TAc				Commercial feed
	SO	CO	PO	POWC	
α-TAc *	176	176	176	176	0
α-T	2	13	20	18	22
α-T_3	0	0	14	13	5
γ-T	36	2	0	0	0
γ-T_3	0	0	22	20	16
δ-T	12	0	0	0	0
δ-T_3	0	1	8	8	5
Total	226	192	240	235	48
% α-T	79	98	82	83	46
$T:T_3$ #	1:nil	1:191	4.4:1	4.7:1	0.85:1
[α]:[γ+δ] #	3.7:1	63:1	7:1	6.1:1	1.3:1

* SO = diet contained soyabean oil, CO = diet contained coconut oil and corn oil, PO = diet contained refined-bleached-deodorized palm olein; diets for SO, CO and PO contained 0.5% cholesterol, POWC = diet contained refined-bleached-deodorized palm olein but without cholesterol supplementation. The weight of *dl*-α-tocopheryl acetate (α-TAc) is given in α-T equivalent.

$T:T_3$ is the ratio of total tocopherols to tocotrienols. [α]:[γ+δ] is the ratio of total α-vitamers to the sum of γ- and δ-vitamers

4.2.3 Analysis of Vitamin E

At the end of each dietary treatment, blood samples were collected from the overnight-fasted animals. Vitamin E compounds were extracted from the plasma according to the method of Handelman *et al.* [242], and vitamin E in the liver was extracted using the procedure of Lang *et al.* [372]. Analyses of tocotrienols and tocopherols were carried out by normal phase high performance liquid chromatography (HPLC) using a 250 x 4.6 mm, 5 μm silica column and a fluorescence detector (Waters 470); hexane-tetrahydrofuran-isopropanol (973.5:25:1.5) was used as the mobile phase.

Statistical analysis

Student's t test [592] was used for statistical evaluation of the results, differences at $p < 0.05$ were considered significant.

4.2.4 *In Vitro* Experiments with Rabbit Liver Microsomal Fraction

The following *in vitro* experiment was also attempted to investigate if any structural change (e.g. methylation) occurred when δ-tocotrienol (δ-T_3) was incubated with rabbit's liver microsomal fraction. The liver, excised from anaesthetized rabbit fed with commercial feed, was quickly washed with ice-cold saline solution and homogenized at 4°C in 10 volumes of ice-cold 0.05 M Tris buffer (pH 7.4) containing 0.3 M sucrose, 10 mM EDTA, 50 mM NaCl and 1 mM dithiothreitol, using a motor-driven tissue homogenizer [111]. The homogenates were centrifuged at 600xg for 5 minutes to obtain the supernatant. The microsomal fraction was prepared by differential centrifugation at 15,000xg for 15 minutes at 4°C using a Beckman L8-80M ultracentrifuge equipped with a swing bucket rotor SW28 to remove mitochondria and cell debris; the supernatant was further centrifuged at 105,000xg for 60 minutes to obtain a sediment microsomal pellet, which was resuspended in 0.05 M Tris buffer, pH 7.4, at a concentration of 1.0 g equivalent of liver tissues per mL of suspension. Two mL of the microsomal suspension was mixed with 2 mg δ-T_3 in 100 μl 0.00625% Tween 20 (0.25% w/v in acetone), 2.2

mg ATP, 3.1 mg NADPH and S-adenosyl-^{14}C-methyl-L-methionine 0.1 μCi (from NEN) [684,685]. The mixture was adjusted to a volume of 5 mL with buffer, incubated and gently agitated for 8 hours at 37°C. Aliquots (2.5 mL) were taken and vitamin E was extracted by ethanol-hexane (1:1). Various vitamin E components were separated by normal phase TLC using hexane-ethyl acetate (9:1) as mobile phase. Radioactivity of samples from each vitamin E fraction was determined using a Packard Liquid Scintillation Analyzer 2500TR.

4.3 RESULTS AND DISCUSSION

4.3.1 Distribution of Vitamin E Components in Guinea Pigs

Results on the distribution of various vitamin E components in the plasma and organs of the guinea pigs are given in Table 4.6. Palm-oil vitamin E (denoted as POVE) as much as 1000 mg per kg body weight per day supplemented to the guinea pigs for two and a half months did not show any toxic effect to the animals. Previous studies with mice and rats have also demonstrated that palm-oil vitamin E did not produce appreciable adverse effects [493]. A high dose of palm-oil vitamin E administration significantly increased the total vitamin E concentrations in the plasma, liver and kidney of the POVE guinea pigs by about 6 to 13 times greater than those in non-treated guinea pigs. For POVE animals, the major vitamin E components were tocopherols accounting for 87%, 99% and 84% of the total vitamin E in plasma, liver and kidney, respectively. More specifically, the α-T levels in plasma, liver and kidney were significantly increased from 0.32 ± 0.22 μg/mL (control) to 4.1 ± 1.9 μg/mL, 5.4 ± 2.9 μg/g (control) to 63.8 ± 28.3 μg/g and 6.0 ± 2.4 μg/g (control) to 32.9 ± 4.7 μg/g, respectively (see Table 4.6). As a consequence of the palm-oil vitamin E supplementation, α-T constituted 83±11%, 99±1% and 83±3% of the total vitamin E and the absolute α-T content was increased by 13-, 12- and 6-fold in the plasma, liver and kidney, respectively, as compared to the controls.

Data given in Table 4.6 show that appreciable amounts of tocotrienols (i.e. α-T_3, 1.5 μg/g; γ-T_3, 4.2 μg/g; δ-T_3, 0.64 μg/g) were present in the kidneys of guinea pigs supplemented with palm-oil vitamin E. Concentrations of tocotrienols, i.e. α-T_3 (0.38 μg/g) and γ-T_3 (0.23 μg/g), accounted for about 1% of the total vitamin E present in their livers were lower than those in the kidneys. Despite a high tocotrienol content in the palm-oil vitamin E concentrate fed to the POVE guinea pigs, a drastic decrease of the tocotrienols was observed in the organs as shown in Fig. 4.2. The ratio of tocopherols to tocotrienols (T:T3), which initially is at 0.75:1 in the supplementary palm oil vitamin E has been found to increase to 6.5:1, 110:1 and 5.2:1 in the plasma, liver and kidney, respectively.

Table 4.6 Vitamin E composition in guinea pig plasma (μg/mL), liver and kidney (μg/g)*

Vitamin E	Plasma		Liver		Kidney	
	Control	POVE	Control	POVE	Control	POVE
α-T	0.32±0.22	4.1±1.9 [a]	5.4±2.9	63.8±28.3 [a]	6.0±2.4	32.9±4.7 [a]
α-T_3	0.06±0.03	0.15±0.06 [a]	0	0.38±0.76	0	1.5±0.6
γ-T	< 0.01	0.02±0.02	0.07±0.03	0.06±0.02	0.08±0.07	0.25±0.10 [a]
γ-T_3	< 0.01	0.24±0.14	0	0.23±0.15	0	4.2±0.8
δ-T	0	0.09±0.04	0	< 0.01	0.01±0.03	0.01±0.02
δ-T_3	0	0.11±0.11	0	0	0	0.64±0.23
Total	0.38±0.22	4.8±1.7 [a]	5.5±2.9	64.5±28.8 [a]	6.1±2.5	39.6±5.7 [a]
% T	81±9	87±10	100	99±1	100	84±3
% α-T	80±9	83±11	99±1	99±1	99±1	83±3 [a]

* Results are expressed as averages ± standard deviations, POVE guinea pigs were fed palm-oil vitamin E (1000 mg per kg body weight per day), control guinea pigs were not fed palm-oil vitamin E. T = tocopherols, T_3 = tocotrienols. The vitamin E content in the commercial pellets was 40 ppm.

[a] Values statistically significant different ($p < 0.01$) from the corresponding control values.

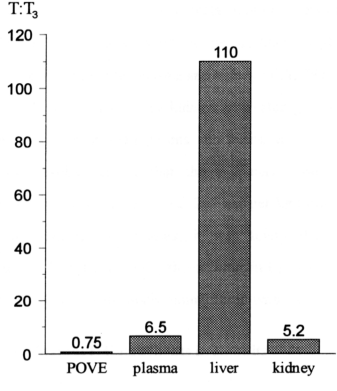


Fig. 4.2 **Ratio of the tocopherols to tocotrienols ($T:T_3$) in the plasma, liver and kidney of guinea pigs orally administered with palm-oil vitamin E (POVE).**

tocotrienol supplementation, the vitamin E components were mainly α-T and a little of γ-T and δ-T also present, but tocotrienols were undetectable in their kidneys and livers. One of the surprising observations is that δ-T_3 was undetectable in the livers of untreated animals and even the animals fed δ-T_3 as much as 105 mg per kg body weight per day, whereas substantial amounts were detected in the plasma and kidney of the POVE guinea pigs. The concentration of γ-T (0.25±0.10 μg/g) in the kidneys of treated guinea pigs was found to be significantly higher than that in their plasma and livers, and also higher than in the control animals, these results suggest that the γ-T may arise from reduction of ingested γ-T_3 which was fed at a high dose, i.e. 267 mg per kg body weight per day for 2½ months. In considering the α-vitamers, it was found that the average ratio of α-T_3:α-T was reduced from 1:2.1 (as in palm-oil vitamin E) to 1:17, 1:111 and 1:21 in the plasma, liver and kidney of animals orally administered with palm-oil vitamin E.

4.3.2 Distribution of Vitamin E Components in Rabbits

Experiment 1

These are preliminary feeding experiments to determine the distribution of vitamin E components in rabbits fed palm-oil vitamin E. Results on the distribution of vitamin E components in the plasma of rabbits fed various diets are given in Table 4.7, and only the distribution data for organs of SO and PO+E rabbits are shown in Table 4.8. In the case where a high dose of palm-oil vitamin E (approximately 0.2 g per kg body weight per day) was fed to the rabbit, a careful HPLC analysis was made to compare the vitamin E content in the rabbit faeces with that in the semi-synthetic diet; data on the distribution of various vitamin E components in feed (Table 4.3) and faeces (Table 4.7) show that the ratio of tocopherols to tocotrienols are comparable, indicating that the absorption of tocotrienols is similar to the tocopherols. The compositional data on unabsorbed vitamin E also indirectly showed that there was no obvious discriminative absorption among the various tocotrienols. Dietary administration of high level of palm-oil vitamin E for 30 days did not give rise to any toxic effect or lead to any abnormality of the organs (by visual

Table 4.7 Vitamin E components in rabbit plasma*

Vitamin E	Plasma (μg/mL)				Faeces (μg/g)
	CP	SO	PO	PO+E	PO+E
α-T	2.34	2.32	3.44	15.8	368
α-T_3	0	0	0	0	67
γ-T	1.26	2.52	0.03	0	0
γ-T_3	0.01	< 0.01	0	0.12	326
δ-T	0.10	0.07	0.07	0	0
δ-T_3	0.10	0	0.02	0	358
Total	3.81	4.92	3.56	15.9	1119
$T:T_3$ #	34:1	> 491:1	177:1	132:1	0.49:1
[α]:[γ+δ] #	1:0.63	1:1.1	1:0.03	1:0.0076	1:1.6

* CP = commercial pellets, SO = semi-synthetic diet contained soyabean oil, PO = semi-synthetic diet contained refined-bleached-deodorized palm olein without palm-oil vitamin E supplementation, PO+E = semi-synthetic diet contained refined-bleached-deodorized palm olein and 1% of palm oil vitamin E.

$T:T_3$ is the ratio of total tocopherols to tocotrienols, [α]:[γ+δ] is the ratio of the total α-vitamers to the sum of γ- and δ-vitamers.

Table 4.8 Vitamin E composition in organs of rabbits (μg/g tissue)

Vitamin E	Liver	Stomach	Heart	Kidney	Brain	Spleen	Lungs	Adipose*
(a) SO								
α-T	12.6	5.9	7.8	4.2	8.7	13.3	4.0	14.0
γ-T	2.2	0.5	2.1	0.9	0.7	2.3	1.1	2.0
δ-T	0.3	1.7	0.2	0	0	0	0	0
Total	15.1	8.1	10.1	5.1	9.4	5.6	5.1	16.0
α-T %	83.4	72.8	77.2	82.4	92.6	85.3	78.4	87.5
[α]:[γ+δ]#	5.0:1	2.7:1	3.4:1	4.7:1	12:1	5.8:1	3.6:1	7:1
(b) PO+E								
α-T	884	41	82	20	15	79	35	83
α-T_3	24	40	67	58	23	92	71	28
γ-T	< 1	5	7	39	6	13	6	5
γ-T_3	2	4	14	1	0	11	3	24
δ-T	2	< 1	6	2	0	28	1	< 1
δ-T_3	1	0	5	0	0	0	0	1
Total	914	91	181	120	44	223	116	142
α-T %	97	45	45	17	34	35	49	58
T:T_3 *	33:1	1.1:1	1.1:1	0.97:1	0.91:1	1.17:1	0.57:1	1.7:1
[α]:[γ+δ]#	151:1	8.1:1	4.7:1	1.9:1	6.3:1	3.3:1	11:1	3.6:1

* Adipose is abdominal; T:T_3 is the ratio of total tocopherols to tocotrienols.

[α]:[γ+δ] is the ratio of the total α-vitamers to the sum of γ- and δ-vitamers.

examination). Dietary vitamin E supplementation elevated the plasma total vitamin E content by about 5-fold from 3.56 μg/mL for the PO rabbit to 15.9 μg/mL for the PO+E rabbit (see Table 4.7). The major plasma vitamin E component was α-T, whereas the tocotrienols were detected at surprisingly low levels despite their preponderance in the feed. Despite a high level of tocotrienol supplementation in the PO+E diet, i.e. about 140 mg per kg body weight per day, the plasma level of the tocotrienols remained very low, with γ-T_3 at 0.12 μg/mL and both α-T_3 and δ-T_3 were not detectable.

Data shown in Table 4.8 indicate that the concentration of vitamin E in tissues increased significantly after dietary supplementation of palm-oil vitamin E. Among the organs studied, the liver had the highest vitamin E level (i.e. 914 μg/g), in which α-T constituted about 97% of the total. While most of the vitamin E components retained in liver is in the α-T form, only very little of tocotrienols were present in the liver as compared to other organs. The tocopherols:tocotrienols (T:T_3) ratios in stomach, heart, kidney, testes, brain, spleen, lungs and adipose tissues are variable, range from 0.57:1 to 1.7:1 (see Table 4.8), but these values are in all cases considerably lower than the value in feed, i.e. 0.44:1 (see Table 4.3). As shown in Table 4.8, the concentrations of vitamin E in tissues of the PO+E group of rabbits given supplementary palm-oil vitamin E were generally higher than those for the SO group of rabbits without vitamin E supplementation. While the proportion of tocotrienols present in the liver was relatively small, i.e. T:T_3 = 33:1, only small differences between the amounts of tocopherols and tocotrienols, i.e. T:T_3 ≈ 1:1, were found in most of the other organs, the tocotrienols present in all of the organs were mainly α-T_3.

Despite a high dose of tocotrienols as much as 0.15 g per kg body weight per day (≡ 150 μg/g/day) being supplemented to the rabbits, tocotrienols were not appreciably elevated in the plasma and liver. Previously, low levels of tocotrienols in the plasma of human and organs of hamsters after a moderate supplementation of palm oil vitamin E (which contained both tocotrienols and tocopherols) have also been noted by Hayes and

co-workers [253,254,384], the disappearance of the tocotrienols from the plasma could be partially attributed to their deposition in the adipose tissues. Tocotrienols incorporated into nascent lipoprotein circulate and exchange between tissues and thus give rise to low concentration of tocotrienols in organs. It is expected that vitamin E deposited in adipose tissue has more tocotrienols than tocopherols (in hamsters supplemented with tocotrienols) [254], because the turnover or release rate of vitamin E in adipose tissue is much slower than in other organs [392,635]. The present study showed that the apparent disappearance of tocotrienols and a relative large elevation of α-T levels in the plasma and tissues of the rabbit fed high dose of palm-oil vitamin E. The results suggest that there is a clearance of the tocotrienols from plasma, possibly mediated by the liver, followed possibly by a rapid bioconversion of tocotrienols to α-T which subsequently is resecreted in nascent hepatic lipoproteins. However, the experimental verification of bioconversion was not achieved with isotopic labels.

Experiment 2

In this experiment, the content of α-T in refined-bleached-deodorized palm olein was reduced to less than 15 mg per kg diet for the PO+γ-T_3, PO+δ-T and PO-E groups of rabbits. In order to study the effect of a normal dose of γ-T_3 and high dose of δ-T on the plasma vitamin E composition, vitamin mixtures without *dl*-α-tocopheryl acetate was used in the preparation of semi-synthetic diets. Results on the concentrations of vitamin E components in the plasma of rabbits fed semi-synthetic diets enhanced with various vitamin E supplementation are presented in Table 4.9. Rabbits fed semi-synthetic diets fortified with various vitamin E components (i.e. PO+γ-T_3, PO+δ-T and PO+α-T groups) showed elevated levels of plasma total vitamin E which were 4- to 5-fold higher than for the rabbits without vitamin E supplementation (PO–E group), the increase being mainly contributed by tocopherols, particularly α-T which constituted more than 94% of the plasma total vitamin E. For rabbits fed a diet deficient in α-T but enriched with γ-T_3 (i.e. PO+γ-T_3 group), the compositions of vitamin E components were drastically altered

Table 4.9 Compositions of the vitamin E components in rabbit plasma (μg/mL)

Vitamin E	Dietary group *			
	PO+γ-T_3 n = 5	PO+δ-T n = 6	PO+α-T n = 6	PO–E n = 5
α-T	20.8 ± 4.3	26.1 ± 13.8	27.5 ± 4.2	5.4 ± 2.2
α-T_3	0	0	0	0
γ-T	0.40 ± 0.26 [a,c]	1.1 ± 0.4 [b,c]	0.08 ± 0.06	0
γ-T_3	0.04 ± 0.02	0	< 0.01	0
δ-T	0.03 ± 0.03 [a]	0.40 ± 0.14 [b]	0.02 ± 0.02	0
δ-T_3	< 0.01	0	0	0
Total	21.2 ± 4.5	27.6 ± 14.3	27.6 ± 4.2	5.4 ± 2.2
% α-T	98 ± 1	94 ± 1	99 ± 0.2	100
T:T_3 #	> 424:1	1:nil	>2759:1	1:nil

* *PO+γ-T_3 = diet enhanced with 186 mg γ-T_3 per kg diet, (b) PO+δ-T = diet enhanced with 863 mg δ-T per kg diet, (c) PO+α-T = diet enhanced with dl-α-tocopheryl*

Table 4.10 Compositions of the vitamin E components in the liver of rabbits (μg/g)

Vitamin E	Palm-oil semi-synthetic diets *			
	PO+γ-T_3 n = 5	PO+δ-T n = 6	PO+α-T n = 6	PO–E n = 5
α-T	29.8 ± 8.9 [a]	53.7 ± 15.1 [a]	36.4 ± 16.6	3.3 ± 0.9
α-T_3	0	0	0	0
γ-T	0.56 ± 0.55 [b,e]	2.52 ± 0.72 [c,e]	0	0
γ-T_3	0.02 ± 0.04 [b]	0.03 ± 0.05	0	0
δ-T	0.01 ± 0.03 [f]	1.37 ± 0.48 [c,f]	0	0
δ-T_3	0	0	0	0
Total	30.4 ± 9.3 [d]	57.6 ± 15.8 [d]	36.4 ± 16.6	3.3 ± 0.9
% α-T	98 ± 1	93 ± 1	100	100
T:T_3 #	1520:1	1920:1	1:nil	1:nil

* PO+γ-T_3 = diet enhanced with 186 mg γ-T_3 per kg diet, (b) PO+δ-T = diet enhanced with 863 mg δ-T per kg diet, (c) PO+α-T = diet enhanced with *dl*-α-tocopheryl acetate and (d) PO–E = palm oil diet depleted of α-T and without vitamin E enhancement. The vitamin E contribution from the commercial pellets (5% w/w) is estimated to be 2 mg/kg diet.

\# Values are averages ± standard deviations. n is the number of rabbits in each group. T:T_3 is the ratio of total tocopherols to tocotrienols. Statistically significant difference at $p < 0.05$ [a-d] or $p < 0.01$ [e, f].

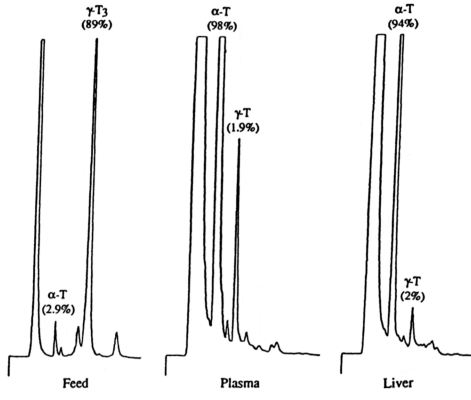


Fig. 4.3. HPLC profiles of vitamin E components in the feed, plasma and liver of rabbits treated with PO+γ-T_3 diet. Values are average (weight percent) of individual vitamin E components in the samples.

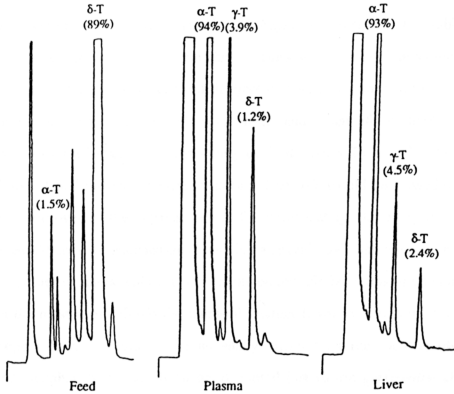


Fig. 4.4 HPLC profiles of vitamin E components in the feed, plasma and liver of PO+δ-T group of rabbits. Values are the average (weight percent) of individual vitamin E components in the samples.

rapid reduction of the unsaturated side chain of the ingested γ-T_3 to give rise to γ-T. Furthermore, using rabbits fed with semi-synthetic diets depleted of α-T but supplemented with high content of δ-T, remarkable alteration of the ratio α-T:γ-T:δ-T = 100:227:5753 in the diet to 100:4.1:1.5 in the plasma and 100:4.7:2.6 in the liver was observed. The plasma and liver levels of γ-T were significantly higher than that of δ-T in rabbits fed with δ-T but with very little of γ-T. Again these results suggest that biomethylation of δ-T to α-T *via* γ-T had apparently occurred. If these biotransformations occur, it is likely that a vitamin E binding protein existed in the liver, and when all of these receptors are saturated by α-T (including those apparently derived from γ-T_3 or δ-T), the methylation from δ-T to γ-T and further methylation from γ-T to α-T would be slowed down in view of the slow turnover of the accumulated α-T. Unfortunately, these hypotheses have not been proven. Alternative explanations involving a specific α-T binding protein and a rapid turnover of other vitamers will show the results on the relative ratios of vitamins.

In the plasma of animals fed palm-oil vitamin E which is rich in tocotrienols, the total tocopherols were found to be unusually higher than the tocotrienols. This high content of tocopherols is unlikely to be attributed just to the preferential absorption or retention of tocopherols over tocotrienols [253] or due to differences in the transport of various vitamin E components to the organs tested. The present data are in agreement with previous report on rats indicating that both tocotrienols and tocopherols were adequately absorbed from the gastrointestinal tract of animals [253]. After a 12-week supplementation of γ-T_3 and δ-T to the rabbits, these vitamin E compounds apparently "disappeared" while α-T accumulated. Despite of semi-synthetic diets depleted of α-T being fed to the rabbits (the estimated amount of α-T contributed by commercial pellets was less than 2 mg/kg diet), there was a relative increase of α-T in the plasma. It is also noted that there was a remarkable accumulation of α-T in the liver even when γ-T_3 was supplemented as the major vitamin E in the feed. The concentration of α-T in the liver of the PO+δ-T group of rabbits (fed 4 times more vitamin E) was about 2-fold higher than

that in the PO+γ-T_3 group of rabbits indicating that the increase of α-T is disproportionately dose-dependent, or the vitamin E storage in the liver of the PO+δ-T group of rabbits has approached a saturation. Similar HPLC profiles of vitamin E components in the liver and plasma (Figs. 4.3 and 4.4) obtained in the present studies suggest that the vitamin E components and their quantities incorporated into the plasma lipoproteins may be a reflection of the vitamin E composition in the liver but not just a preferential incorporation mechanism.

The present results are in agreement with previous studies [28,36,37,51,104] that the plasma content of α-T is a few times higher than the γ-T despite the diets containing more γ-T than α-T. The observation that α-T supplementation could decrease human plasma γ-T level [242], need not necessarily be explained by a competition for intestinal binding in the process of absorption. Previous reports on the absorption of all-*rac*-α-T, *RRR*-γ-T, and the corresponding radiolabelled tocols showed that there was no discriminative absorption of the vitamers [51,637]. However, the hypothesis implying displacement of γ-vitamer by α-vitamer is inadequate to explain present results. A specific α-T binding protein which has a higher affinity for α-T than other vitamin E components has also been reported to be present in the liver [33,96,430,442], and this seems to regulate the preferential incorporation of α-T rather than γ-T into the nascent lipoproteins as well as the secretion of α-T from the liver [637]. In addition, it has been reported that secretion of γ-T in chylomicrons was unaffected by the excess of α-T dosage [634]. If the incorporation of γ-T into lipoproteins is less preferred than the α-T, then a distinct metabolism of the γ- and δ-vitamers might have happened so that they are removed from the plasma; however, this has still to be studied. It has been reported that, after a single dose of α-T and γ-T supplementation (300 mg each) to human, the level of α-T a few times higher than γ-T was simultaneously observed in the plasma and bile; these results therefore do not support the preferential excretion of γ-T (or absorption of α-T) into bile [637]. The observations of low levels of the γ- and δ-vitamers in organs especially liver

have been attributed to the displacement of these vitamers by the elevated level of α-T [372,637]. The preferential accumulation of α-T over γ-T in humans or hamster liver has been observed before [242,634].

Experiment 3 Effects of dietary cholesterol and fat on vitamin E levels

The effects of cholesterol and high fat diets have been investigated using soyabean oil, coconut oil and palm oil and the results are shown in Table 4.11. The data show that a high fat dietary treatment did not raise the plasma vitamin E level in the POWC-rabbits (i.e. 3.9±1.3 μg/mL) as compared to the rabbits fed commercial feed (i.e. 3.2±1.3 μg/mL). An obvious alteration was observed when cholesterol was given in the palm-oil semi-synthetic diet, with plasma vitamin E level elevated to 27.6±4.2 μg/mL. High fat diets supplemented with cholesterol effectively increased the plasma vitamin E in all of the rabbits (i.e. SO, CO and PO) and the vitamin E was mainly α-T. Despite comparable amounts of vitamin E fed to the SO, CO and PO rabbits, the palm oil-fed rabbits appeared to have higher plasma vitamin E levels than rabbits fed with soyabean oil (11.3±3.7 μg/mL) or coconut oil (14.6±5.3 μg/mL), this may play an antioxidative role to account for the relatively higher oxidative stability of LDL obtained from the rabbits fed PO as compared to SO (see Chapter 3).

Supplementation of palm-oil vitamin E, by oral administration or given in the diet, effectively increased the vitamin E levels in the plasma and various organs of animals (rabbits and guinea pigs). In the multiple-step pathway for vitamin E delivery to tissues [641], most of the vitamin E is transported by lipoproteins, mainly LDL and HDL and to a lesser extent by VLDL and the intermediate density lipoprotein (IDL). It is widely accepted that high-cholesterol and high-fat diets tend to raise the level of plasma lipoproteins which are the major carriers of vitamin E [34]. Consequently, the level of vitamin E to be transported would also be elevated in the plasma of rabbits fed supplementary cholesterol as compared to those without supplementary cholesterol. Subsequent to the treatment with hypercholesterolaemic diets in the present study, the

Table 4.11 Compositions of vitamin E components in the plasma of rabbits (μg/mL)

Vitamin E	Semi-synthetic diets enhanced with *dl*-α-TAc *				Commercial feed n = 3
	SO n = 6	CO n = 5	PO n = 6	POWC n = 5	
α-T	10.5 ± 3.6 *a*	13.7 ± 5.4	27.5 ± 4.2 *a,b*	3.9 ± 1.3 *b*	3.2 ± 1.3
α-T_3	0	0	0	0	0
γ-T	0.71 ± 0.19	0.77 ± 0.57	0.08 ± 0.06	< 0.01	0.03 ± 0.01
γ-T_3	0	0	< 0.01	0	< 0.01
δ-T	0.01 ± 0.01	0.1 ± 0.1	0.02 ± 0.02	< 0.01	< 0.01
δ-T_3	0	0	0	0	0
Total	11.3 ± 3.7	14.6 ± 5.3	27.6 ± 4.2	3.9 ± 1.3	3.2 ± 1.3
% α-T	93 ± 1	93 ± 5	99.6 ± 0.2	99.7 ± 0.3	98.9 ± 0.5
T:T_3 #	1:nil	1: nil	>2759:1	1:nil	>320:1

* SO = diet containing soyabean oil supplemented with *dl*-α-tocopheryl acetate (*dl*-α-TAc), CO = diet containing soyabean oil supplemented with *dl*-α-TAc, PO = diet containing refined-bleached-deodorized palm olein supplemented with *dl*-α-TAc, POWC = diet containing refined-bleached-deodorized palm oil supplemented with *dl*-α-TAc but without cholesterol; n = number of rabbits in each group. Values are averages ± standard deviations.

\# T:T_3 is the ratio of total tocopherols to tocotrienols.

*a,b*Values are statistically significant different at $p < 0.01$

plasma vitamin E level in the rabbits fed palm oil tend to be higher than that in rabbits fed soyabean oil and coconut oil. However, present results do not provide simple explanation as to how the palm oil constituents affect the regulation of the plasma vitamin E level.

4.3.3 *In Vitro* Experiments

In order to verify whether biotransformation of tocotrienols to tocopherols occurs in the rabbits, *in vitro* experiments have been carried out using the rabbit's liver microsomal fraction, radiolabelled *S*-adenosyl-^{14}C-methyl-methionine was used as the tracer and δ-T_3 as the substrate. The results are shown in Table 4.12. These preliminary results indicate there was no obvious increase of radioactivity in the α-T and other vitamin E components.

4.3.4 Does Bioconversion of Tocotrienols to Tocopherols Occur in Animals?

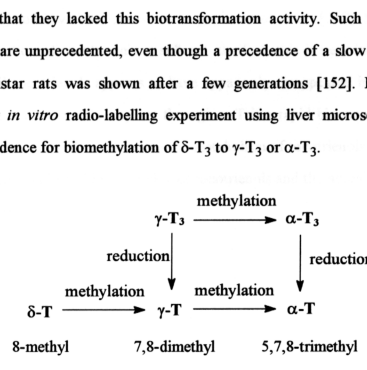
There are some reports indicating that discrimination of tocopherol vitamers in liver may be based on the structure of the chromanyl ring and stereochemistry of phytyl tail side chain [290,639,640]. In contradiction to the extrahepatic organs, a sharp decline in the α-T_3:α-T ratio has occurred in the liver of tocotrienol-fed rabbits, and such a decline was similar to that observed in rat's liver which was just attributed to a more effective retention of the α-T [505]. However, to date there is still inadequate evidence to support that a specific binding protein exists which act to highly discriminate among α-T, α-T_3 and other vitamers which have similar chromanyl moieties but only structurally different in their side chains and the degree of methyl substitution. Another explanation as suggested from present results is that it reflects a possible bioconversion of tocotrienols to tocopherols (although this could not be verified experimentally), which might have taken place in the liver since very low levels of α-T_3 were found in the liver, whereas a comparable amount of α-T_3 and α-T present in other organs (particularly adipose tissues) suggest that they lacked of this activity.

Table 4.12 Incubation of liver microsomal fraction with *S*-adenosyl-^{14}C-methyl-methionine

Vitamin E fraction	TLC R_f	Radioactivity (count per min)*	
		without δ-T_3	with δ-T_3
α-T	0.65	15 ± 10	77 ± 12
α-T_3	0.54	27 ± 14	17 ± 13
γ-T	0.42	30 ± 15	94 ± 18
γ-T_3	0.30	243 ± 30	134 ± 15
δ-T	0.20	19 ± 8	21 ± 10
δ-T_3	0.11	57 ± 15	39 ± 14

* Values are averages ± standard deviations, value for a blank solution is 21 ± 12, for radiolabelled *S*-adenosyl-^{14}C-methyl-methionine (0.1 μCi) is 1.02 x 10^5.

Discriminative absorption, incorporation, secretion and specific binding activity are inadequate to account for the high content of α-T in the liver and plasma which may be reflected by a rapid biomethylation of 8-monomethyl (i.e. δ-vitamer) and 7,8-dimethyl chromanols (i.e. γ-vitamer) to the 5,7,8-trimethyl chromanols (i.e. α-vitamer). A slow methylation of γ-T to α-T in Wistar rats was observed byElmadfa *et al.* [152] but only after a few generations. Present results seem to suggest that a bioconversion, involving biomethylation and reduction of tocotrienols to tocopherols, have occurred in the liver in which α-T remains as the exclusively dominant vitamer despite the high proportion of tocotrienols that had been consumed by the animals. The bioconversion process appeared to be unidirectional as there seems to be no conversion of α-T to γ-T_3 or δ-T when the diets were supplemented with *dl*-α-tocopheryl acetate. Since α-T_3 was undetectable in the plasma and liver of rabbits fed γ-T_3, the possible reduction (γ-T_3 to γ-T) is expected to precede biomethylation (γ-T to α-T), this would seem to be different from the biosynthetic pathway of α-T from precursor (i.e. δ-T_3) in plants [677]. Another possible explanation is that the reduction of α-T_3 to α-T is a very fast step. In contrast to very low level of α-T_3 found in the liver, comparable amounts of α-T_3 and α-T present in other organs indicating that they lacked this biotransformation activity. Such rapid transformations postulated are unprecedented, even though a precedence of a slow methylation of γ-T to α-T in Wistar rats was shown after a few generations [152]. However, the present preliminary *in vitro* radio-labelling experiment using liver microsomal fraction did not provide evidence for biomethylation of δ-T_3 to γ-T_3 or α-T_3.



Assuming that the hypothesis of biomethylation and bioreduction of tocotrienols occur, bound vitamin E compounds (including tocotrienols) in circulating chylomicrons or lipoproteins are retained or returned to the site of bioconversion, possibly the liver, and would be subjected to methylation to form α-T and resecreted into nascent lipoproteins. Continuous biomethylation will eventually give rise to an α-T level much higher than the γ-T. However, mild accumulation of the γ-T, i.e. 2.0 - 4.5% compared to 93-98% α-T in the plasma and liver of the rabbits fed γ-T_3 and δ-T may have resulted due to saturation of the binding site involved in the biomethylation. The bioconversion hypothesis does not pose contradiction to the other mechanisms for the bioavailability of vitamin E components. In fact, it may provide additional basis to support the α-T specific binding mechanisms in order to explain the apparent disappearance of tocotrienols and an unusual accumulation of α-T in the plasma and liver. The above discussion has yet to be verified and the possibility of the simple explanation of a high specific α-T binding protein causing these observation is still possible.

Some biological functions of α-T including anticancer activity have been related to its antioxidant properties. Palm-oil tocotrienols are also potential radical-scavenging agents [219] and have been shown to be good antioxidants [567]. However, the mode of biological action of tocotrienols is still not clarified, this is very important since most of the ingested tocotrienols appears to have apparently disappeared in the plasma and liver. However, the tocotrienols may act through α-T if a rapid bioconversion of γ-T_3 to α-T occurs in animals. Further studies on the metabolism of tocotrienols in other organs would be needed to justify the bioavailability of tocotrienols and the actual mechanism of their *in vivo* activities.