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Natural vitamin E α-tocotrienol: Retention in vital organs in response to long-term oral supplementation and withdrawal

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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; Stroke 36:2258). The report that the affinity of TTP to bind α-TCT is an order of magnitude lower than that for α-TCP questions the bioavailability of orally taken TCT to tissues. Oral supplementation of TCT for 3 years in nine generations of female and male rat was studied. Ten vital organs were examined. To gain insight into the turnover of α-TCT in tissues, a subset of supplemented rats was moved to vitamin E deficient diet for 7 weeks. Orally supplemented α-TCT was delivered to all vital organs including the brain and spinal cord in significant amounts. In organs such as the skin, adipose and gonads the maximum level of α-TCT achieved in response to supplementation was folds higher than baseline values of α-TCP in rats maintained on laboratory chow. Females had higher levels of α-TCT compared to matched tissues of corresponding males. To gain insight into how quickly α-TCT is metabolized in the tissues, washout of α-TCT from vital organs was examined. α-TCT accumulated in vital organs over more than 2 years was almost completely lost in less than 2 months when the supplementation was stopped. This is in sharp contrast with findings related to α-TCP retention. The ability of long term oral supplementation to maintain and elevate α-TCT levels in vital organs together with the rapid elimination of the intact vitamin from all organs studied underscores the need for continuous oral supplementation of TCT.

Keywords: *Tocotrienol, neurodegeneration, nutrition, stroke, supplementation, tocopherols*

Introduction

Vitamin E refers to all tocopherols and their derivatives exhibiting the biological activity of RRR-α-tocopherol (TCP), the naturally occurring stereo-isomer compounds with vitamin E activity [1–3]. In nature, the vitamin E family consists of α-, β-, γ-, and δ-TCP and α-, β-, γ-, and δ-tocotrienol (TCT). TCT, formerly known as ϵ, ζ, η-TCP, are similar to TCP except that they have an isoprenoid tail with three *trans* double bonds instead of a saturated phytyl tail. Primary sources of TCP include vegetable oils (olive, sunflower, safflower oils), nuts, whole grains, and green leafy vegetables, while TCT is the primary form of vitamin E in seed endosperms of most monocots, including cereal grains such as wheat, rice, and barley. Palm oil represents a major source of natural TCT.

Compared to TCP, TCT have been poorly studied [4]. However, recently interest in TCT has risen sharply because of the discovery of unique characteristics of TCT not shared by the better known TCP [1,2,5]. TCT possesses neuroprotective, antioxidant, anticancer, and cholesterol lowering properties that

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differ from the properties of TCP [1,2]. Micromolar amounts of TCT suppress the activity of HMG-CoA reductase, the hepatic enzyme responsible for cholesterol synthesis [6,7]. TCT are thought to have more potent antioxidant properties than TCP [8,9]. Unsaturation in the side chain of α-TCT confers a different three-dimensional molecular structure compared to α-TCP changing the orientation and organization of membrane phospholipids. Compared to α-TCP, α-TCT possess higher mobility through membranes and, therefore, better lipidphase antioxidant potency [10]. Recently, an anti-angiogenic function of TCT has been reported [11,12].

During the last two decades, efforts to understand the transport of dietary vitamin E to tissues have focused on α-TCP transport [13–16]. α-Tocopherol transfer protein (TTP) has been identified to mediate α-TCP secretion into plasma while other TCP-binding proteins seem to play a less important role [14]. α-TCP selectively binds to TTP. The observation that the affinity of TTP to bind α-TCT is an order of magnitude lower than that for α-TCP raised concerns questioning the bioavailability of orally taken TCT to tissues [3,17,18]. Such concerns were strengthened by the report that TCT supplemented in laboratory chow does not reach the brain [19]. Later, we observed that TCT, in the free phenol form, was unstable when added to laboratory chow. Our experiments revealed that even short-term oral supplementation is effective for delivering TCT to the brain provided appropriately stored TCT is gavaged daily [20]. More recently, we have reported the first evidence that oral supplementation of TCT is effective in delivering TCT to vital organs [4]. Furthermore, TCT transport *in vivo* was noted to be TTP-independent [4].

Our striking observation that α-TCT is potently neuroprotective *in vitro* as well as *in vivo* [21–23] led us to revisit tissue uptake of α-TCT orally supplemented on a long-term basis. In humans subjected to oral supplementation, plasma α-TCT rises to micromolar concentration [24,25], an order of magnitude in excess of the concentration required for complete neuroprotection [21,22]. The standard laboratory chow contains excessive amounts of α-TCP [4,26] but negligible amounts of TCT. Long term lack of TCT in the diet may repress any putative TCT transport mechanism *in vivo*. The present study builds on our previous observation reporting the effects of TCT supplementation for 60 weeks [4]. Here, we present first evidence reporting the results of oral supplementation of TCT for 3 years. Ten vital organs were studied. The rate of elimination of vitamin E from the tissue provides important information related to the metabolic turnover of the molecule. For the first time, we sought to examine the rate of elimination of intact α-TCT from vital organs enriched in that isoform of natural vitamin E by long-term oral supplementation.

Materials and methods

Animals and supplementation protocol

The supplementation protocol was identical to that of our previous study [4]. In brief, female rats (Harlan, Indianapolis, IN) were maintained on vitamin E deficient diet (TD 88163, Harlan) supplemented (5d/week) with TCT (5 mg/kg body weight). TCT (90% α-TCT; free of TCP; residual 10% made up of β-, γ-, and δ-T3) was provided by Carotech Sdn Bhd, Perak, Malaysia. TCT was suspended in E-deficient corn oil (Harlan) for feeding. 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 nursed from their mother until 4 weeks of age. On the fifth week of age, the offspring were weaned and supplemented with TCT for 1 week. This was followed by tissue harvest for vitamin E analyses. The protocol described above was utilized to generate second, third, and fourth generation rats. The 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 TCT for a period of 4 weeks. On the eighth week of age, tissues were harvested for vitamin E analyses. The protocol described above was utilized to generate sixth and seventh generation rats. Seventh 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 TCT for a period of 28 weeks. On the thirty-third week of age, tissues were harvested for vitamin E analyses. Eighth 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 split into two groups: (a) TCT supplemented for a period of 7 weeks as described above, or (b) washout group maintained on vitamin E deficient diet for the same length of time. On the twelfth week of age, tissues were harvested from the supplemented and washout group for vitamin E analyses. For all generations, 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.

Vitamin E extraction and analyses. Vitamin E extraction and analyses were performed as described previously [4]. Tissues excised from rats were rinsed with icecold phosphate buffered saline (PBS) to remove blood,

blotted to remove excess water and promptly snap-frozen in liquid nitrogen. For all tissues except adipose, 150–200 mg of tissue was ground in liquid nitrogen. PBS (0.01 ml/mg tissue) and butylated hydroxytoluene (0.5 μl/mg tissue; stock 10 mg/ml of ethanol) were added to the ground sample. The ground tissue was then homogenized on ice for 30 s, after which 0.1 M SDS (0.01 ml/mg tissue) was added to the sample. This was followed by homogenization for 30 s. HPLC grade ethanol (0.02 ml/mg tissue) was added to the homogenate followed by vortexing for 30 s. Next, following addition of hexane (0.02 ml/mg tissue) the sample was again vortexed for 30 s. The resultant mixture was sonicated on ice for 1 min in 20 s intervals. For adipose tissue, sonication occurs before hexane is added to prevent the formation of aerosols.

The sample was transferred to glass tubes, shaken for 15 min in +4°C and centrifuged at 1000*g* for 5 min at +4°C. The hexane supernatant (0.015 ml/mg tissue) was collected and evaporated under nitrogen gas. Mobile phase B (78% methanol, 20% *n*-propanol and 2% 1 M ammonium acetate) was added to the sample after evaporation. The samples were shaken for 5 min at +4°C, and the tubes were then vortexed for 30 s. The mobile phase B was then filtered (centrifugation for 2 min at 16,000*g*) through 0.22 micron micro-spin (LSPI Filtration Products) columns. The extracts were stored at −80°C in sealed tubes. 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-TCT was detected on a channel set at 600 and 700 mV as described previously [4,20,22,27].

Data presentation. Results are illustrated as mean ± SD. The significance of difference between genders in the same generation, the significance of difference between generations for the same gender, and the significance of difference between washout group and supplemented group for the same gender were examined by ANOVA. $p < 0.05$ was considered to indicate statistically significant difference between means.

Results

This work represents the continuation of our effort to investigate the tissue availability of α-TCT in response to long-term oral supplementation. Furthermore, the washout of α-TCT after long-term oral supplementation was examined. A fundamental consideration that influenced the design of this study was that the standard laboratory chow contains excessive amounts of α-TCP [4,26]. In light of the knowledge that natural analogs of vitamin E may compete for specific transporting mechanisms [17], we chose to use vitamin E deficient standardized laboratory chow for this study. Animals maintained on such a diet were gavaged with known amounts α-TCT throughout the 3 year period (Figure 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 newborns may occur in

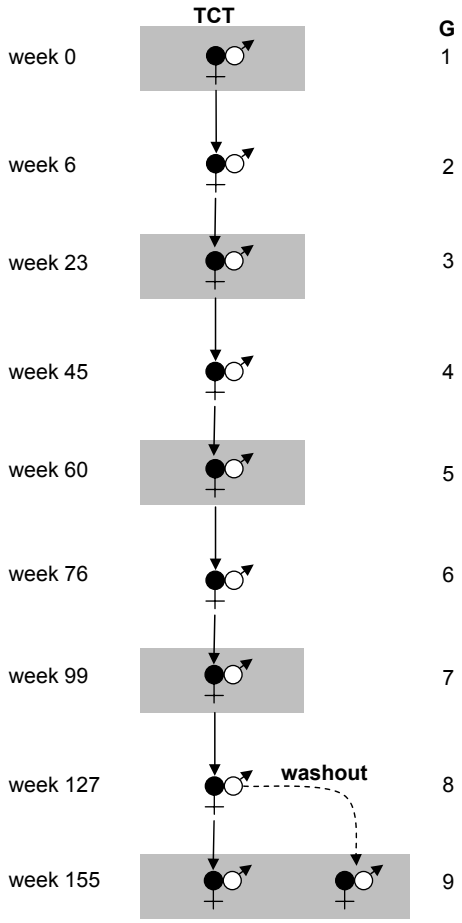

Figure 1. Schematic representation of the study design aimed at examining the long-term effects of oral supplementation of TCT in rats. Females rats were maintained on vitamin E-deficient diet (TD 88163, Harlan) and supplemented (5 days/week) with: TCT (5 mg/kg body weight). These rats were identified as first generation breeders, i.e. $G = 1$. Ninth generation offspring were divided in to two groups: (i) supplemented with TCT as described above, and (ii) washout group, maintained on a 7 week vitamin E-deficient diet (represented by dotted line). Offspring from supplemented groups nursed from their mother until 4 weeks of age. On the fifth week of age, the offspring were weaned and supplemented with TCT for 1 week for $G = 2$, 3, and 5; 28 weeks for $G = 8$; and 7 weeks for $G = 9$. This was followed by tissue harvest in $G = 2$, 3, 5, 8, and 9. The mean duration taken to generate each generation is indicated in weeks against each G row. Sample size for $G = 2$, $n = 3$ M and 3 F. For $G = 3$, $n = 3$ M and 3 F. For $G = 5$, $n = 4$ M and 4 F. For $G = 8$, $n = 3$ F. For $G = 9$, $n = 6$ M TCT, $n = 4$ M washout, $n = 6$ F TCT, and $n = 4$ F washout. M, male; F, female; G, generation.

response to gavaging of pregnant mother rats [20]. 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 (Figure 1).

Skin was noted to be most efficient in receiving orally supplemented TCT (Figure 2). Longer supplementation resulted in a marked increase in the α-TCT levels in the skin indicating a cumulative build-up of α-TCT over time. This observation indicates the presence of an effective transport mechanism delivering α-TCT to the skin and efficient retention of α-TCT in the skin over time. However, the build-up of α-TCT in the skin over time was rapidly obliterated when oral vitamin E supplies were withdrawn for only 7 weeks. Results from the third, fifth, and ninth generations indicate higher levels of α-TCT in the skin of females compared to that of male rats. Adipose tissue serves as a reservoir of vitamin E in the body [28]. Analysis of α-TCT content in adipose tissue of fifth, eighth, and ninth generation rats revealed accumulation of α-TCT over time (Figure 3). α-TCT enriched in the adipose tissue over time was sharply eliminated in 7 weeks. α-TCT levels in the washout group were consistently multifold lower compared to their supplemented counterparts. Consistent with the observation in the skin, levels of α-TCT were strikingly higher in the adipose tissue of females compared to that of males. This gender-dependent observation led us to examine the vitamin E levels in the gonads. Of note, the level of α-TCT was approximately one magnitude higher in ovaries than in the testes (Figure 3). Elimination of α-TCT from the enriched ovaries, under conditions of vitamin E free diet, was observed to be much sharper compared to that from the testes. After 7 weeks of washout, gonadal levels of α-TCT were comparable in both genders.

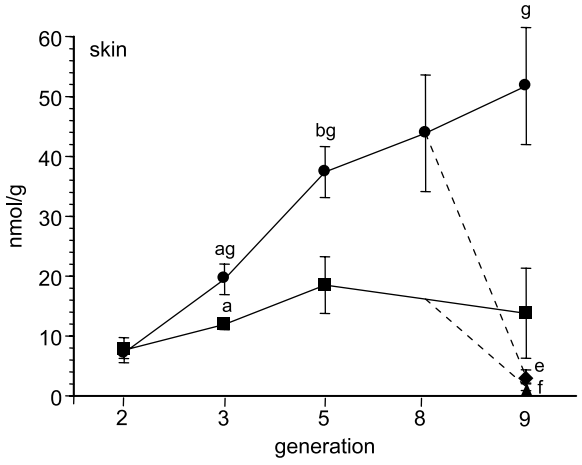

Figure 2. α-Tocotrienol levels in the skin. Animals were maintained on vitamin E-deficient diet and supplemented with TCT. Square represents α-TCT level in TCT supplemented males. Circle represents α-TCT level in TCT supplemented females. Triangle represents α-TCT level in male washout group. Diamond represents α-TCT level in female washout group. Dotted line represents the effect of α-TCT washout. a, Higher than in corresponding gender-matched supplemented rats in *G2*; b, higher than in corresponding gender-matched supplemented rats in *G3*; e, lower in female washout group than corresponding female TCT supplemented rats; f, lower in male washout group than corresponding male TCT supplemented rats; g, higher in females compared to corresponding males in the same generation and supplementation group.

Unlike that for the transport of α-TCP, the liver does not seem to serve as the hub for α-TCT distribution in the body. Levels of a-TCT in the liver were much lower than the levels noted in most organs studied. The level of α-TCT in the liver of TCT supplemented female rats steadily increased in response to longer supplementation (Figure 4). Male rats maintained a steady α-TCT level in the liver from the second to the ninth generation. Seven weeks of TCT withdrawal resulted in near-total loss of α-TCT from the liver (Figure 4). Next, we evaluated the vital organs heart and lungs. The level of α-TCT in the heart of TCT supplemented rats increased in response to longer supplementation (Figure 5). Delivery of oral α-TCT to the heart of females was more efficient compared to that of males. The observed depression in the level of α-TCT in female hearts of the eighth generation, compared to the fifth generation, was not significant. The build-up of α-TCT in heart over time was obliterated during 7 weeks when the sub-group of rats was subjected to vitamin E deficient diet. α-TCT levels in the hearts of the washout group were strikingly low and comparable to pre-supplementation baseline values reported earlier [4]. Observations in the lung were consistent with those in the heart. The level of α-TCT in the lungs of TCT supplemented female rats increased in response to longer supplementation (Figure 6). α-TCT, accumulated in the lung in the lungs over 2.5 years, was sharply lower in the ninth generation washout group. Levels of α-TCT were higher in the lungs of supplemented females compared to that of supplemented males in the third and fifth generations (Figure 6).

The pattern of α-TCT accumulation in the skeletal muscle was different from what was noted in the heart and lungs. In the skeletal muscle, the maximum of α-TCT accumulated was lower than levels noted in the heart and lungs. Levels of α-TCT were higher in the vastus lateralis muscle of supplemented females compared to that of supplemented males. Interestingly, after 60 weeks of supplementation, the tissue levels seemed to plateau (Figure 7). Consistent with the observation in the heart and lungs, however, the elimination of α-TCT from the skeletal muscle was rapid (Figure 7). Oral supplementation of TCT clearly elevated α-TCT levels in tissues of the central nervous system such as the brain and spinal cord (Figure 8). Consistent with the observation in the skeletal muscle, the levels of α-TCT leveled off in the brain and spinal cord after 60 weeks of

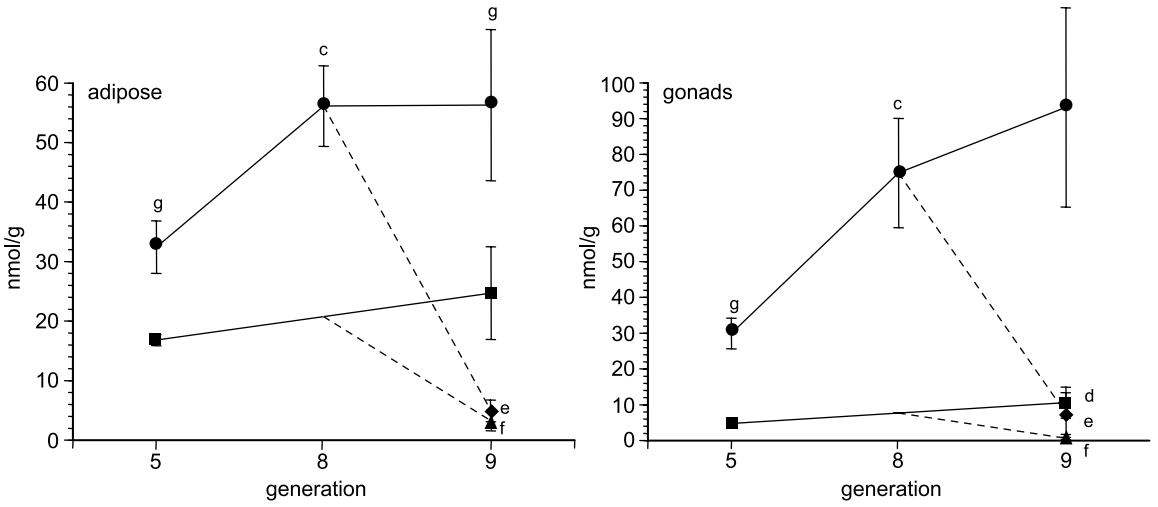

Figure 3. α-Tocotrienol levels in the adipose and gonads. Animals were maintained on vitamin E-deficient diet and supplemented with TCT. Square represents α-TCT level in TCT supplemented males. Circle represents α-TCT level in TCT supplemented females. Triangle represents α-TCT level in male washout group. Diamond represents α-TCT level in female washout group. Dotted line represents the effect of α-TCT washout. c, higher than in corresponding gender-matched supplemented rats in *G*5; d, higher than in corresponding gender-matched supplemented rats in *G*8 for females and *G*5 for males; e, lower in female washout group than corresponding female α-TCT supplemented rats; f, lower in male washout group than corresponding male α-TCT supplemented rats; g, higher in females compared to corresponding males in the same generation and supplementation group.

supplementation (Figure 8). Further supplementation beyond that period did sustain the tissue α-TCT level but not elevate it. Females had higher levels of α-TCT compared to males. Withdrawal of TCT supplementation for 7 weeks sharply depleted α-TCT levels in the brain as well as in the spinal cord (Figure 8). The baseline circulating level of α-TCT in the blood of TCT-supplemented female and male rats increased in response to longer supplementation (Figure 9). Of note, the circulatory α-TCT levels reported are from 24 h after the last supplementation representing steady-state circulatory levels. Post-washout levels of α-TCT in the blood were virtually non-detectable. Supplemented females had a higher α-TCT than supplemented males in the third, fifth, and ninth generations.

Discussion

Our recent works have provided striking new results supporting that nanomolar concentrations of α-TCT, but not α-TCP, is potently protective against stroke related neurodegeneration [1,21–23]. Delivery of orally taken vitamin E to vital organs is a key determinant of the overall efficacy of vitamin E in those tissues. Although interest in vitamin E transport to tissues is a matter of active investigation [3,16], information related to TCT transport is scanty. This work represents the maiden long-term effort where the effect of TCT supplementation was studied over a period of 3 years. Orally supplemented α-TCT reached all vital organs. As a general trend, tissues of females had higher levels of α-TCT compared to matched tissues of corresponding males. This finding is consistent with that of our previous study [4]. Gender-based differences in the transport of dietary vitamins are known to exist in specific cases [29].

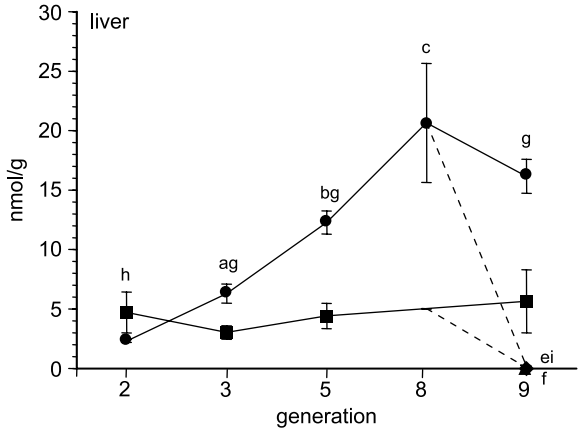

Figure 4. α-Tocotrienol levels in the liver. Animals were maintained on vitamin E deficient diet and supplemented with TCT. Square represents α-TCT level in TCT supplemented males. Circle represents α-TCT level in TCT supplemented females. Triangle represents α-TCT level in male washout group. Diamond represents α-TCT level in female washout group. Dotted line represents the effect of α-TCT washout. a, Higher than in corresponding gender-matched supplemented rats in *G*2; b, higher than in corresponding gender-matched supplemented rats in *G*3; c, higher than in corresponding gender-matched supplemented rats in *G*5; e, lower in female washout group than corresponding female α-TCT supplemented rats; f, lower in male washout group than corresponding male α-TCT supplemented rats; g, higher in females compared to corresponding males in the same generation and supplementation group; h, higher in males compared to corresponding females in the same generation and supplementation group; i, higher in female washout compared to corresponding male washout.

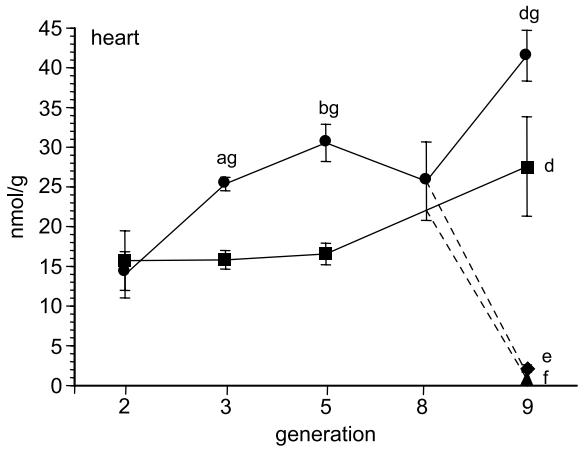

Figure 5. α-Tocotrienol levels in the heart. Animals were maintained on vitamin E-deficient diet and supplemented with TCT. Square represents α-TCT level in TCT supplemented males. Circle represents α-TCT level in TCT supplemented females. Triangle represents α-TCT level in male washout group. Diamond represents α-TCT level in female washout group. Dotted line represents the effect of α-TCT washout. a, higher than in corresponding gender-matched supplemented rats in *G*2; b, higher than in corresponding gender-matched supplemented rats in *G*3; d, higher than in corresponding gender-matched supplemented rats in *G*8 for females and *G*5 for males; e, lower in female washout group than corresponding female α-TCT supplemented rats; f, lower in male washout group than corresponding male α-TCT supplemented rats; g, higher in females compared to corresponding males in the same generation and supplementation group.

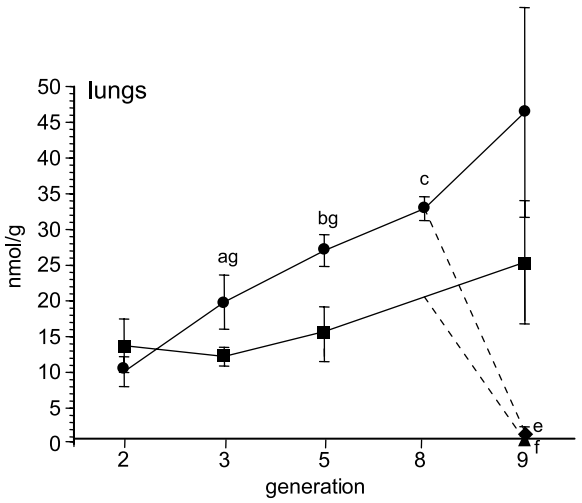

Figure 6. α-Tocotrienol levels in the lungs. Animals were maintained on vitamin E-deficient diet and supplemented with TCT. Square represents α-TCT level in TCT supplemented males. Circle represents α-TCT level in TCT supplemented females. Triangle represents α-TCT level in male washout group. Diamond represents α-TCT level in female washout group. Dotted line represents the effect of α-TCT washout. a, higher than in corresponding gender-matched supplemented rats in *G*2; b, higher than in corresponding gender-matched supplemented rats in *G*3; c, higher than in corresponding gender-matched supplemented rats in *G*5; e, lower in female washout group than corresponding female α-TCT supplemented rats; f, lower in male washout group than corresponding male α-TCT supplemented rats; g, higher in females compared to corresponding males in the same generation and supplementation group.

Although the effect of several physiological factors on vitamin E transport has been studied, the gender factor remains to be specifically addressed [30]. The gender-specific effect was most prominent in response to long-term supplementation. Of interest, gonads of the fifth and ninth 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 corresponding male rats. In the ovary, TCP is known to accumulate via a lipoprotein receptor dependent mechanism [31]. Whether α-TCT shares that mechanism remain to be tested.

This study establishes that not only does orally supplemented α-TCT reaches the vital organs but that it is delivered in significant amounts indicating the presence of effective α-TCT transport systems *in vivo*. The maximum levels of α-TCT in the brain and spinal cord of supplemented rats were roughly half to a fourth of the baseline levels of α-TCP in those organs [4]. Considering the α-TCT is orders of magnitude more effective in neuroprotection, the observed levels of α-TCT in the tissues of the nervous system are noteworthy. Complete prevention of stroke-related neurodegeneration can be achieved by nanomolar concentration of α-TCT [21–23]. The steady-state level of α-TCT in blood of supplemented rats was an order of magnitude higher than the concentration necessary for complete neuroprotection. Indeed, oral supplementation of α-TCT has been shown to significantly minimize stroke-related injury of the brain *in vivo* [23]. In response to oral supplementation, α-TCT levels were highest in the skin. The maximum levels of α-TCT in the skin of supplemented rats were roughly fourfold higher than the baseline levels of α-TCP in those organs [4]. The adipose and gonads represented the other organs in which supplementation resulted in levels of α-TCT higher than the baseline levels of α-TCP found in these rats [4]. In vital organs such as the heart, lung, skeletal muscle and liver long-term supplementation resulted in levels of α-TCT that were comparable to the levels of α-TCP in rats fed with standard rat chow containing excessive α-TCP [4].

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 [3]. 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 [3]. Results of the current study show that α-TCT levels in the liver of rats were much lower

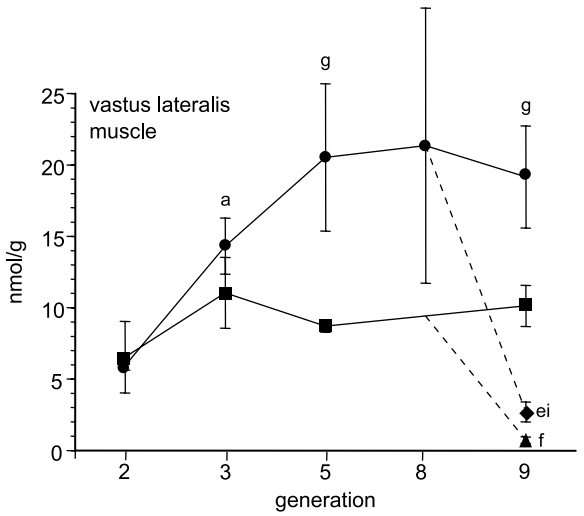

Figure 7. α-Tocotrienol levels in the vastus lateralis skeletal muscle. Animals were maintained on vitamin E-deficient diet and supplemented with TCT. Square represents α-TCT level in TCT supplemented males. Circle represents α-TCT level in TCT supplemented females. Triangle represents α-TCT level in male washout group. Diamond represents α-TCT level in female washout group. Dotted line represents the effect of α-TCT washout. a, higher than in corresponding gender-matched supplemented rats in *G2*; e, lower in female washout group than corresponding female α-TCT supplemented rats; f, lower in male washout group than corresponding male α- TCT supplemented rats; g, higher in females compared to corresponding males in the same generation and supplementation group; i, higher in female washout compared to corresponding male washout.

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. Supplementation for over 2 years was necessary to reach a plateau in the levels of α-TCT in the liver. This observation is consistent with our previously stated hypothesis that long-term lack of TCT in the standard laboratory diet may repress any putative TCT transport mechanism *in vivo* [4]. Supplementation of the rats with TCT for 2 years may have recruited the dormant TCT transport mechanisms *in vivo*. 3-Hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase activity in the liver represents a major source of cholesterol biosynthesis in the body [32]. The purported cholesterol-lowering property of TCT depends on the ability of this form of natural vitamin E to inhibit HMG-CoA reductase activity [6,7]. Results of clinical studies testing the effect of TCT supplementation in diabetics indicate that the limited level of TCT in the liver is sufficient to prevent and treat hyperlipidemia and atherosclerosis [33].

Elimination of TCP from organs is known to be a very slow process [34,35]. For example, it took over 5 months to deplete 50% of brain TCP [35]. To gain insight into how quickly α-TCT is metabolized in the tissues, washout of α-TCT from vital organs was examined. TCP and TCT are metabolized to carboxyethyl hydroxychroman as final products. TCT and TCP are metabolized by essentially the same mechanism, involving an initial ω-hydroxylation followed by five cycles of β-oxidation [36]. However, identification of an intermediate metabolite of TCT metabolism, carboxymethylbutyl hydroxychroman, lacking the double bond present in a precursor suggests that the metabolism of TCT is more complex than that of TCP [36]. α-TCT accumulated in vital organs over more than 2 years was almost completely lost in 7 weeks.

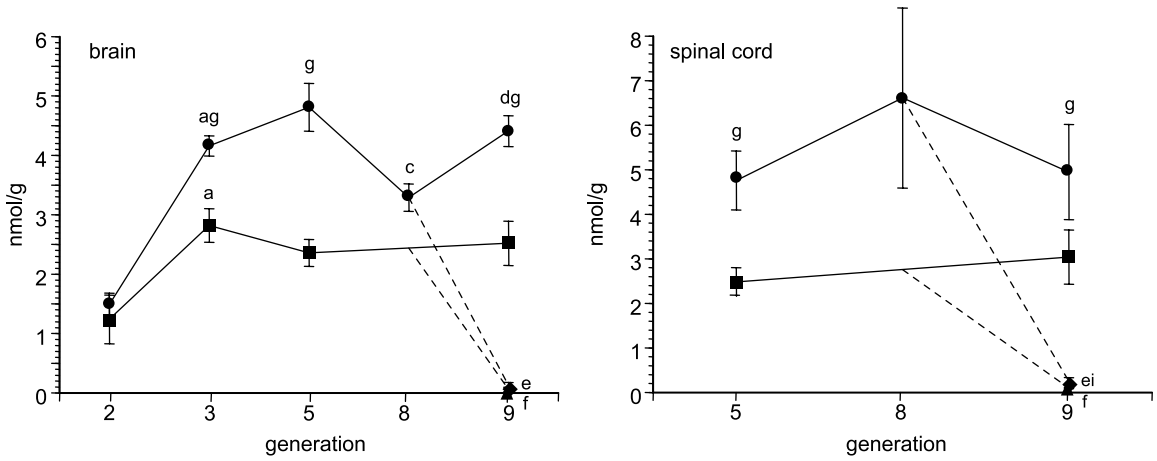

Figure 8. α-Tocotrienol levels in the brain and spinal cord. Animals were maintained on vitamin E-deficient diet and supplemented with TCT. Square represents α-TCT level in TCT supplemented males. Circle represents α-TCT level in TCT supplemented females. Triangle represents α-TCT level in male washout group. Diamond represents α-TCT level in female washout group. Dotted line represents the effect of α-TCT washout. a, higher than in corresponding gender-matched supplemented rats in *G2*; c, lower than in corresponding gender-matched supplemented rats in *G5*; d, higher than in corresponding gender-matched supplemented rats in *G8* for females and *G5* for males; e, lower in female washout group than corresponding female α-TCT supplemented rats; f, lower in male washout group than corresponding male α-TCT supplemented rats; g, higher in females compared to corresponding males in the same generation and supplementation group; i, higher in female washout compared to corresponding male washout.

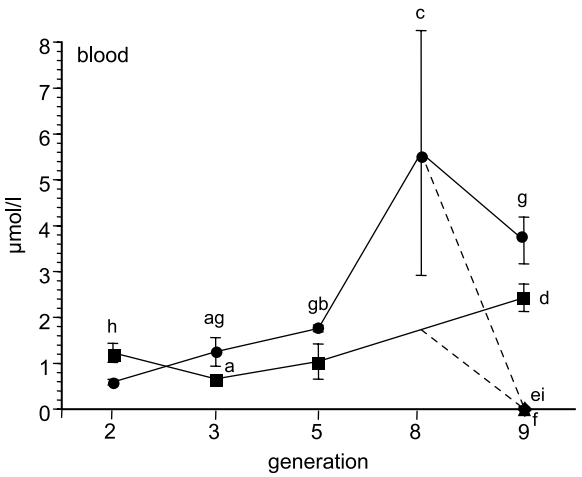

Figure 9. α-Tocotrienol levels in the blood of rats. Animals were maintained on vitamin E-deficient diet and supplemented with TCT. Square represents α-TCT level in TCT supplemented males. Circle represents TCT level in TCT supplemented females. Triangle represents α-TCT level in male washout group. Diamond represents α-TCT level in female washout group. Dotted line represents the effect of α-TCT washout. a, higher or lower than in corresponding gender-matched supplemented rats in *G*2; b, higher than in corresponding gender-matched supplemented rats in *G*3; c, higher or lower than in corresponding gender-matched supplemented rats in *G*5; d, higher than in corresponding gender-matched supplemented rats in *G*8 for females and *G*5 for males; e, lower in female washout group than corresponding female α-TCT supplemented rats; f, lower in male washout group than corresponding male α-TCT supplemented rats; g, higher in females compared to corresponding males in the same generation and supplementation group; h, higher in males compared to corresponding females in the same generation and supplementation group; i, higher in female washout compared to corresponding male washout.

This striking observation is in sharp contrast to findings with TCP [34,35]. The findings are indicative of low tissue retention and rapid metabolic turnover of α-TCT as previously speculated [37]. Indeed, TCT have been shown to be degraded to a larger extent than TCP [36]. The *in vivo* elimination half life of TCT has been shown to be much shorter than that of α-TCP [38]. The shorter elimination half life of TCT than that of TCP seems to be dependent on its higher metabolic rate and antioxidant property [10,36].

Vast majority of the current literature on vitamin E addresses α-TCP [2,4]. As we learn more about the unique functions of the other forms of vitamin E and about the potential safety issues of α-TCP [39,40], current interest focuses on the non-α-TCP forms of vitamin E [1,2,41,42]. Nanomolar α-TCT, but not α-TCP, is potently neuroprotective [21–23]. On a concentration basis, this finding represents the most potent of all biological functions exhibited by any natural vitamin E molecule. In a recent human study, we have noted that the maximal α-TCT concentrations in supplemented individuals average approximately 3 μM in blood plasma, 1.7 μM in LDL, 0.9 μM in triglyceride rich lipoprotein and 0.5 μM in HDL. The current study, albeit logistically challenging because of the 3 years duration, provided novel information about the maximum achievable levels of α-TCT in vital organs. While in organs such as the brain and skeletal muscle that level was reached in just over a year, it took over 2 years for the liver. The colony of rats supplemented for 3 years, therefore, may serve as a useful tool to delineate the mechanisms of TCT transport in the body. α-TCT accumulated in organs for over 1 year was almost completely lost from the tissues in 7 weeks. The ability of long term oral supplementation to maintain and even increase α-TCT levels in vital organs together with the rapid elimination from all organs studied underscores the need for continuous oral supplementation of TCT. Further investigation aimed at the identification of specific α-TCT transport mechanisms *in vivo* is warranted.

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