δ-Tocopherol Slightly Accumulates in the Adipose Tissue of Mice

Chikako Kiyose1*, Hiroaki Nishikawa1, Mana Nagase1, Rieko Tanaka-Yachi2, and Chie Takashashi-Muto3

1 Department of Nutrition and Life Science, Kanagawa Institute of Technology, 1030 Shimo-ogino, Atsugi-shi, Kanagawa 243-0292, JAPAN
2 Department of Pharmacology, National Center for Child Health and Development, Tokyo 157-8535, JAPAN
3 Department of Clinical Nutrition, Kitasato Junior College of Health and Hygienic Sciences, Niigata 949-7241, JAPAN

Abstract: This study aimed to compare the distribution of vitamin E analogs, particularly α-tocopherol and δ-tocopherol, in mice fed with a normal diet and a high-fat and high-sucrose diet separately. We used male C57BL/6Jcl strain mice, which were divided into six groups (control [C], Cα, Cδ, high-fat and high-sucrose [H], Ha, and Hδ groups) and bred for 4 weeks. The additional quantity of α-tocopherol or E-mix D (containing 86.7% δ-tocopherol) into diet was 800 mg/kg diet. The final body weight was significantly higher in the H group than in the C group. However, the effects of vitamin E analog intake had no significant difference, with no synergy between vitamin E and diet. Similar results were obtained in epididymal fat weight. Moreover, α-tocopherol was mainly distributed in the liver in both the Ca group and Ha group, whereas δ-tocopherol was mostly accumulated in the epididymal fat, in both the Cδ group and Hδ group. Also, δ-tocopherol was detected in all tissues in both groups. Both the α-tocopherol and δ-tocopherol levels in the epididymal fat were significantly lower in the H group than in the C group. In conclusion, our results suggest that a portion of δ-tocopherol was incorporated into the adipose tissue by chylomicron before arriving at the liver, and then it is metabolized in the liver.

Key words: vitamin E analogs, δ-tocopherol, distribution, adipose tissue, mice

1 Introduction

Vitamin E is the fat-soluble vitamins found in vegetable oils, seeds, and nuts. This vitamin has eight different naturally occurring forms or analogs: four tocopherols (α-, β-, γ-, and δ-tocopherols) and four tocotrienols (α-, β-, γ-, and δ-tocotrienols). These analogs differ in the number and position of methyl groups on the chroman ring. Tocopherol (T-)’s have saturated tails, whereas tocotrienol (T3-)’s have three double bonds in their phytyl tails. Among the eight analogs, α-T is widely known as a major antioxidant for protecting cellular membranes1. The other vitamin E analogs have also been reported about the biological function. For example, we previously investigated on the effect of γ-T on the primary hepatocytes and liver in rats; we found that γ-T3 can inhibit inflammation in such tissues2. In addition, Jiang et al. reported that γ-T significantly decreased 8-isoprostane, which is a biomarker of lipid peroxidation that can be increased by inflammation in rats3. Some reports also showed the biological function of δ-T in vivo. Wang et al. observed that δ-T, but not α-T, reduced the phosphorylation of Akt in the prostate of the mice4. Bak et al. showed that the final tumor weight in γ-T, δ-T and γ-TmT (T rich mixture T) groups was significantly decreased compared with control group, respectively in the estrogen-induced MCF-7 xenograft model mice5. Thus, the reports indicating the inhibitory effect of δ-T on the development of cancer are increasing recently. On the other hand, we have recently demonstrated that α-T increases the expression of the UCP1 and PGC-1a genes in the 3T3-L1 cells of mice and rats fed with a high-fat diet6. Furthermore, we clarified that δ-T can express thermogenic genes in 3T3-L1 cells more than α-T7. Vitamin E analogs absorbed from the small intestine are transported to various tissues by lipoprotein. Those analogs incorporated into the liver are discriminated into α-T and non-α-T by α-tocopherol transfer protein (α-TTP), which reportedly binds to α-T preferentially in rat liver8 and in humans9. Biodiscrimination by α-TTP in rat liver is related to the bioavailability of each T, and the relative affinity of α-TTP to α-T, β-T, γ-T and δ-T is 100%, 38%, 9% and 2%.
metabolized and excreted in urine\(^{10}\). This \(\alpha\)-TTP catalyzes the secretion of vitamin E analogs via a novel non–Golgi-mediated pathway in rat liver cells; subsequently, \(\alpha\)-T is incorporated into VLDL preferentially and transported to various tissues by lipoprotein\(^{11}\). Either excess \(\alpha\)-T or non–\(\alpha\)-T, such as \(\gamma\)-T, \(\delta\)-T and T3s, is rapidly metabolized and excreted in urine\(^{12}\) or bile\(^{13}\). Therefore, contrary to non–\(\alpha\)-T, only \(\alpha\)-T can accumulate in each tissue. However, some reports showed novel effects of \(\alpha\)-T, such as \(\alpha\)-T, only contrarily to non–\(\alpha\)-T, such as \(\alpha\)-T, \(\delta\)-T, \(\gamma\)-T, \(\beta\)-T, \(\alpha\)-T, \(\gamma\)-T, \(\delta\)-T, \(\beta\)-T, \(\alpha\)-T or non–\(\alpha\)-T, \(\gamma\)-T, \(\delta\)-T and T3s, is rapidly metabolized and excreted in urine\(^{12}\) or bile\(^{13}\). Therefore, contrary to non–\(\alpha\)-T, only \(\alpha\)-T can accumulate in each tissue.

2 Experimental Procedures

2.1 Materials

In this study, \(\alpha\)-T and E-mix D (\(\delta\)-tocopherol rich) were donated by Mitsubishi Chemical Foods Inc. E-mix D (\(\alpha\)-T, 0.5%; \(\beta\)-T, 0.1%; \(\gamma\)-T, 3.9%; \(\delta\)-T, 86.7%) was 91.2% pure.

2.2 Experimental procedure

All experiments were conducted according to the Guide for the Care and Use of Laboratory Animals at Kanagawa Institute of Technology.

We used male C57BL/6Jcl strain mice (3 weeks old, \(n = 31\)), which were purchased from CLEA Japan, Inc. These mice were housed individually in plastic cages and were kept in an environment controlled at 23°C ± 2°C and 55% ± 5% humidity, with 12 h/12 h light/dark cycle. They were initially fed with a basic diet for 1 week to allow them to adapt to the new environment. Thereafter, they were divided into six groups (control[C], \(\alpha\), \(\gamma\), \(\delta\), high-fat and high-sucrose[H], \(\rm H_{\alpha}\), and \(\rm H_{\delta}\) groups) according to their average weight to avoid differences. \(\text{Table 1}\) presents the diet composition of each group. The feed and water were supplied ad libitum for 4 weeks. After a 16 h fast, all the mice were sacrificed under isoflurane anesthesia, and the arterial blood and each tissue were extracted for analysis.

2.3 Extraction of vitamin E analogs from mice tissues

Vitamin E analogs in each tissue were measured using Ueda’s method\(^{14}\). A 0.1 g sample of each tissue was homogenized with 0.9 mL of 0.9% NaCl solution (wt/vol). The homogenate solution (0.1 mL) was then pipetted into a 10 mL centrifuge tube, with 0.1 mL of 2,2,5,7,8-pentamethyl-6-hydroxycromanol (100 ng/mL) as an internal standard. Subsequently, we added 1.0 mL of ethanolic pyrogallol (6%, wt/vol) to each tube while stirring. After adding 0.2 mL of KOH solution (60%, wt/vol) to each tube, we saponified the contents at 70°C for 30 min. After cooling, vitamin E analogs were extracted using 4.5 mL of NaCl solution (1%, wt/vol) and 3.0 mL of 10% ethyl acetate/n-hexane solution and then centrifuged at 3,000 rpm at 4°C for 5 min. A 2.0 mL aliquot of the upper layer was evaporated, dissolved in 0.1 mL of n-hexane, and subjected to HPLC.

2.4 Measurements of vitamin E analogs by using HPLC

The HPLC system consists of an LC-20AD pump, a DGU-20A3 degasser, a CTO-20A column oven, and an RX-10AXL fluorescence detector (Shimadzu Co., Kyoto, Japan). The analytical conditions were as follows: column, Capcellpak NH\(_2\) column (4.6 mm ID × 250 mm; FUJIFILM Wako Pure Chemical Co., Osaka, Japan); column temperature, 40°C; mobile phase, n-hexane/isopropanol ratio of 98:2; flow rate, 2.0 mL/min.

| Group                                      | C  | \(\alpha\) | \(\gamma\) | H  | \(\alpha\) | \(\delta\) |
|--------------------------------------------|----|-----------|-----------|----|-----------|-----------|
| Cornstarch                                 | 400| 400       | 400       | 84 | 84        | 84        |
| Vitamin free-casein                        | 200| 200       | 200       | 200| 200       | 200       |
| \(\alpha\)-Cornstarch                      | 132| 132       | 132       | 28 | 28        | 28        |
| Sucrose                                    | 100| 100       | 100       | 312| 312       | 312       |
| Lard                                       | 0  | 0         | 0         | 208| 208       | 208       |
| vitamin E-deficient stripped corn oil      | 70 | 70        | 70        | 70 | 70        | 70        |
| Cellulose                                  | 50 | 50        | 50        | 50 | 50        | 50        |
| Mineral mix (AIN-93G)                      | 35 | 35        | 35        | 35 | 35        | 35        |
| vitamin E-deficient vitamin mix            | 10 | 10        | 10        | 10 | 10        | 10        |
| \(\upsilon\)-Cystine                       | 3  | 3         | 3         | 3  | 3         | 3         |
| \(\upsilon\)-butylhydroquinone             | 0.014| 0.014| 0.014| 0.014| 0.014| 0.014|
| +\(\alpha\)-tocopherol                     | 0  | 0         | 0         | 0  | 0.8       | 0         |
| +E-mix D                                   | 0  | 0         | 0.8       | 0  | 0         | 0.8       |
| Total energy (kcal/kg diet)                | 3950| 3950      | 3950      | 5830| 5830      | 5830      |
Distribution of δ-Tocopherol in Each Mouse Tissue

J. Oleo Sci.

1.0 mL/min; and detection wavelength, 298 nm (excitation) and 325 nm (emission).

2.5 Statistical analysis

All data were expressed as the mean ± SD. Vitamin E concentration of each tissue was statistically analyzed by *t*-test. The final body weight, food intake, energy intake, epididymal fat weight and epididymal fat weight/100 g body weight were statistically analyzed by two-way ANOVA, followed by the Tukey–HSD post-hoc test. All statistical data were analyzed using the SPSS for Windows (Tokyo, Japan). Differences were considered significant at *p* < 0.05.

3 Results

3.1 Final body weight, food intake, energy intake, epididymal fat weights and epididymal fat weights per 100 g body weight in each group

Table 2 shows the final body weight, food intake, energy intake, epididymal fat weights and epididymal fat weights per 100 g body weight of mice in each group. The final body weight was significantly higher in the H group than in the C group. However, the effects of vitamin E analogs intake revealed no significant differences, and no synergistic effects were found between diet and vitamin E. Furthermore, food intake was significantly lower in the H group than in the C group. However, therefore, high-fat and high-sucrose diet induced obesity for 4 weeks. Because energy intake was markedly higher in the H group than in the C group.

No significant difference was found for each tissue weight (brain, heart, lung, liver, kidney, testes, and skeletal muscle), except for the epididymal fat weights in all groups (data not shown). However, the epididymal fat weights were significantly higher in the H group than in the C group. However, the effects of vitamin E analog intake were not significantly different. Moreover, similar results were obtained in epididymal fat weight/100 g body weight.

3.2 α-T concentration in the mouse tissue of the C and H groups

Figure 1 illustrates the concentration of α-tocopherol in each tissue of the control and high-fat and high-sucrose groups. C: control diet (*n* = 6), H: high-fat and high-sucrose diet (*n* = 5). The data are presented as mean ± SD.

|        | C          | Cα         | Cδ         | H          | Hα         | Hδ         |
|--------|------------|------------|------------|------------|------------|------------|
| Final body weight (g) | 24.5±1.28   | 23.8±0.86  | 24.4±2.87  | 26.3±3.16  | 26.4±0.76  | 26.9±2.52  |
| Dietary intake (g/day) | 3.28±0.28   | 3.26±0.21  | 3.12±0.42  | 2.52±0.13  | 2.57±0.15  | 2.58±0.38  |
| Energy intake (kcal/day) | 13.0±1.10   | 12.9±0.84  | 12.3±1.66  | 14.7±0.77  | 15.0±0.87  | 15.0±2.20  |
| Epididymal fat (g) | 0.35±0.10   | 0.36±0.06  | 0.38±0.12  | 0.59±0.32  | 0.56±0.11  | 0.64±0.23  |
| Epididymal fat (g/100g body weight) | 1.43±0.34   | 1.47±0.23  | 1.54±0.31  | 2.15±0.87  | 2.11±0.35  | 2.33±0.56  |

1. *Values are mean±SD, n=5-6.
2. NS, not significant.

**Table 2** Final body weight, dietary intake, energy intake and epididymal fat weight in each group

3.3 δ-T concentration in the mouse tissue of the C and H groups

In the Cδ and Hδ groups, α-T was detected in all tissues. However, the α-T level tended to be lower in the Hδ group than in the Cδ group in all tissues (Fig. 3).

Figure 4 shows the δ-T concentration in each tissue of
Fig. 2 Concentration of α-tocopherol in each tissue of the Cα and Hα groups. Cα: control diet + α-tocopherol (n = 5), Hα: high-fat and high-sucrose diet + α-tocopherol (n = 5). The data are presented as mean ± SD. Statistical analysis was performed by Student’s t-test (Cα vs. Hα). *; p < 0.05.

Fig. 3 Concentration of α-tocopherol in each tissue of the Cδ and Hδ groups. Cδ: control diet + E-mix D (n = 5), Hδ: high-fat and high-sucrose diet + E-mix D (n = 5). The data are presented as mean ± SD.

Fig. 4 Concentration of δ-tocopherol in each tissue of the Cδ and Hδ groups. Cδ: control diet + E-mix D (n = 5), Hδ: high-fat and high-sucrose diet + E-mix D (n = 5). The data are presented as mean ± SD. Statistical analysis was performed by Student’s t-test (Cδ vs. Hδ). *; p < 0.05.

The Cδ and Hδ groups. In both groups, δ-T was detected in all tissues. Furthermore, δ-T accumulated most of epididymal fat in two groups. Furthermore, the concentration of δ-T in epididymal fat of Cδ group was significantly higher than that of Hδ group. Even, we did not write the plasma data into the figure because the data had too few numbers (n = 2-3). So, the concentration of δ-T in the plasma of each group is as follows: C, not detected; Cα, not detected; Cδ, 0.063 ± 0.003 μg/mL; H, not detected; Hα, not detected; and Hδ, 0.106 ± 0.016 μg/mL.

4 Discussion
This study investigated the effects of vitamin E analogs on mice fed with a high-fat and high-sucrose diet. We found that the final body weights and epididymal fat weights were significantly higher in the H group than in the C group. However, the effects of vitamin E analog intake were not significantly different, with no synergy between diet and vitamin E (Table 2). Zhao et al. found that the body weight gain of mice fed with a high-fat diet + γ-tocotrienol was drastically lower than that of mice fed with merely a high-fat diet. Furthermore, Burdeos et al. reported that the body weight between the high-fat diet group and the high-fat diet + 10 mg rice bran tocotrienol group was not significantly different; however, the epididymal fat weight was significantly lower in the high-fat diet + 10 mg rice bran tocotrienol group than in the high-fat diet group. Therefore, tocotrienols can potentially prevent or attenuate obesity. On the other hand, we have described that α-T reportedly cannot decrease the body weight of mice fed with a high-fat diet even though α-T increases the expression of the UCP1 and PGC-1α genes of adipose tissues in rats fed with a high-fat diet. Moreover, in the present data, we were not able to prove the anti-obesity effect of δ-T. Therefore, we suggested that the anti-obesity effects of α-T and δ-T are not revealed in this study, probably because of the short feeding period (4 weeks). Hence, further examination is needed.

Next, we measured the distribution of α-T and δ-T in the tissues of mice fed with a diet containing vitamin E analogs. Results showed that α-T accumulates mostly in the liver (Fig. 2). Generally, α-T is transported to the liver and then to various tissues with VLDL again. Therefore, vitamin E stays in the liver once. However, a few numbers of δ-T remain in the liver (Fig. 4). Hence, we presumed that δ-T was metabolized more quickly than α-T in the liver. Regarding vitamin E metabolism, these analogs first undergo
Distribution of δ-Tocopherol in Each Mouse Tissue

J. Oleo Sci.

ω-oxidation by the cytochrome P450 (CYP) enzyme, followed by degradation of the side chain by β-oxidation; finally, they are converted into carboxyethyl hydroxychroman (CEHC) and excreted into urine. In the present study, we did not measure α-CEHC and δ-CEHC in rat urine, but we suggested that most of the δ-T was converted into δ-CEHC and rapidly excreted into urine. In the future, we will measure the metabolites of vitamin E in rat tissues for confirmation.

Interestingly, δ-T accumulated in the epididymal fat more than in the liver, as shown in Fig. 4. Among all vitamin E analogs, α-T accumulates the most in the body. However, vitamin E, except α-T, is characterizedly distributed. Ikeda et al. reported that α-T3 and γ-T3 were detected in substantial amounts in the skin of nude mice, hairless mice, and wistar strain rats administered with vitamin E mixtures. These T3s have low affinity with vitamin E analogs, except α-T, and were accumulated into the adipose tissue more than other tissues. Therefore, we suggested that some of the δ-tocopherol was incorporated into the adipose tissue by chylomicron before arriving at the liver, and then the remaining δ-tocopherol was metabolized in the liver.

5 Conclusion

In mice separately fed with a normal diet and a high-fat and high-sucrose diet, α-tocopherol mostly accumulated in the liver, whereas δ-tocopherol mostly accumulated in the adipose tissue but in minimal amounts. Therefore, we suggested that some of the δ-tocopherol was incorporated into the adipose tissue by chylomicron before arriving at the liver, and then the remaining δ-tocopherol was metabolized in the liver.

Acknowledgment

This work was supported by in part of MEXT-Supported Program for the Strategic Research Foundation at Private Universities 2015-2019 (S1511019L).

References

1) Traber, M.G. Determinants of plasma vitamin E concentration. Free Radical Biol. Med. 16, 229-239 (1994).
2) Yachi, R.; Muto, C.; Ohtaka, N.; Aoki, Y.; Koike, T.; Igarashi, O.; Kiyose, C. Effects of tocotrienol on tumor necrosis factor-α/D-galactosamine-induced steatohepatitis in rats. J. Clin. Biochem. Nutr. 52, 146-153 (2013).
3) Jiang, Q.; Ames, B.N. γ-Tocopherol, but not α-tocopherol, decreases proinflammatory eicosanoids and inflammation damages in rats. FASEB J. 17, 816-822 (2003).
4) Wang, H.; Yang, X.; Liu, A.; Wang, G.; Bosland, M.C.; Yang, C. δ-Tocopherol inhibits the development of prostate adenocarcinoma in prostate specific Pten-/ mice. Carcinogenesis 39, 158-169 (2017).
5) Bak, M.J.; Gupta, S.D.; Wahler, J.; Lee, H.J.; Li, X.; Lee, M.J.; Yang, C.S.; Suh, N. Inhibitory effects of γ- and
δ-tocopherols on estrogen-stimulated breast cancer in vitro and in vivo. Cancer Prev. Res., 10, 188-197 (2017).
6) Tanaka-Yachi, R.; Takahashi-Muto, C.; Adachi, K.; Tanimura, Y.; Aoki, Y.; Koike, T.; Kiyose, C. Promoting effect of α-tocopherol on beige adipocyte differentiation in 3T3-L1 cells and rat white adipose tissue. J. Oleo Sci. 66, 171-179 (2017).
7) Tanaka-Yachi, R.; Shirasaki, M.; Otsu, R.; Takahashi-Muto, C.; Inoue, H.; Aoki, Y.; Koike, T.; Kiyose, C. δ-Tocopherol promotes thermogenic gene expression via PGC-1α upregulation in 3T3-L1 cells. Biochem. Biophys. Res. Commun. 506, 53-59 (2018).
8) Sato, Y.; Hagiwara, K.; Arai, H.; Inoue, K. Purification and characterization of the α-tocopherol transfer protein from rat liver. FEBS Lett. 288, 41-45 (1991).
9) Arita, M.; Sato, Y.; Miyata, A.; Tanabe, T.; Takahashi, E.; Kayden, H.J.; Arai, H.; Inoue, K. Human α-tocopherol transfer protein: cDNA cloning, expression and chromosomal localization. Biochem. J. 306, 437-443 (1995).
10) Hosomi, A.; Arita, M.; Sato, Y.; Kiyose, C.; Ueda, T.; Igarashi, O.; Arai, H.; Inoue, K. Affinity for α-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. FEBS Lett. 409, 105-108 (1997).
11) Traber, M.G.; Arai, H. Molecular mechanisms of vitamin E transport. Annu. Rev. Nutr. 19, 343-355 (1999).
12) Chiku, S.; Hamamura, K.; Nakamura, T. Novel urinary metabolite of d-δ-tocopherol in rats. J. Lipid Res. 25, 40-48 (1984).
13) Kiyose, C.; Saito, H.; Kaneko, K.; Hamamura, K.; Tomioka, M.; Ueda, T.; Igarashi, O. α-Tocopherol affects the urinary and biliary excretion of 2,7,8-trimethyl-2-(2-carboxyethyl)-6-hydroxycromane, γ-tocopherol metabolite, in rats. Lipids 36, 467-472 (2001).
14) Ueda, T.; Igarashi, O. Determination of vitamin E in biological specimens and food by HPLC-pretreatment of samples and extraction of tocopherols. J. Micronutr. Anal. 7, 79-96 (1990).
15) Zhao, L.; Kang, I.; Fang, X.; Wang, W.; Lee, M.A.; Holins, M.R.; Marshall, M.R.; Chung, S. Gamma-tocotrienol attenuates high-fat diet-induced obesity and insulin resistance by inhibiting adipose inflammation and M1 macrophage recruitment. Int. J. Obes. 39, 438-446 (2015).
16) Burdeos, G.C.; Nakagawa, K.; Kimura, F.; Miyazawa, T. Tocotrienol attenuates triglyceride accumulation in HepG2 cells and F344 rats. Lipids 47, 471-481 (2012).
17) Birringer, M.; Drogan, D.; Brigelius-Folhe, R. Tocopherols are metabolized in HepG2 cells by side chain α-oxidation and consecutive β-oxidation. Free Radical Biol. Med. 15, 226-232 (2001).
18) Ikeda, S.; Niwa, T.; Yamashita, K. Selective uptake of dietary tocotrienols into rat skin. J. Nutr. Sci. Vitam. 46, 141-143 (2000).
19) Ikeda, I.; Inasato, Y.; Sasaki, E.; Sugano, M. Lymphatic transport of alpha-, gamma- and delta-tocotrienols and alpha-tocopherol in rats. Int. J. Vitam. Nutr. Res. 66, 217-221 (1996).
20) Traber, M.G.; Kayden, H.J.; Green, J.B.; Green, M.H. Absorption of water-miscible forms of vitamin E in a patient with cholestasis and in thoracic duct-cannulated rats. Am. J. Clin. Nutr. 44, 914-923 (1986).
21) Abe, C.; Ikeda, S.; Uchida, T.; Yamashita, K.; Ichikawa, T. Triton WR1339, an inhibitor of lipoprotein lipase, decreases vitamin E concentration in some tissues of rats by inhibiting its transport to liver. J. Nutr. 137, 345-350 (2007).
22) Traber, M.G.; Olivecrona, T.; Kayden, H.J. Bovine milk lipoprotein lipase transfers tocopherol to human fibroblasts during triglyceride hydrolysis in vivo. J. Clin. Invest. 75, 1729-1734 (1985).
23) Schmolz, L.; Birringer, M.; Lorkowski, S.; Wallet, M. Complexity of vitamin E metabolism. World J. Biol. Chem. 26, 14-43 (2016).