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Free Radical Biology & Medicine 39 (2005) 1310 – 1319

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Original Contribution

Delivery of orally supplemented α-tocotrienol to vital organs of rats and tocopherol-transport protein deficient mice

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Received 18 May 2005; revised 15 June 2005; accepted 21 June 2005

Abstract

The natural vitamin E tocotrienol (TCT) possesses biological properties not shared by tocopherols (TCP). Nanomolar α-TCT, not α-TCP, is potently neuroprotective (JBC 275:13049; 278:43508). Tocopherol-transport protein (TTP) represents the primary mechanism for maintaining normal α-TCP concentrations in plasma and extrahepatic tissues. TTP primarily transports α-TCP and has low affinity for α-TCT. There are no studies that have investigated tissue delivery of α-TCT when orally gavaged on a long-term basis. A long-term study was conducted to examine the effects of α-TCT or α-TCP supplementation, either alone or in combination, on tissue levels. Rats were maintained on a vitamin E-deficient diet and gavaged with α-TCT or α-TCP alone or in combination. Five generations of rats were studied over 60 weeks. TTP-deficient mice were supplemented with TCT and bred to examine tissue delivery of oral α-TCT. Orally supplemented α-TCT was effectively delivered to most tissues over time. When co-supplemented, α-TCP outcompeted α-TCT for transport systems delivering vitamin E to tissues. To evaluate the significance of TTP in α-TCT delivery to tissues, tissue levels of α-TCT in supplemented TTP-deficient mice were studied. α-TCT was transported to several vital organs in TTP-deficient mice. α-TCT restored fertility in TTP-deficient mice. In sum, orally supplemented α-TCT was successfully delivered to several vital organs. The transport efficiency of α-TCT to tissues may be maximized by eliminating the co-presence of α-TCP in the oral supplement. Examination of whether α-TCT may benefit humans suffering from neurological disorders because of congenital TTP deficiency is warranted.
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Keywords: Antioxidant; Ataxia; Vitamin E; Neuroprotection

Introduction

Vitamin E represents all tocopherols and their derivatives having the biological activity of RRR-α-tocopherol (TCP), the naturally occurring stereoisomer compounds with vitamin E activity. In nature, there are eight members in the vitamin E family: α-, β-, γ-, and δ-TCP, and α-, β-, γ-, and δ-tocotrienol (TCT). TCT, formerly known as ζ, ε, or η-TCP, are similar to TCP except that they have a isoprenoid tail with three *trans* double bonds instead of a saturated phytyl tail. TCP represents the natural form of vitamin E in green leafy vegetables while TCT are the primary form of vitamin E in the seed endosperm of most monocots, including cereal grains such as wheat, rice, and barley. Palm oil represents a major source of natural TCT.

TCT possess powerful neuroprotective, antioxidant, anticancer, and cholesterol lowering properties that often differ from the properties of TCP [1]. Such observations, mostly in vitro, warrant in vivo studies testing the effects of TCT in a disease setting. Micromolar amounts of TCT suppress the activity of HMG-CoA reductase, the hepatic enzyme responsible for cholesterol synthesis [2,3]. TCT are thought to have more potent antioxidant properties than

Abbreviations: TCP, tocopherol; TCT, tocotrienol; TTP, tocopherol transfer protein.

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doi:10.1016/j.freeradbiomed.2005.06.013

TCP [4,5]. Unsaturation in the side chain of α-TCP confers a different three-dimensional molecular structure to α-TCT changing the orientation and organization of membrane phospholipids. Compared to α-TCP, α-TCT possess higher mobility through membranes and therefore better lipid-phase antioxidant potency [6]. Recently, an antiangiogenic function of TCT has been reported in vitro [7,8]. Like TCP, TCT have been identified to possess distinct functions that may benefit human health [1,9,10], yet TCT accounts for a very small fraction of overall vitamin E research.

During the last two decades, efforts to understand how dietary vitamin E is transported to the tissues have focused on α-TCP transport [11–13]. α-Tocopherol transfer protein (TTP) has been identified to mediate α-TCP secretion into the plasma while other TCP-binding proteins seem to play a less important role [11]. α-TCP selectively binds to TTP. The affinity of α-TCT to bind TTP has been estimated to be almost an order of magnitude less compared to the affinity for α-TCP [14,15]. The lack of relative specific affinity of TTP for TCT led to the notion that availability of dietary TCT to vital organs is negligible. Indeed it was reported that TCT, supplemented to laboratory chow, does not reach the brain [16]. Later, we observed that even short-term oral supplementation is effective for delivering TCT to the brain provided appropriately stored TCT is gavaged daily. The effect was more pronounced in the fetal brain when pregnant rats were fed with TCT [17]. We noted that TCT, in the free phenol form, was unstable when added to laboratory chow.

Our striking observation that α-TCT, but not α-TCP, is potently neuroprotective at nanomolar concentration in vitro [18,19] led us to revisit tissue uptake of orally supplemented α-TCT. In humans subjected to oral supplementation, plasma α-TCT rises to a micromolar concentrations [20], 10 times in excess of the concentration required for complete neuroprotection [18,19]. The standard laboratory chow contains excessive amounts of α-TCP [21] but negligible amounts of TCT. Long-term lack of TCT in the diet may repress any putative TCT-transport mechanism in vivo. We sought to conduct a long-term study examining the effects of TCT or TCP supplementation, either alone or in combination, on tissue levels. To evaluate the significance of TTP in α-TCT delivery to tissues, we studied the tissue levels of α-TCT in TTP-deficient mice orally supplemented with TCT on a long-term basis.

Materials and methods

Animals and supplementation protocol

Sprague-Dawley rats

Female rats (Harlan, Indianapolis, IN) were maintained on vitamin E-deficient diet (TD 88163, Harlan) and divided into the following four groups supplemented (5 days/week) with: (i) α-TCT (5 mg/kg body weight) (ii) α-TCP (5 mg/kg body weight), (iii) α-TCT + α-TCP (2.5 + 2.5 mg/kg body weight), and (iv) placebo vitamin E-stripped corn oil (volume matched). These rats were identified as first-generation breeders. The female breeders received supplementation through pregnancy. Supplementation, however, was suspended for a period of 1 week after the birth of second-generation litter. During this time, handling of mother rats for supplementation often resulted in killing of the pups by the mother. Offspring from all groups nursed from their mother until 4 weeks of age. On the fifth week of age, the offspring were weaned and supplemented with their respective isoform of vitamin E for 1 week. This was followed by tissue harvest for vitamin E analysis. α-TCT (90%; free of TCP; residual 10% made up of β-, γ-, and δ-T3) and α-TCP (100%) were provided by Carotech Sdn Bhd, Perak, Malaysia. Vitamin E was suspended in E-deficient corn oil (Harlan) for feeding.

Second-generation females from each group were bred. All rats received their designated supplementation through pregnancy. As in the case of first-generation rats, supplementation was suspended for 1 week after the birth of third-generation rats. After 4 weeks of age, the offspring were supplemented with their respective isoform(s) of vitamin E for 1 week. This was followed by tissue harvest of the third-generation rats and vitamin E analyses. The protocol described above was utilized to generate fourth-generation rats. The placebo group, fed with E-deficient corn oil alone, lost fertility and did not breed (Fig. 1). The remaining three groups of fourth-generation females were bred with a supplementation protocol similar to that used for the previous generations. On the fifth week of age, the offspring were weaned and supplemented with their respective isoform(s) of vitamin E for a period of 4 weeks. On the eighth week of age, tissues were harvested for vitamin E analyses. Access to diet was denied to the rats 12 h before harvest. Rats were not supplemented on the day of harvest. The last supplementation was performed 24 h before tissue harvest. Whole blood was drawn from the hepatic vein.

Tocopherol transfer protein-deficient mice

TTP knockout mice [22] were provided by Chugai Research Institute for Medical Science, Japan. At the quarantine facility, breeder pairs were not available for daily gavaging. The mice were fed with standard laboratory chow enriched with TCT (1 g Tocomin 50% per kg diet). As reported previously [17], 1 g of Tocomin 50% (Carotech Sdn Bhd, Perak, Malaysia) contains a mixture of 110 mg α-TCP and 119 mg of α-TCT. After 3 weeks of supplementation on Tocomin-enriched powder diet, two of the three females received from Japan were pregnant. Healthy pups were born 3 weeks after pregnancy. The pups and the mother passed quarantine check and were moved to the laboratory animal facility of the investigator. At this facility, the pups were gavaged five times a week with Tocomin in vitamin E-stripped corn oil (Harlan) at a dose of 250 mg/kg body weight for 7 months. These mice were then bred to

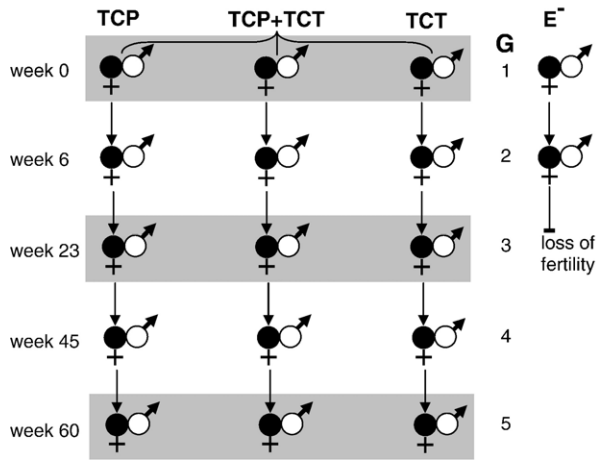

Fig. 1. Schematic representation of the study design aimed at examining the long-term effects of oral vitamin E supplementation in rats. Female rats were maintained on vitamin E-deficient diet (TD 88163, Harlan) and divided into the following four groups supplemented (5 days/week) with: (i) α-TCT (5 mg/kg body weight) (ii) α-TCP (5 mg/kg body weight), (iii) α-TCT + α-TCP (2.5 + 2.5 mg/kg body weight), and (iv) placebo vitamin E-stripped corn oil (volume matched). These rats were identified as first-generation (G) breeders, i.e., G = 1. Offspring from all groups nursed from their mother until 4 weeks of age. On the fifth week of age, the offspring were weaned and supplemented with their respective isoform of vitamin E for 1 week. This was followed by tissue harvest from vitamin E-supplemented rats in G = 2, 3, and 5. The placebo group females, fed with E-deficient corn oil alone, lost fertility and did not produce, G = 3. The mean duration taken to generate each generation is indicated in weeks against each G row. Sample size: For G = 2, α-TCP n = 3 M and 4 F, α-TCT + α-TCP n = 4 M and 3 F, and α-TCT n = 3 M and 3 F. For G = 3, α-TCP n = 4 M and 3 F, α-TCT + α-TCP n = 4 M and 0 F (no females were born), α-TCT n = 3 M and 3 F. For G = 5, α-TCP n = 4 M and 6 F, α-TCT + α-TCP n = 4 M and 4 F, α-TCT n = 4 M and 4 F. M, male; F, female.

obtain next (second) generation pups. The pups (5 males and 3 females) were weaned on the fourth week of age and gavaged with Tocomin 50% (250 mg/kg body weight) for 1 week. On Week 5 of age, the mice were killed to harvest tissues for vitamin E analysis. Access to diet was denied to the mice 12 h before harvest.

Vitamin E extraction and analyses

Excised tissues were cut into small pieces, rinsed in phosphate-buffered saline to remove blood, and stored in liquid nitrogen until analyses. Vitamin E extraction was performed as described previously [23]. Vitamin E analysis was performed using a HPLC-coulometric electrode array detector (CoulArray Detector Model 5600 with 12 channels; ESA Inc., Chelmsford, MA). This system uses multiple channels with different redox-potentials. α-TCP was detected on a channel set at 200 mV. α-TCT was detected on a channel set at 600 and 700 mV as described previously [17,18,23].

Data presentation

Results are illustrated as means ± SD. ANOVA was used to compare differences between groups in rats. For data collected from TTP-deficient mice, the significance of difference between α-TCP and α-TCT values in the same tissue was examined by t test. $P < 0.05$ was considered to indicate statistically significant difference between means.

Results

This work represents a maiden effort to investigate the tissue availability of α-TCT in response to long-term oral supplementation. A fundamental consideration that influenced the design of this study was that the standard laboratory chow contains excessive amounts of α-TCP [21]. In light of the knowledge that natural analogs of vitamin E may compete for specific transporting mechanisms [15], we chose to use vitamin E-deficient standardized laboratory chow for this study. Animals maintained on such diet were gavaged with known amounts of specific forms of vitamin E (Fig. 1). Another consideration that influenced the study design was our own previous observation that although incorporation of orally supplemented vitamin E into tissues is a slow and progressive process, rapid incorporation of the supplement into tissues of newborn may occur in response to gavaging of pregnant mother rats [17]. To generate proof of principle testing whether dietary α-TCT is capable of being transported to vital organs in vivo, we combined long-term oral supplementation with breeding (Fig. 1). While second-generation rats in the vitamin E-deficient group lost fertility and failed to reproduce when bred for over 4 months, rats on TCT supplementation maintained fertility and continued to reproduce comparable to the reference group supplemented with TCP (Fig. 1).

Baseline levels of α-TCT in the skin of TCP-fed rats that never received any TCT supplementation were negligible. Orally supplemented TCT was rapidly taken up by the skin. Already in second-generation rats, α-TCT levels in the skin of TCT-supplemented rats exceeded twice the α-TCP levels in that organ. Of note, the α-TCT level in the skin matched the α-TCP level in the skin of rats fed with a comparable amount of TCP. When TCT and TCP were co-supplemented, the uptake of α-TCT by the skin was clearly blunted. In this group, α-TCT levels were lower than α-TCP levels in the skin, suggesting a direct competition between orally taken TCT and TCP for delivery to the skin (Fig. 2). Longer supplementation resulted in a marked increase in the α-TCT levels in the skin of TCT fed rats, indicating a buildup of α-TCT over time. Interestingly, the levels of α-TCT in the skin of these rats were folds higher than the α-TCP level in the skin of TCP fed rats. This observation suggests the presence of an effective transport mechanisms delivering α-TCT to the skin and efficient retention of α-TCT in the skin over time. In the case of TCT as well as of TCP feeding, results from third and fifth-generation rats indicate higher levels of vitamin E in the skin of female compared to that of male rats. Co-supplementation of TCT

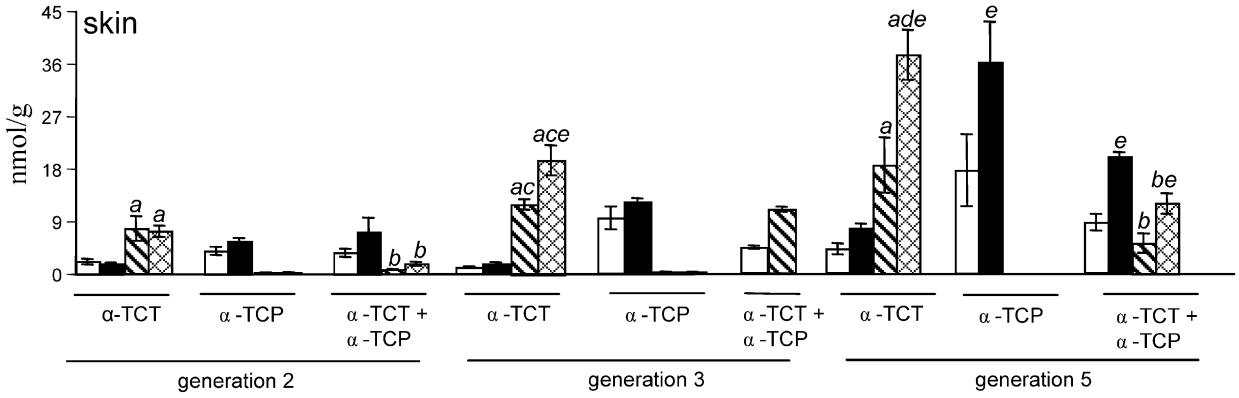

Fig. 2. α-Tocotrienol and α-tocopherol levels in the skin of rats. Animals were maintained on vitamin E-deficient diet and supplemented with either α-TCT, α-TCP, or co-supplemented with α-TCT + α-TCP as indicated in the figure. Open and closed bars represent α-TCP data from male and female rats, respectively. Hatched and cross-hatched bars represent α-TCT data from male and female rats, respectively. Data represent mean ± SD. $P < 0.05$ is designated by letters a–e: a, higher than in corresponding gender-matched group in the same generation; b, lower than in corresponding gender-matched group in the same generation; c, higher than in corresponding gender-matched and supplementation-matched group in G2; d, higher than in corresponding gender-matched and supplementation-matched group in G3; e, higher in females compared to corresponding males in the same generation and supplementation group. G, generation.

and TCP demonstrated favorable uptake of α-TCP than α-TCT. Adipose tissue serves as a storage organ for vitamin E [24]. Analysis of adipose tissue vitamin E content of fifth-generation rats revealed substantially more accumulation of α-TCT in that tissue than α-TCP (Fig. 3). In the co-supplemented group, tissue levels of α-TCT and α-TCP were comparable (Fig. 3). Consistent with the observation in the skin, levels of both forms of vitamin E were higher in the adipose tissue of females compared to that in the males (Fig. 3). This observation led us to examine the vitamin E level in the gonads of available rats. Indeed, the levels of both forms of vitamin E studied were significantly higher in ovaries than in the testes (Fig. 3). This gender-dependent effect was more striking for α-TCT than for α-TCP. In rats co-supplemented with TCT and TCP, α-TCP outcompeted α-TCT for delivery to the gonads (Fig. 3).

The α-TCT level in the heart, lungs, and skeletal muscle of TCP-supplemented rats may be considered as baseline for α-TCT in the heart of rats not supplemented with TCT (Figs. 4–6). Compared to that baseline, the level of α-TCT in the heart, lungs, and skeletal muscle of second-generation TCT-fed rats was substantially higher establishing that oral TCT does get delivered to these organs. The level of α-TCT in these organs of TCT-fed rats was lower than the level of α-TCP in TCP-fed rats, indicating that in these rats, the efficiency to deliver oral TCT to the respective organs was lower than that for oral TCP delivery. Co-supplementation of TCT and TCP clearly compromised delivery of α-TCT to all three organs (Figs. 4–6). The level of α-TCT in the heart, lungs, and skeletal muscle of TCT-fed female rats increased in response to a longer supplementation. Third and fifth-generation females had significantly higher levels of α-TCT in the heart of TCT-fed group (Fig. 4). Co-supplementation of TCT and TCP compromised delivery of α-TCT to the lung and skin (Figs. 2 and 5). The extent of compromise was most in the second-generation rats. In the third and fifth

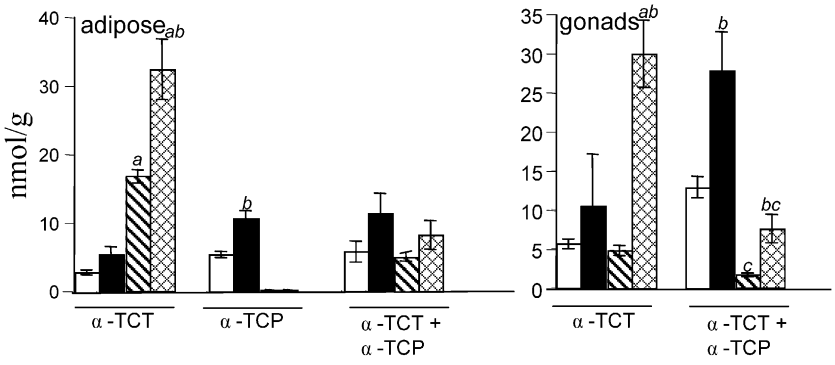

Fig. 3. α-Tocotrienol and α-tocopherol levels in abdominal adipose and gonads of rats. Animals were maintained on vitamin E-deficient diet and supplemented with α-TCT, α-TCP, or co-supplemented with α-TCT + α-TCP as indicated in the figure. Data from G5 are shown. Open and closed bars represent α-TCP data from male and female rats, respectively. Hatched and cross-hatched bars represent α-TCT data from male and female rats, respectively. Data represent mean ± SD. $P < 0.05$ is designated by letters a–c: a, higher than in corresponding gender matched in the same supplementation group; b, higher in females compared to corresponding males in the same generation and supplementation group; c, lower compared to α-TCP levels in the corresponding gender-matched co-supplemented group. G, generation.

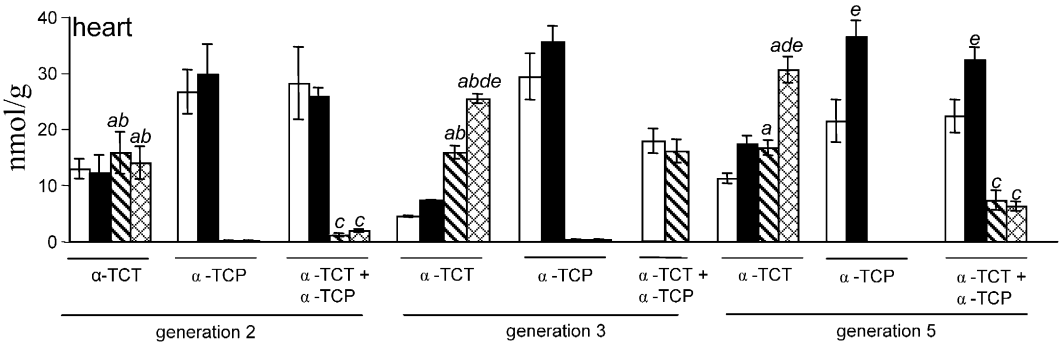

Fig. 4. α-Tocotrienol and α-tocopherol levels in the heart of rats. Animals were maintained on vitamin E-deficient diet and supplemented with α-TCT, α-TCP, or co-supplemented with α-TCT + α-TCP as indicated in the figure. Open and closed bars represent α-TCP data from male and female rats, respectively. Hatched and cross-hatched bars represent α-TCT data from male and female rats, respectively. Data represent mean ± SD. $P < 0.05$ is designated by letters a–e: a, higher than in corresponding gender-matched α-TCP supplemented group in the same generation; b, lower than α-TCP levels in the corresponding gender-matched α-TCP supplemented group in the same generation; c, lower than corresponding gender-matched α-TCP levels in the same tissue in co-supplemented rats; d, higher than corresponding supplementation-matched females in G2. e, higher in females compared to corresponding males in the same generation and supplementation group. G, generation.

generations, rats co-supplemented with TCT and TCP had higher levels of α-TCT in the lung compared to the corresponding rats in the second generation (Fig. 5). In third- and fifth-generation rats, the levels of α-TCT were significantly higher in females than in males. Of note, in the third- and fifth-generation rats the α-TCT levels in TCT-fed group were comparable to the α-TCP levels in the TCP-fed group, indicating comparable delivery and retention of the two forms of vitamin E in the tissue (Figs. 2–9).

In tissues of the central nervous system, brain, and spinal cord, TCT feeding increased the levels of α-TCT compared to baseline levels detected in rats never supplemented with any TCT (Fig. 7). In second-generation rats, α-TCT was appreciably detected. However, the level was folds lower than the α-TCP level in the brain of TCP-fed rats. This observation indicates that oral α-TCT is delivered to the central nervous system but the delivery system is much weaker than the system to deliver α-TCP. Co-supplementation of rats with TCT and TCP resulted in lower α-TCT delivery to the brain as well as to the spinal cord compared to α-TCP levels. Longer term supplementation resulted in higher levels of brain α-TCT in the third- and fifth-generation rats compared to animals of the second generation. In both third- and fifth-generation rats fed with TCT, females had higher α-TCT in the brain than males. This was also evident in the spinal cord of fifth-generation rats (Fig. 7).

In the blood, baseline α-TCT levels in rats never fed with TCT (i.e., TCP group) were negligible (Fig. 8). TCT supplementation increased the levels of circulatory α-TCT even 12 h after the last supplementation. The level of α-TCT detected in the circulation of TCT-fed rats under comparable conditions for the second, third, and fifth generation were similar. α-TCT levels in the blood of TCT-fed were roughly a magnitude lower than the levels of α-TCP in TCP-fed rats. Co-supplementation of TCT and

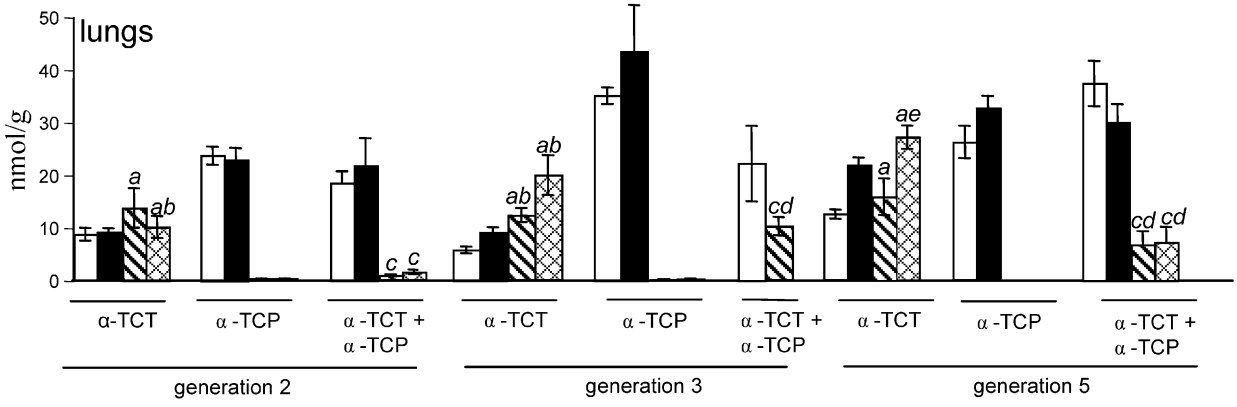

Fig. 5. α-Tocotrienol and α-tocopherol levels in the lungs of rats. Animals were maintained on vitamin E-deficient diet and supplemented with α-TCT, α-TCP, or co-supplemented with α-TCT + α-TCP as indicated in the figure. Open and closed bars represent α-TCP data from male and female rats, respectively. Hatched and cross-hatched bars represent α-TCT data from male and female rats, respectively. Data represent mean ± SD. $P < 0.05$ is designated by letters a–e: a, higher than in corresponding gender-matched α-TCP-supplemented group in the same generation; b, lower than α-TCP levels in the corresponding gender-matched α-TCP-supplemented group in the same generation; c, lower than in corresponding gender-matched α-TCP levels in the same tissue of co-supplemented rats; d, higher than in corresponding gender-matched co-supplemented rats in G2; e, higher in females compared to corresponding males in the same generation and supplementation group. G, generation.

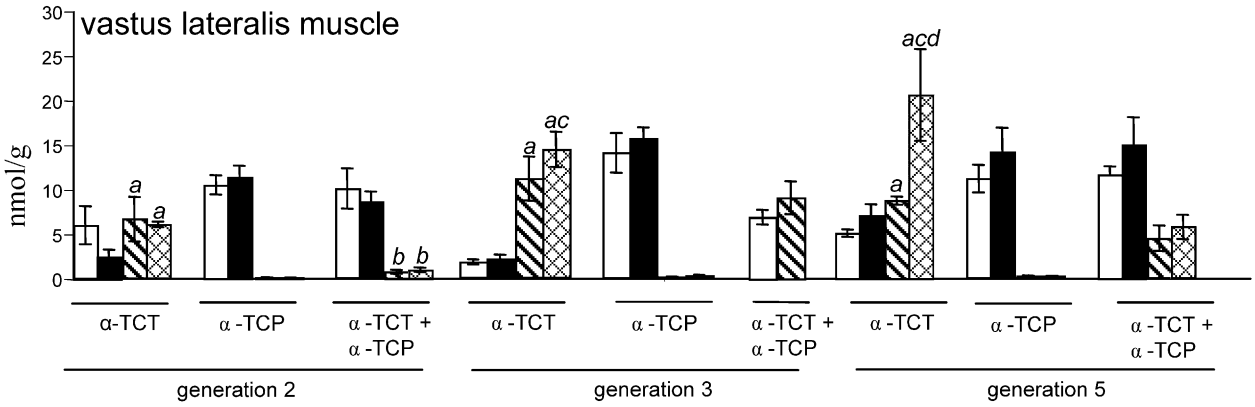

Fig. 6. α-Tocotrienol and α-tocopherol levels in the vastus lateralis skeletal muscle of rats. Animals were maintained on vitamin E-deficient diet and supplemented with α-TCT, α-TCP, or co-supplemented with α-TCT + α-TCP as indicated in the figure. Open and closed bars represent α-TCP data from male and female rats, respectively. Hatched and cross-hatched bars represent α-TCT data from male and female rats, respectively. Data represent mean ± SD. $P < 0.05$ is designated by letters a–d: a, higher than in corresponding gender-matched α-TCP-supplemented group in the same generation; b, lower than in corresponding gender-matched α-TCP levels in the same tissue of co-supplemented rats; c, higher than in corresponding gender-matched α-TCT-supplemented rats in G2; d, higher in females compared to corresponding males in the same generation and supplementation group. G, generation.

TCP resulted in higher levels of blood α-TCP than α-TCT, indicating more efficient uptake and retention of oral α-TCP in the circulation than that for α-TCT. In third- and fifth-generation TCT-fed rats, females had higher blood α-TCT levels than males (Fig. 8).

In rats never supplemented with TCT, baseline α-TCT levels in the liver were negligible (Fig. 9). TCT supplementation increased hepatic α-TCT concentration. However, in TCT-fed rats hepatic α-TCT content was only half of the α-TCP levels in the liver of TCP-fed second-generation rats. Co-supplementation of TCT and TCP resulted in preferential uptake of α-TCP by the liver. In the third- and fifth-generation TCT-fed rats, hepatic α-TCT concentration was higher in the females than in the males. While long-term TCT-supplementation had little effect on the α-TCT levels in male rats, in fifth-generation female rats significantly elevated levels of α-TCT were noted (Fig. 9).

TTP-deficient mice are known to be embryonic lethal because of their inability to deliver TCP to tissues [22,25]. Oral TCT supplementation, but not TCP, restored reproductive capability in these mice (Fig. 10). Next, we tested whether delivery of oral α-TCT to vital organs is dependent on TTP. Orally supplemented α-TCT was effectively delivered to several vital organs in TTP-deficient mice. While these results do not rule out the possibility that TTP may contribute to TCT transport in the body, it was clear that TTP does not represent a major or sole mechanism of α-TCT transport in the body. In TTP-deficient mice, the adipose tissue represented a major destination for orally consumed α-TCT. Long-term TCT supplementation to mice resulted in adipose tissue α-TCT levels that were folds higher than α-TCP levels. Consistent with the observation in our rat study, the skin and skeletal muscle were observed to be efficient in accumulating dietary α-TCT. In these organs, α-TCT levels were folds higher than that of α-TCP. In TTP-deficient mice supplemented with TCT, α-TCT levels were significantly higher in the heart than α-TCP levels. In the lung and brain of these mice, however, α-

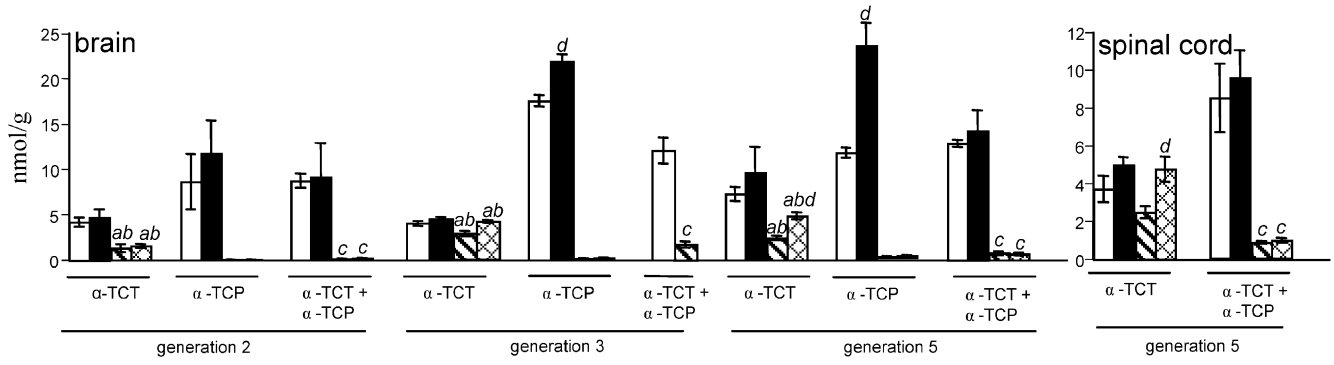

Fig. 7. α-Tocotrienol and α-tocopherol levels in the brain and spinal cord of rats. Animals were maintained on vitamin E-deficient diet and supplemented with α-TCT, α-TCP, or co-supplemented with α-TCT + α-TCP as indicated in the figure. Open and closed bars represent α-TCP data from male and female rats, respectively. Hatched and cross-hatched bars represent α-TCT data from male and female rats, respectively. Data represent mean ± SD. $P < 0.05$ is designated by letters a–d: a, higher than in corresponding gender-matched α-TCP-supplemented group in the same generation; b, lower than α-TCP levels in the corresponding gender-matched α-TCP-supplemented group in the same generation; c, lower than in corresponding gender-matched α-TCP levels in the same tissue of co-supplemented rats; d, higher in females compared to corresponding males in the same generation and supplementation group. G, generation.

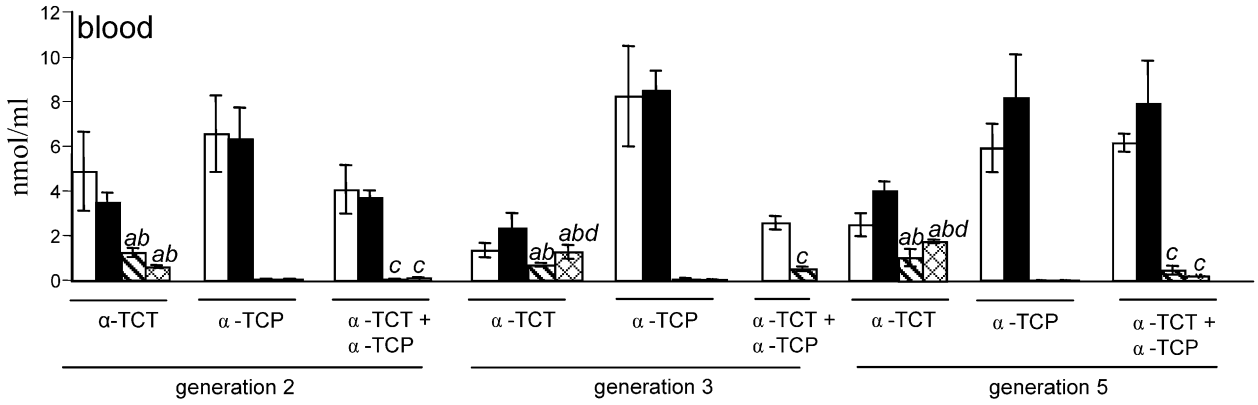

Fig. 8. α-Tocotrienol and α-tocopherol levels in the blood of rats. Animals were maintained on vitamin E-deficient diet and supplemented with α-TCT, α-TCP, or co-supplemented with α-TCT + α-TCP as indicated in the figure. Open and closed bars represent α-TCP data from male and female rats, respectively. Hatched and cross-hatched bars represent α-TCT data from male and female rats, respectively. Blood was collected 12 h after last supplementation. Data represent mean ± SD. $P < 0.05$ is designated by letters a–d: a, higher than in corresponding gender-matched α-TCP-supplemented group; b, lower than in corresponding gender-matched α-TCP levels in the TCP-supplemented group in the same generation; c, lower than in corresponding gender-matched α-TCP levels in the same tissue in co-supplemented rats; d, higher in females compared to corresponding males in the same generation and supplementation group.

TCP and α-TCT levels were not significantly different. It was clear that oral TCT was indeed delivered to both lung and brain. Consistent with the observation in rats, mice never supplemented with TCT show negligible α-TCT levels in all organs (not shown). In the spinal cord, α-TCP levels were comparable to those in the brain. TCT supplementation was more effective in raising the α-TCT level in the spinal cord than that of the brain. α-TCT levels in the spinal cord were multifold higher than the levels of α-TCP. Although the levels of α-TCT were remarkably high in tissues such as the fat, skin, and muscle, hepatic α-TCT concentration in TCT-supplemented TTP-deficient mice was folds lower than the corresponding TCP levels. In TTP-deficient mice with compromised ability to traffic α-TCP from the liver to the peripheral tissues, hepatic α-TCP content is known to accumulate. In the blood, both α-TCP and α-TCT were detected even 12 h after the last supplementation. α-TCT levels in the blood were significantly lower than circulating α-TCP levels (Fig. 10).

Discussion

Delivery of orally taken vitamin E to vital organs is a key determinant of the overall efficacy of vitamin E in those tissues. Thus, mechanisms responsible for the transfer of absorbed vitamin E to the tissues have been the subject of active investigation [26]. TTP has emerged as the major intracellular transport protein for vitamin E, mediating α-TCP secretion into the plasma via a non-Golgi-dependent pathway [11]. It has been estimated that TTP has 8.5-fold lower affinity to transport α-TCT than α-TCP [15]. Inefficiency to transport α-TCT to vital organs represents one of the key concerns that have limited enthusiasm for this

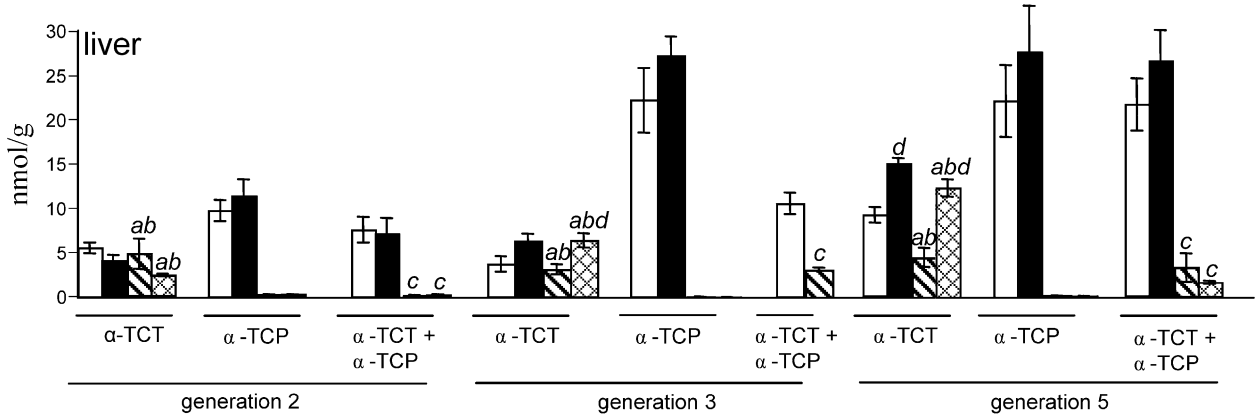

Fig. 9. α-Tocotrienol and α-tocopherol levels in the liver of rats. Animals were maintained on vitamin E-deficient diet and supplemented with α-TCT, α-TCP, or co-supplemented with α-TCT + α-TCP as indicated in the figure. Open and closed bars represent α-TCP data from male and female rats, respectively. Hatched and cross-hatched bars represent α-TCT data from male and female rats, respectively. Data represent mean ± SD. $P < 0.05$ is designated by letters a–d: a, higher than in corresponding gender-matched α-TCP-supplemented group; b, lower than α-TCP levels in the corresponding gender-matched α-TCP-supplemented group; c, lower than corresponding gender-matched α-TCP levels in the same tissue of co-supplemented rats; d, higher in females compared to corresponding males in the same generation and supplementation group.

form of natural vitamin E. Recently, we have reported the first evidence demonstrating the biological effects of trace concentrations of vitamin E [1,18,19]. Nanomolar concentration α-TCT, but not α-TCP, was potently neuroprotective [1,18,19]. Results of the current study represent the first evidence addressing the effect of long-term TCT supplementation on tissue α-TCT levels. Dietary α-TCT was effectively delivered to several vital organs among which the skin, adipose, ovaries, and the heart seemed to be preferred destinations within the body. Oral α-TCT was also delivered, albeit to a lesser extent, to vital organs such as the brain, lung, testes, and skeletal muscle.

Gender-based differences in the transport of dietary vitamins are known to exist in specific cases [27]. Although the effect of several physiological factors on vitamin E transport has been studied, the gender factor remains to be specifically addressed [28]. Recently it has been demonstrated that γ-TCP is more rapidly metabolized in women than in men [29]. In all organs tested, we consistently observed higher tissue levels of α-TCT in females than in males. This effect was most prominent in response to long-term supplementation. Of interest, gonads of the fifth-generation rats exhibited the most striking difference. The level of α-TCT in the ovary was over fivefold higher than that in the testes from the corresponding males rats. In the ovary, TCP is known to accumulate via a lipoprotein receptor-dependent mechanism [30]. Whether TCT share that mechanism remain to be tested.

TTP is a soluble 32-kDa protein expressed in liver that selectively binds α-TCP. TTP maintains the concentration of serum α-TCP by facilitating α-TCP export from the liver. TTP is required to maintain normal α-TCP concentrations in plasma and extrahepatic tissues [26]. Although TTP is known to bind to α-TCT with a 8.5-fold lower affinity than that for α-TCP [15], it is not clear whether, or to what extent, the delivery of orally supplemented α-TCT to vital organs is dependent on TTP. Previously it has been reported that TTP-deficient females are infertile presumably because of vitamin E deficiency [25]. This important observation was confirmed in another lineage of TTP-deficient mice, the one used in the current study. Placentas of pregnant TTP-deficient females were severely impaired with marked reduction of labyrinthine trophoblasts, and the embryos died at mid-gestation even when fertilized eggs of TTP-containing wild-type mice were transferred into TTP-deficient recipients [22]. In our study, TTP-deficient mice fed a standard laboratory chow containing TCP were infertile, consistent with previous observations. Even in the presence of dietary TCP, TTP knockout mice are known

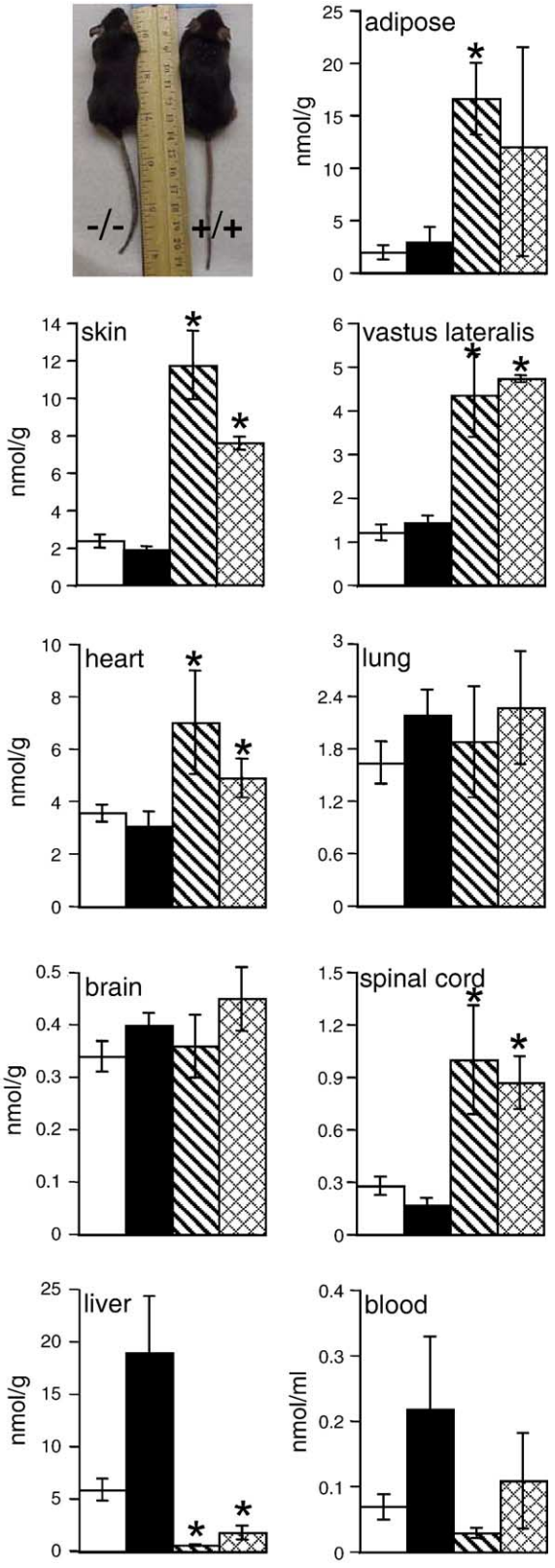

Fig. 10. α-Tocotrienol and α-tocopherol levels in tocotrienol-supplemented TTP-deficient mice. Top left: An adult (7 week old) TTP-deficient (−/−) mouse born from homozygous parents maintained on tocotrienol supplementation compared to a wild-type (+/+) mouse of the same background. Mice were maintained on long-term tocotrienol supplementation as described in Materials and methods. Open and closed bars represent α-TCP data from male and female mice, respectively. Hatched and cross-hatched bars represent α-TCT data from male and female mice, respectively. Data represent mean ± SD. *, $P < 0.05$, represents significantly higher (lower, in the case of liver) than α-TCP level in the corresponding gender-matched tissue.

to suffer from TCP deficiency [22,25]. Oral supplementation of the female mice with α-TCT restored fertility, suggesting that TCT was successfully delivered to the relevant tissues and that α-TCT supported reproductive function under conditions of α-TCP deficiency. This observation was consistent with our observation in the rats where α-TCT supplementation spared loss of fertility caused by long-term vitamin E deficiency in the diet.

Accumulation of α-TCT in several vital organs of the TTP-deficient mice indicates that the delivery of oral α-TCT to these tissues occurs independent of TTP. Heritable mutations in the TTP gene are incident in humans and display low plasma vitamin E levels and pathological conditions such as autosomal recessive Friedreich-like ataxia and retinitis pigmentosa subsequent to the onset of ataxia. Neurological symptoms included ataxia, dysarthria, hyporeflexia, and decreased proprioceptive and vibratory sensations. TTP deficiency in humans specifically affects the central axons of dorsal root ganglion cells and the retina, with minor involvement of the peripheral sensory nerve, optic nerve, and pyramidal tract [31,32]. Previously we have observed that the neuroprotective properties of α-TCT are more potent than that of α-TCP [18,19]. In the present study, we observe that orally supplemented α-TCT may be transported to tissues even in the absence of TTP. Taken together, these findings warrant clinical studies testing the efficacy of oral α-TCT supplementation in humans suffering from inherited mutations in the gene encoding TTP and also in those that are TTP-sufficient but suffer from or are at a high risk of neurodegenerative diseases.

Vitamin E enters the circulation from the intestine in chylomicrons. The conversion of chylomicrons to remnant particles results in the distribution of newly absorbed vitamin E to all of the circulating lipoproteins and ultimately to tissues. This enrichment of lipoproteins with vitamin E is a key mechanism by which vitamin E is delivered to tissues [26]. In the liver, newly absorbed dietary lipids are incorporated into nascent very low density lipoproteins. The liver is responsible for the control and release of α-TCP into blood plasma. In the absence of α-TTP, α-TCP is not secreted back into the plasma. Excess vitamin E is not accumulated in the liver, but is excreted, mostly in bile [26]. Results of the current study show that α-TCT levels in the liver of rats and of TTP-deficient mice were much lower than the levels of this vitamin E isoform in most peripheral tissues studied. Such observation argues against a central role of the liver in delivering oral α-TCT to peripheral tissues. TTP has the ability to bind to both α-TCP as well as α-TCT. The affinity to bind α-TCP is several-fold higher than that for α-TCT [15]. Thus under conditions of coexistence, α-TCP is expected to clearly outcompete α-TCT for binding. Although our studies with the TTP-deficient mice indicate the existence of TTP-independent mechanisms for the tissue delivery of oral α-TCT, observations in the rat indicate that the mechanisms for transporting α-TCP and α-TCT seem to compete such that transport of α-TCP is favored. Thus, co-supplementation of α-TCP and α-TCT is likely to compromise tissue delivery of α-TCT.

TCP and TCT are metabolized by side-chain degradation initiated by cytochrome P450 (CYP)-catalyzed ω-hydroxylation followed by β-oxidation. CYP3A4 and CYP4F2 are involved in the degradation of TCP. Both TCP and TCT in particular induce the expression of CYP3A4 and CYP3A5 by activating the pregnane × receptor (PXR), a nuclear receptor regulating a variety of drug-metabolizing enzymes [33]. Quantitatively, TCT are degraded to a larger extent than their counterparts with saturated side chains. The pronounced quantitative differences in the metabolism between individual TCP as well as between TCT and TCP in vitro suggest a corresponding lack of equivalence in vivo [34].

The efficiency of α-TCP as chain-breaking antioxidants, combined with its prevalence in the human body led biologists to almost completely discount the “minor” vitamin E molecules as topics for basic and clinical research. Recent discoveries have led to a serious reconsideration of this conventional wisdom [35]. All eight tocols in the vitamin E family share close structural similarity and hence possess comparable antioxidant efficacy. Yet, current studies of the biological functions of vitamin E indicate that members in the vitamin E family possess unique biological functions often not shared by other family members [36–39]. Numerous beneficial functions of tocotrienol have been reported during the last two decades [1,9,39]. The current work represents the first evidence documenting the tissue distribution of α-TCT following long-term supplementation. It is clear that orally taken α-TCT may be successfully delivered to several vital organs. This transport efficiency seems to be down-regulated under conditions of α-TCP co-supplementation. Studies with TTP-deficient mice revealed that α-TCT may be transported to tissues by α-TTP-independent mechanisms. Given that α-TCT exhibit potent biological functions at nanomolar concentrations [18,19], further investigation aimed at the identification of specific α-TCT-transport mechanisms in vivo is warranted.

Acknowledgments

We thank Christina Phillips and Sumona Ghosh for their assistance in gavaging animals and tissue processing, respectively. This work was supported by NIH-NINds RO1 NS42617 to CKS.

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