Serial Article

New Findings on the Relationship between Aging and Oxidative Stress

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Short-term intake of delta-tocotrienol on lipid profiles in healthy subjects

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Delta-tocotrienol (δ-T3) are beneficial for lipid metabolism. However, there are almost no reports on the specific numerical values of changes in blood tocotrienol levels when Japanese people consume tocotrienol. To observe the effect on blood δ-T3 and lipid concentrations after taking δ-T3, a crossover test was conducted with a control group, alpha-tocopherol (α-TP) group (264 mg/day), and δ-T3 group (250 mg/day). Participants took the target supplements for 14 days. This study revealed that plasma δ-T3 concentrations in healthy men and women increased significantly even with short-term intake (pre; 0.11 ± 0.03 to post; 0.40 ± 0.19 μmol/L). The results of this study showed that α-TP and δ-T3 intake did not significantly affect any of the lipid profiles. However, the intake of δ-T3 tended to increase Low-density lipoprotein cholesterol (HDL-C) levels (pre: 59 ± 17 to post: 64 ± 20 mg/dl). These results provide new evidence on changes in blood δ-T3 concentrations with δ-T3 intake in young people and suggest the potential of δ-T3 for the prevention of atherosclerosis.

***Key Words*:** **short-term intake, delta-tocotrienol, lipid profiles, blood delta-tocotrienol level**

Cardiac and cerebrovascular disease are endpoints of lifestyle-related diseases, and atherosclerosis is one of the factors contributing to these diseases. Atherosclerosis often develops with age from maturity onwards; therefore, prevention from a young age is essential. Statins used to treat hypercholesterolemia competitively inhibit the binding of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, thereby inhibiting cholesterol synthesis and promoting low-density lipoprotein (LDL-C) receptor synthesis.(1) Conversely, tocotrienol (T3) is involved in the post-transcriptional repression of HMG-CoA reductase, which inhibits cholesterol synthesis without affecting the substrate of HMG-CoA reductase.(2,3) Delta tocotrienol (δ-T3) has greater inhibition of HMG-CoA reductase than any other T3.(4) However, while such beneficial reports have been made, the oral bioavailability of δ-T3 is reported to be as low as 8.5%.(5) Furthermore, few reports show specific blood δ-T3 levels in healthy Japanese individuals after short-term continuous intake of δ-T3. This study aimed to determine blood δ-T3 levels in healthy young people who consume δ-T3 continuously. Moreover, this study examined whether δ-T3 intake alters lipid metabolism in young Japanese individuals.

Material and Methods

Study design. This was a placebo-controlled crossover study. The study was conducted in accordance with Ethical Guidelines for Medical and Biological Research Involving Human Subjects set out in the Declaration of Helsinki, in accordance with Ethical Regulations for Medical Research Involving Human Subjects at Toyo University. This study was approved by the Ethics and Research Committee of Toyo University (Approval number: TU2019-023 Approval date: 21 October 2019). Written consent was obtained from all participants. The study period was nine months, from January to October 2020, with three cycles of 14 days of vitamin intake per period. The control, alpha-tocopherol (α-TP) intake period, and δ-T3 intake periods were set for 14 days each. Blood samples were collected before and after each period. Physical measurements were performed after urination and defecation. Physical measurements and blood samples were obtained from Toyo University. The washout period was at least 28 days between the control period and the α-TP intake and δ-T3 periods (Fig. 1). To demonstrate that the inhibition of CoA reductase by δ-T3 among the vitamin E homologs is beneficial for lipid metabolism, an α-TP group of vitamin E homologs was established in addition to the control group for comparison.

Participants. The sample size was $\alpha = 0.05$, power = 0.8, and was calculated from figures in previous reports.(6,7) The study included 21 participants; however, due to the COVID-19 epidemic, 10 participants (six males and four females) were able to participate in all experimental periods. The participants had *ad libitum* access to food and drinking water during the experimental period. Alcohol consumption, overeating, and excessive exercise were restricted during each study period. The participants were not smokers.

Supplement intake. Participants took α-TP at 267 mg/day [Allergy Research Group, Vitamin E (D-α-tocopherol acid

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(*n*=10)	STEP 1	Wash-out	STEP 2	Wash-out	STEP 3
Days	1・・・・・・・・・・・・14 15	≥28 days	1・・・・・・・・・・・・14 15	≥28 days	1・・・・・・・・・・・・14 15
Collecting blood (Fasted morning)	○ ○		○ ○		○ ○
Questionnaire (BDHQ)	○		○		○
Group 1	Control		α-TP		δ-T3
Group 2	α-TP		δ-T3		Control
Group 3	δ-T3		Control		α-TP

Fig. 1. Experimental protocol: The participants were randomly divided into three groups. The experimental period was divided into three periods: Steps 1–3, a control period, an α-TP intake period, and a δ-T3 intake period. Fasting blood was collected in the early morning of the first day of each period and in the early morning of the day following the last day.

Table 1. Weight and Height change of participants

(*n* = 10)	Control		α-TP		δ-T3	
	Pre	Post	Pre	Post	Pre	Post
Height (cm)	169.3 ± 8.0	169.4 ± 8.1	169.5 ± 8.1	169.5 ± 8.1	169.4 ± 8.2	169.5 ± 8.2
Weight (kg)	58.9 ± 9.8	58.8 ± 10.1	59.4 ± 9.7	59.2 ± 10.2	59.3 ± 10.2	59.2 ± 10.3
BMI	20.4 ± 2.0	20.3 ± 2.1	20.6 ± 2.1	20.5 ± 2.2	20.5 ± 2.2	20.5 ± 2.2

The following table shows the changes in height and weight of the participants during each period. No significant changes were observed before, during, or after each period or between any of the three periods (Paired samples *t* test, one-way analysis of variance).

succinicinate)] or δ-T3 (250 mg/day) [Allergy Research Group, δ-Fraction Tocotrienols; Tocotrienols (as DeltaGOLDR containing 90% δ-Tocotrienols and 10% γ-Tocotrienols)]. They were set at amounts within a range that considered the Dietary Reference Intakes (2020) and previous studies.[8,9] During the vitamin intake period, the participants took one tablet of the supplement after breakfast and dinner (two tablets per day).

Sample and data collection. Height and weight were measured using a body composition analyzer (Body Composition Analyzer Inbody 770; Inbody Japan Inc., Tokyo, Japan). Early morning fasting blood samples were collected before and after each study period, that is, immediately before supplement intake and on the morning of the day after the intake ended (Fig. 1). The participants fasted for 12 h before blood collection. Blood was drawn from a vein on the upper arm of each participant. Dietary records for each study period were examined using a brief self-administered diet history questionnaire (BDHQ). Data analysis of the BDHQ, which is a reliable questionnaire on dietary intake with equal or slightly higher validity than similar questionnaires, was conducted at the DHQ Support Center.[10,11] The measured parameters were α-TP, δ-T3, total cholesterol (TC), triglycerides (TG), free cholesterol (FC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). α-TP and δ-T3 were analyzed using liquid chromatography: high-performance liquid chromatography (HPLC). The other analytes were analyzed and measured by LSI Medience Corporation.

δ-T3 and α-TP analysis for HPLC. Serum samples were analyzed using HPLC [Pump; EP-700 LIQUID CHROMATOGRAPH PUMP (Eicom, Kyoto, Japan), Auto sampler; AS-4050 (JASCO Corporation, Tokyo, Japan), Detector; FP 2025 Plus Intelligent Fluorescence Detector (JASCO Corporation)]. The standard reagents were used *d*-α-tocopherol 250 mg for vitamin E determination (Mitsubishi Chemical Foods Corporation, Tokyo, Japan), *d*-*δ*-tocotrienol 100 mg and dl-tocol 100 mg (Tama Biochemical Co., Ltd., Tokyo, Japan). Analyses were performed using the internal standard method. The analysis conditions were as follows: The column was a COSMOSIL Packed column 5PFP 4.6 mm I.D. × 250 mm, and the pre-column was an Eicom PC-04 4.0 mmφ × 5 mm. The column temperature was 40°C, the wavelength was EX 292 nm/EM 325 nm, the mobile phase was Methanol: MilliQ (v/v) = 9:1, the flow rate was 700 μl/min, and the injection volume was 20 μl.

Statistical analysis. The data obtained were analyzed using the statistical software SPSS (Statistical Package for the Social Sciences ver. 26). Values are expressed as mean ± SD. Pre- and post-intervention comparisons were made using a test of normality (Shapiro–Wilk test), followed by a corresponding *t* test for those for which normality was confirmed. A Wilcoxon signed-rank test was performed for data for which normality was not observed. For changes in concentration, a one-way analysis of variance (Kruskal–Wallis test, one-way nonparametric) was performed based on the results of the significance probability after the test of normality (Shapiro–Wilk) was conducted. All tests were two-tailed, with a significance level of <5%.

Results

Physical characteristics. Changes in the height and weight of the participants during each period are represented (Table 1). Six male and four female participants took part in all periods. No significant changes were observed in height or weight before or after each period or between any of the three periods. The participants' BMI were within the "normal weight" range of between 18.5 and 25, as defined by the Japan Society for the Study of Obesity.

Energy and nutrient intake. The participants' energy intakes were 1,861 ± 769 kcal, 1,812 ± 441 kcal, and 1,947 ± 487 kcal in the control, α-TP, and δ-T3 intake periods, respectively. The PFC (protein, fat, and carbohydrate) ratios were within the ranges of 13% to 20% for P, 20% to 30% for F, and 50% to 65% for C for all periods (Supplemental Table 1*). No significant changes were observed in energy and nutrient intake in the control, α-TP, and δ-T3 intake periods, respectively. The vitamin E (V.E) intake met the recommended dose (RDA).[12]

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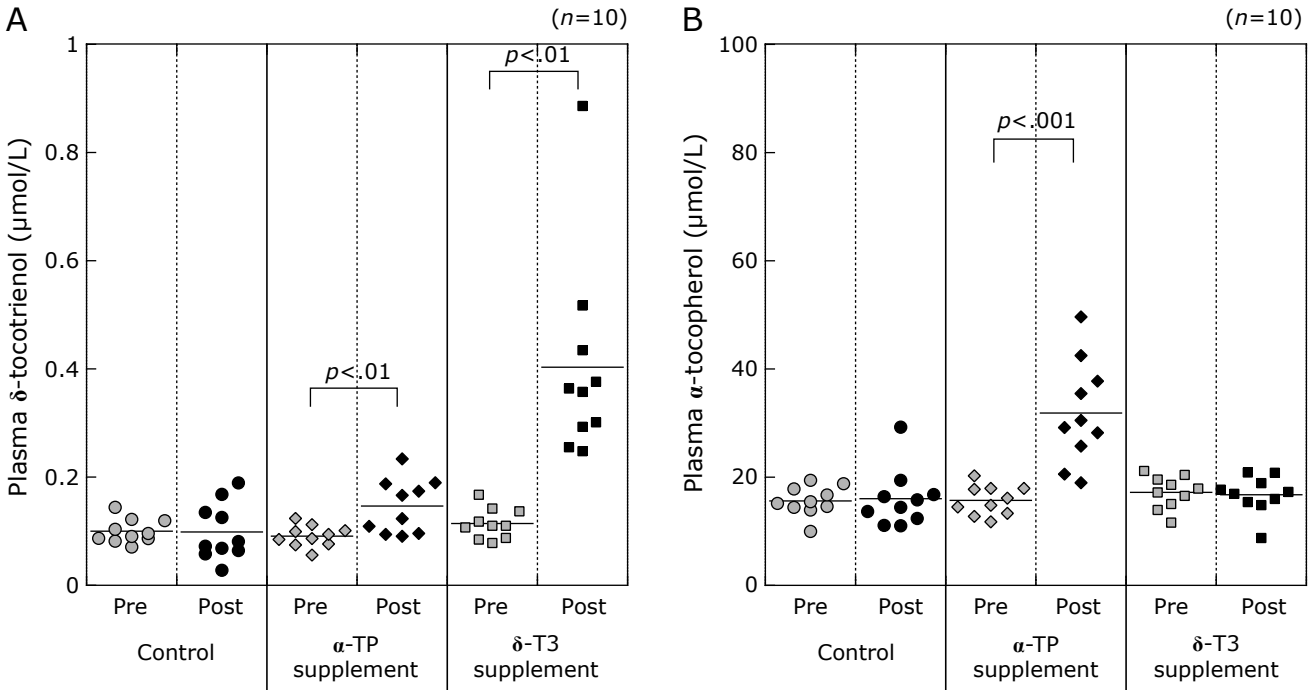

Fig. 2. Variations of concentration in plasma analysis items after α-tocopherol and δ-tocotrienol supplementation. (A) Change in plasma δ-T3 concentration; δ-T3 supplementation significantly increased plasma δ-T3 concentration (Pre; 0.11 ± 0.03, Post; 0.40 ± 0.19 μmol/L). Plasma δ-T3 was significantly increased by the intake of α-TP (Pre; 0.09 ± 0.02, Post; 0.15 ± 0.05 μmol/L). Statistical analyses were performed using a paired samples *t* test. (B) Change in plasma α-TP concentration; α-TP supplementation significantly increased plasma α-TP concentration (Pre; 15.7 ± 2.7, Post; 31.8 ± 9.6 μmol/L). The reference values for plasma α-TP concentrations are usually between 17.4 and 32.7 μmol/L. Statistical analyses were performed using a paired samples *t* test.

V.E intake met the recommended intake (RDA values) under conditions that did not include α-TP or δ-T3 supplements.

Plasma δ-T3 concentration and lipid profile. δ-T3 supplementation significantly increased plasma δ-T3 concentrations from 0.11 ± 0.03 μmol/L to 0.40 ± 0.19 μmol/L (p<0.01). α-TP supplementation significantly increased plasma δ-T3 concentration from 0.09 ± 0.02 μmol/L to 0.15 ± 0.05 μmol/L (p<0.01) (Fig. 2A). α-TP supplementation significantly increased plasma α-TP concentrations from 15.7 ± 2.7 μmol/L to 31.8 ± 9.6 μmol/L (p<0.001). (Fig. 2B). Variations in plasma δ-T3 concentrations before and after each supplementation were compared among the three groups. The change in plasma δ-T3 concentration (0.29 ± 0.19 μmol/L) was significantly increased by δ-T3 supplementation compared to the control (0.00 ± 0.06 μmol/L) and α-TP groups (0.06 ± 0.04 μmol/L) (p<0.001, p<0.01, respectively). No significant correlations were observed for lipid items, though a trend towards increased HDL-C was observed with δ-T3 intake (p = 0.063) (Table 2).

Discussion

Several reports have shown changes in blood δ-T3 levels with δ-T3 supplementation.[6,13,14] However, the bioavailability of δ-T3 is low, and it has been said to be excreted faster than TP. This is because α-TP has a higher affinity for hepatic α-tocopherol transfer protein (αTTP) than T3, and α-TP is preferentially released into the blood or T3 is converted to TP *in vivo*.[15,16] In a report on oral administration of 300 mg of mixed T3 (α-T3 29%, γ-T3 55%, and δ-T3 14%) to healthy volunteers, the mean apparent elimination half-life (T1/2) values for α-T3, γ-T3, and δ-T3 were 4.4, 4.3, and 2.3 h, respectively. That is 4.5 to 8.7 times shorter than α-TP.[17] Therefore, there are few reports on blood δ-T3 levels in healthy individuals. Fourteen days after δ-T3 administration to healthy young men and women, which has rarely been reported, this study provided evidence of a significant increase in δ-T3 concentrations with short-term ingestion and its specific blood δ-T3 levels. Despite the low bioavailability of δ-T3, plasma δ-T3 concentrations were significantly increased by the short-term intake of δ-T3 (250 mg/day) by approximately 4-fold (0.40 ± 0.19 μmol/L) compared to before the intake of δ-T3 in this study. In one previous report, blood δ-T3 levels increased by approximately 100 ng/dl (0.25 μmol/L) in 36 men (21–30 years of age) treated for two months with 320 mg/day of a T3 mixed supplement containing 50 mg/day as δ-T3.[13] In addition, a study in which 250 mg of δ-T3 supplementation was administered to patients with hypercholesterolemia for eight weeks increased blood δ-T3 levels by 0.096 ± 0.068 μmol/L compared to placebo.[6] Under conditions of 300 mg or 600 mg of δ-T3 for 12 weeks in postmenopausal women, blood δ-T3 levels after 12 weeks were approximately 3 μmol/L for both doses.[14] From these reports, there was a significant variation in the post-ingestion values. The differences in post-intake plasma δ-T3 concentrations may be attributed to differences in participant characteristics. However, the results suggest that plasma δ-T3 concentrations are largely independent of the amount ingested and the duration of ingestion. The results of this study indicate that a short-term, two-week intake of δ-T3 (250 mg/day) is sufficient to increase plasma δ-T3 concentrations in healthy young men and women. We considered that the maintenance of blood δ-T3 levels, which has a rapid half-life, may depend on the frequency of δ-T3 intake. Although the ideal blood δ-T3 levels have not been determined based on previous reports and the results of this study, results from patients with hypercholesterolemia who were administered δ-T3 doses of 125, 250, 500, and 750 mg/day every 4 weeks in a stepwise fashion with an AHA diet showed that 250 mg/day of δ-T3 lowered the blood LDL-C the most.[18] In addition, a δ-T3 intake of 250 mg per day was most effective in reducing oxidative stress parameters and

Table 2. Comparison of changes in blood lipid levels after supplementation with α-tocopherol and δ-tocotrienol

(*n* = 10)	Control	α-TP supplementation	δ-Tocotrienol supplementation	Standard value
TC (mg/dl)				
Pre	175 ± 26	171 ± 31	176 ± 30	120 to 219
Post	180 ± 32	181 ± 26[a]	179 ± 30	
Variations	5.2 ± 19.8	10.8 ± 11.9	2.8 ± 10.9	
TG (mg/dl)				
Pre	60 ± 19	62 ± 29	76 ± 25	30 to 149
Post	62 ± 22	71 ± 29	69 ± 29	
Variations	2.5 ± 18.0	9.9 ± 15.3	−6.8 ± 22.9	
FC (mg/dl)				
Pre	43 ± 7	41 ± 8	42 ± 7	34 to 66
Post	42 ± 8	43 ± 7	44 ± 8	
Variations	−0.2 ± 3.9	1.9 ± 2.9	1.7 ± 3.5	
HDL-C (mg/dl)				M 40 to 85
Pre	61 ± 17	60 ± 16	59 ± 17	
Post	64 ± 16	63 ± 15	64 ± 20	F 40 to 95
Variations	2.7 ± 4.0	2.5 ± 5.8	4.3 ± 6.4	
LDL-C (mg/dl)				
Pre	98 ± 16	95 ± 23	98 ± 21	65 to 139
Post	101 ± 24	101 ± 23[a]	101 ± 18	
Variations	2.8 ± 16.3	6.6 ± 9.0	2.7 ± 10.1	

The table shows the changes and increases/decreases in blood parameters due to supplement intake. In addition, a comparative study of pre- and post-supplementation intake (Paired samples *t* test) and a comparison of the three groups are represented (one-way analysis of variance). Significant differences are indicated using the following letters: [a]A significant difference was observed before and after the supplemental intake ($p<0.05$).

inflammatory biomarkers (resistin, IL-1α, IL-12, FGF-b, and PDGF) associated with cardiovascular disease.[8] Therefore, we considered that 14 days of δ-T3 (250 mg/day) intake may have triggered oxidative stress marker lowering and inflammatory cytokine suppression in the human body in the present study.

The finding that α-TP intake increases plasma δ-T3 concentrations is new. However, the mechanism is difficult to interpret solely based on the current results.

The next research question is to quantify oxidative stress markers and inflammatory cytokines after short-term δ-T3 intake and to investigate their correlation with blood δ-T3 levels.

Positive by-product results showed an increase in blood α-TP levels as a result of "short-term" α-TP supplementation. Thirty-six men and women took 400 IU of α-TP for eight weeks and showed a significant increase from 22.7 ± 4.8 μmol/L to 35.1 ± 5.95 μmol/L.[19] In a study of 3 weeks of α-TP at 400 IU/day, the shortest duration known to date,[20] α-TP concentrations in the control group after 3 weeks of intake were 21.5 ± 2.7 μmol/L, compared with 33.9 ± 2.9 μmol/L in the α-TP-loaded group. These blood α-TP concentration values are the same level and increase as the 14-day, 264 mg/d α-TP intake in the current study. In other words, the current study showed that the 14-day α-TP intake reached the same blood levels as in these studies.

Moreover, this study examined the relationship between δ-T3 and the lipid profile. Statins are prescribed to improve the high-cholesterol target, HMG-CoA reductase. TRF (a mixture of α-, β-, γ-, and δ-T3) inhibits HMG-CoA reductase, a cholesterol synthase, thereby inhibiting cholesterol production in the body.[21–24] Therefore, we predicted that δ-T3 intake would lower LDL-C levels in this study. However, the results of this study showed no LDL-C lowering effect of δ-T3 (250 mg/day) intake in young men and women. LDL-C levels of the participants in this study were within the reference range and did not vary significantly. In contrast, there was an increasing trend in HDL-C when δ-T3 (250 mg/day) was ingested ($p = 0.063$). This result is similar to several reports of increased HDL-C with δ-T3 intake.[25,26] Furthermore, TG levels showed a decreasing trend with δ-T3 intake. The Framingham Heart Study showed that HDL-C level is a strong predictor of coronary artery disease (CAD) risk,[27] as each 1 mg/dl increase in HDL-C was associated with a 2% reduction in CAD risk in men and 3% in women. Thus, it is clear that the prevention of oxidation and increase in HDL-C and LDL-C levels are effective in preventing atherosclerosis. In the future, δ-T3 may be useful as an antioxidant and a nutrient to increase HDL-C levels. Although statins have intolerance problems, the advantage of improving blood lipid metabolism with δ-T3 is that δ-T3 has no side effects.

As tocotrienols may inhibit the progression of fibrosis during the early non-alcoholic steatohepatitis (NASH) formation process,[28] the mechanism of action on liver function and blood lipid profile needs to be further investigated by δ-T3.

One limitation of this study is that COVID-19 restricted the number of participants who could participate in three consecutive periods. This study has several limitations, though, most studies examining the effects of V.E on lipid parameters have been conducted on the patients, and few experiments have been conducted on healthy men and women, as in this study. This study provides valuable basic data on the effects of δ-T3 on blood lipid levels in humans, which has rarely been reported in Japan or other countries.

Author Contributions

Study concept and design, NS and MO; Acquisition of data, NS, YN, AS, and YM; Analysis and interpretation of data, NS; Drafting of the manuscript, NS; Critical revision of the manuscript for important intellectual content, AM, YM, TY, and MO; Statistical analysis, NS; Obtained funding, NS, TY, and MO; Study supervision, TY and MO. All the authors discussed the results and commented on the manuscript.

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Abbreviations

α-TP	alpha tocopherol
δ-T3	delta tocotrienol
FC	free cholesterol
HDL-C	high-density lipoprotein cholesterol
LDL-C	low-density lipoprotein cholesterol
TC	total cholesterol
TG	triglycerides
$V.B_2$	vitamin B_2
$V.B_6$	vitamin B_6
$V.B_{12}$	vitamin B_{12}
V.D	vitamin D
V.E	vitamin E

Conflict of Interest

No potential conflicts of interest were disclosed.

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