Application of Partial Hydrogenation for the Generation of Minor Tocochromanol Homologs and Functional Evaluation of Hydrogenated Tocotrienol-rich Vitamin E Oil in Diabetic Obese Mice

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Abstract: Recent research has identified minor homologs of vitamin E with one or two double bonds in the side-chain, namely tocomonoenol (T1) and tocodienol (T2), in natural products. We first explored the effectiveness of partial hydrogenation for generating minor tocochromanols from tocotrienol (T3). During hydrogenation with pure α-T3 as a substrate, the side-chain was partially saturated in a time-dependent manner, and a large amount of α-T1 and α-T2 was obtained. To investigate the beneficial effects of the hydrogenated product, we fed diabetic obese KK-øy mice with a hydrogenated T3 mixture (HT3). Feeding HT3 revealed tissue-specific accumulation of tocochromanols, ameliorated hyperglycemia and improved ratio of high-density lipoprotein cholesterol to total cholesterol in serum, with invariant body weight and fat mass. Hence, we propose that hydrogenation is a useful method for generating T1 and T2 homologs, which can be applied to explore the structure-related function of tocochromanols.

Key words: vitamin E, tocomonoenol, tocodienol, partial hydrogenation, metabolic disorder

1 Introduction

Vitamin E (VE) is a naturally occurring lipophilic antioxidant which can be obtained from the diet. Free radical scavenging reactions of VE protect cells from peroxidation of polyunsaturated fatty acids and other components of cell membrane which contributes to maintain cellular homeostasis.

The VE family is divided into tocopherols (Toc) and tocotrienols (T3), which have a saturated and unsaturated phytol side-chain, respectively. Toc and T3 are further classified into α, β, γ and δ types of chromanol structure depending on the number and position of methyl groups in the ring. Generally, Toc and T3 are widely distributed in natural products, such as palm oil in various compositions. In addition to these major tocochromanols (α-, β-, γ-, δ-Toc and T3), minor homologs, namely tocomonoenol (T1) and tocodienol (T2), with one or two double bonds on the side-chain have been recently found in natural products, such as palm oil and plant seeds⁴. The identified structure of T1 exhibits a double bond at the 11-12 position and that of T2 exhibits a double bond at the 3-4 and 11-12 position and the 7-8 position. Furthermore, another type of T1, called marine-derived tocopherol (MDT), with one double bond at the terminal 12-13 position has been found in salmon roe⁵. Given the important biological role of major VE homologs, there has been increasing interest in the health benefits of these minor homologs. However, the low content of minor homologs in natural products makes it difficult to develop analytical methods and investigate their function even in model systems.

Hydrogenation⁶ typically adds a hydrogen atom to a carbon–carbon double bond in a molecule in the presence...
of a catalyst, such as palladium or nickel. Partial hydrogenation is used extensively in many industrial applications, including the food and cosmetics industry. In this reaction, it is possible to control the degree of saturation/hydrogenation by varying the amount of hydrogen, reaction time and temperature. Hence, we hypothesize that partial hydrogenation of T3 could saturate the double bonds in the side-chain. This would effectively and selectively produce α-, β-, γ- and δ-type minor homologs using the corresponding T3 in the reaction.

We also wanted to examine the beneficial effects of the hydrogenated product in the prevention of obesity and metabolic disorders. Generally, obesity-related diseases are complications accompanied by several pathogenic factors, such as oxidative stress and inflammation. Many studies have reported the beneficial effects of VE on hyperglycemia and hyperlipidemia in humans and various other models. In addition to its antioxidant property, VE homologs have been reported to play a direct role in cell signaling and regulation of inflammatory gene expression. Furthermore, comparative studies on VE major homologs have demonstrated that the potency of functional activity differs depending on the structure of the chroman ring or side-chain. However, few studies have investigated the influence of the number of double bonds in the side-chain using T1 and T2. Further studies on the health benefits of minor homologs would provide new insights into the nutritional and physiological properties of VE compounds.

In the present study, we used α-T3 as a substrate during hydrogenation to monitor the production of minor homologs, and we confirmed the generation of α-T1 and α-T2 accompanied with decreasing α-T3 using liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis. The isolation of α-T1 and α-T2 served to describe the calibration curve for the respective homologs. To examine whether the structural properties of the side-chain affect the health benefits of VE, we hydrogenated a T3-enriched VE mixture and examined its effects on the glucose and lipid metabolism of obese/diabetic KK-A mice.

2 Materials and Methods

2.1 Materials

VE standards (α-, β-, γ- and δ-Toc and T3) were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). The T3 mixture (25.8% α-T, 0.6% α-T1, 18.4% α-T3, 42.8% βγ-T3 and 12.4% δ-T3) was kindly provided by MITSUBISHI-CHEMICAL FOODS CORPORATION (Tokyo, Japan). All other chemicals and reagents were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).

2.2 Isolation of α-T3

The T3 mixture underwent silica gel column chromatographic separation. First, α-tocochromanol were eluted with hexane/ethyl acetate (95 : 5, v/v), and then fractionated α-tocochromanol were dried under reduced pressure. Separation was performed using high-performance liquid chromatography (HPLC) with C18 column (Inertsil® ODS-3, 250 × 14 mm, 5 µm; GL Sciences, Inc., Tokyo, Japan), and an ultraviolet (UV) detector was set to 298 nm for detection. Methanol was used as the mobile phase at a flow rate of 10.0 mL/min.

2.3 Preparation of α-T1 and T2

The isolated α-T3 was hydrogenated in a 500 mL stainless-steel reactor (Taiatsu Techno Corp., Tokyo, Japan). Briefly, 50 mg of α-T3 in 50 mL of tetrahydrofuran and 0.5 mg of the palladium-activated carbon ethylenediamine complex (Pd 3.5–6.5%) were mixed in the reactor. Then, the reactor was filled with hydrogen and subjected to the following hydrogenation conditions: 0.4 MPa, 500 rpm at 25°C or 50°C. After the reaction, the hydrogenated product was depressurized, cooled down to room temperature and filtered through a filter paper. Finally, α-T2 and α-T1 were fractionated using the HPLC setup used in the isolation of α-T3.

For the animal experiment, the T3 mixture was used as the substrate for hydrogenation. This mixture was hydrogenated under the conditions described above at 25°C for 72h.

2.4 Preparation of the standard curve for α-tocochromanol using LC-MS/MS

LC-MS/MS analyses were performed using HPLC coupled with a triple quadrupole mass spectrometer (Shimadzu LC-MS/MS-8040) connected to a Shimadzu LC system (Shimadzu, Kyoto, Japan). Briefly, α-tocochromanol in methanol was separated on a Develosil ODS-UG3 column (150 × 2.0 mm, 3 µm) at 40°C. The isocratic mobile phase (methanol/acetonitrile, 60 : 40, v/v) was run at a flow rate of 0.25 mL/min. Double-bond positional isomers of α-T1 and α-T2 were separated using an InertSustain FFP column (150 × 3.0 mm, 3 µm). The mass spectrometer was operated in the negative ion mode of electrospray ionization, and the parameters were as follows: interface voltage, 2,500 V; nebuliser gas (Ar) flow rate, 2 L/min; drying gas (N2) flow rate, 15 L/min; heating block temperature, 400°C; de-solvation line temperature, 250°C; CID gas pressure, 230 kPa.

All tocochromanol were detected in multiple reaction monitoring (MRM) mode. Both the precursor and product ion mass spectra used for the detection are shown in Supplementary Table. The respective calibration curve was constructed using the ratio of peak area to that of tocol, an internal standard.
2.5 Animal care

Four-week-old male KK-A\(^{y}\) mice (CLEA Japan, Inc., Tokyo, Japan) were fed with a normal chow diet during acclimatization. Animals were housed individually in plastic cages in a temperature-controlled environment (22–24\(^{\circ}\)C) with 12 h light/dark cycles, and food and water were available \textit{ad libitum}.

One week later, the mice were divided into four groups with six mice each. Both the hydrogenated T3 mixture (HT3) and the original T3 mixture were used for diet experiments. The mice were given an AIN-93G-based diet supplement with or without 0.5\% \(\alpha\)-Toc, T3 mixture or HT3 (Table 1) for four weeks. To identify the effects of additional HT3 supplement, an \(\alpha\)-Toc group was used. All mice were sacrificed under isoflurane anesthesia on day 29 after 12 h of fasting. Tissue samples were weighed and snap-frozen in liquid nitrogen, and the harvested tissues were stored at \(-80\)\(^{\circ}\)C until analysis. All protocols followed the animal care guidelines of Tokyo University of Marine Science and Technology.

2.6 Measurement of blood glucose and serum lipids

Blood glucose levels were measured on day 26 using a Glutest Neo Alpha blood glucose monitor (Sanwa Kagaku Kenkyusyo Co., Ltd., Aichi, Japan). Blood samples were collected from the tail vein.

Serum levels of triglycerides (TG), total cholesterol and high-density lipoprotein (HDL) cholesterol (HDL-C) were measured using a commercial assay kit (FUJIFILM Wako Pure Chemical Corporation) according to the manufacturer’s instructions.

2.7 Analysis of tocochromanolns in the experimental diet and animal tissue

Total lipids extraction was performed by Folch’s method\(^{10}\). Briefly, the experimental diet or sample tissue with 1.76 \(\mu\)g of tocol was homogenized in phosphate-buffered saline. Lipids were extracted using chloroform/methanol (2 : 1, v/v), followed by centrifugation at 3,000 \(\times\) \(g\) for 10 min. The chloroform layer was collected and stored at \(-30\)\(^{\circ}\)C until analysis.

Tocochromanols were quantitatively analyzed using LC-MS/MS with the corresponding calibration curve. We herein prepared calibration curves for \(\alpha\)-T3, T2, T1 and Toc; \(\gamma\)-T3 and Toc; and \(\delta\)-T3 and Toc. As \(\beta\)- and \(\gamma\)-tocochnanols cannot be separated using the chromatographic method employed here, the content was calculated together as \(\beta/\gamma\)-T3, T2, T1 and Toc. The contents of \(\beta/\gamma\)-type and \(\delta\)-type T2 and T1 were calculated on the basis of the calibration curve constructed using \(\gamma\)-Toc and \(\delta\)-Toc, respectively. The equations were established as follows:

\[
\gamma\text{-Toc}, \quad y = 0.7587x - 0.0615 \quad (R^2 = 0.9956)
\]

\[
\gamma\text{-T3}, \quad y = 1.363x + 3.743 \quad (R^2 = 0.9903)
\]

\[
\delta\text{-Toc}, \quad y = 0.4646x + 0.1372 \quad (R^2 = 0.9993)
\]

\[
\delta\text{-T3}, \quad y = 0.7912x - 0.1552 \quad (R^2 = 0.9977)
\]

2.8 Statistical analysis

Values were represented as the mean \pm standard error (SE) of six mice. Significant differences between control and test groups were assessed using one-way ANOVA followed by Dunnett’s multiple comparisons test. Differences were regarded as significant at \(p<0.05\).

| Table 1 | Tocochromanol content (mg/g diet) and composition (%) in the experimental diet. |
|--------|--------------------------------------------------------------------------------|
|        | Control | \(\alpha\)-Toc | T3 mix | HT3 |
| \(\alpha\)-Toc | 0.096 | 100.0 | 4.810 | 100.0 | 1.132 | 25.8 | 1.129 | 24.4 |
| -T1     | - | - | - | - | 0.027 | 0.6 | 0.107 | 2.3 |
| -T2     | - | - | - | - | - | - | 0.403 | 8.7 |
| -T3     | - | - | - | - | 0.806 | 18.4 | 0.307 | 6.6 |
| \(\beta/\gamma\)-Toc | - | - | - | - | - | - | 0.168 | 3.6 |
| -T1     | - | - | - | - | - | - | 0.222 | 4.8 |
| -T2     | - | - | - | - | - | - | 0.619 | 13.4 |
| -T3     | - | - | - | - | 1.883 | 42.8 | 0.912 | 19.7 |
| \(\delta\)-Toc | - | - | - | - | - | - | 0.003 | 0.06 |
| -T1     | - | - | - | - | - | - | 0.052 | 1.1 |
| -T2     | - | - | - | - | - | - | 0.222 | 4.8 |
| -T3     | - | - | - | - | 0.546 | 12.4 | 0.491 | 10.6 |
| Total   | 0.096 | 100.0 | 4.810 | 100.0 | 4.394 | 100.0 | 4.635 | 100.0 |
3 Results and Discussion

In contrast to the vast knowledge on the physiological functions of Toc and T3, the minor homologs of VE, namely T1 and T2, remain poorly studied, which is mainly because T1 and T2 are not commercially available. Recently, Muller et al. have developed a simple and excellent method to concentrate α-T1 and T2 from palm oil using countercurrent chromatography. In that study, however, the estimated concentration of α-T2 was less than 40 μg/100 g in palm oil, and their low abundance in natural products prevent the characterization of their structure-dependent functional properties. The aim of the present study was to evaluate the effectiveness of partial hydrogenation as a new method to generate T1 and T2 homologs from T3. In addition, we explored the health benefits of hydrogenated products in preventing metabolic disorders in KK-A mice.

We first hydrogenated purified α-T3 as the substrate. As shown in Fig. 1(a), α-T3 was identified at a retention time of 4.8 min, and additional peaks (No.1 and 2) and α-Toc appeared after reaction, suggesting the presence of tocochromanols with one or two double bonds. To identify tocochromanols, we used LC-MS/MS analysis in the negative ion mode. Previous studies have shown that m/z 163 appears as a fragment of the α-type ring and that α-Toc is identified at m/z 429 (Fig. 1(b)) as the molecular ion [M-H]-. The mass spectrum exhibited a fragment ion at m/z 163.2, indicating an α-type ring, in both mass spectra from m/z 425.4 and 427.4 (Figs. 1(c) and 1(d)). A difference of two or four mass unit indicates the saturation of one or two double bonds in the side-chain. Muller et al. identified α-T2 isomers with double bonds at the 3’-4’ and 11’-12’ position or at the 7’-8’ and 11’-12’ position in palm oil. A double bond of α-T1 found in natural products has also been identified at only the 11’-12’ position. Notably, hydrogenation occurs randomly. Therefore, it is likely that one or two double bonds at 3’-4’, 7’-8’ or 11’-12’ were saturated in this study. Indeed, using a pentafluorophenyl (PFP) column, searches for m/z 163.2 from 425.4 and 427.4 showed separate peaks in a narrow time range (Fig. 1(e)), suggesting the presence of double-bond positional isomers. Our results suggest that the hydrogenated product also includes isomers that are not found in natural products.

Next, time-dependent changes in the compositions of homologs during hydrogenation at 25°C and 50°C were monitored (Fig. 2(a)). The amount of individual homologs, as the sum of double-bond positional isomers, was determined using the corresponding standard curves of α-T1 and α-T2 (Fig. 2(b)). When the temperature was set at 25°C, the ratio of α-T3 to total α-tocochromanol decreased in 24 h and almost diminished in 96 h, whereas α-T2 increased from 24 to 96 h. The ratio of α-T1 was only 9.6% under these conditions. In the reaction at 50°C, approximately 50% of α-T3 reacted with hydrogen in 3 h, and its ratio decreased to less than 20% in 12 h. The generation of α-T1 and α-T2 occurred in a time-dependent manner, and the tocochromanol composition after 12 h was as follows: α-Toc, 0.7%; α-T1, 18.0%; α-T2, 64.0%; α-T3, 13.3%. Interestingly, the production of α-Toc was very low, possibly because of the high stability of the side-chain double bond rather than the degradation of α-Toc during the reaction.

Fig. 1 HPLC-UV profiles of hydrogenated α-T3 and LC-MS/MS analysis of α-tocochromanols. (a) Chromatograms of purified α-T3 before (left) and after (right) hydrogenation. (b) Typical fragmentation pattern of α-Toc in LC-MS/MS in the negative mode. Chromatogram and MS/MS spectrum of corresponding product peaks No. 1 (c) and No. 2 (d) in the HPLC-UV chromatogram. (e) Precursor ion scan chromatogram eluted with a PFP column.
This is supported by a previous report\(^{15}\), which investigated the changes in the Toc content in hydrogenated plant oil. Their results showed that α-Toc and γ-Toc are highly stable during hydrogenation even at 150–210°C. Although further consideration of the type of reaction catalyst is needed to establish a more effective method, the reaction speed and homolog composition can be easily controlled by varying the temperature and time in the proposed method. Our results suggest that T3 hydrogenation is a useful method to produce T1 and T2.

In the animal experiment, we hydrogenated the T3 mixture at 25°C for 72 h (Fig. 3). LC-MS/MS analysis in the MRM mode revealed four types of tocopheranols in HT3. Table 1 shows the composition of tocopheranols of the experimental diets.

To assess the physiological function of HT3, we used obese/obese KK-A^y mice. These mice are characterized by a marked increase in visceral white adipose tissue (WAT) accumulation during growth after weaning, and they develop hyperglycemia accompanied with insulin resistance\(^{16}\). A marked increase in body weight was consistently observed during the experiment. However, no difference was observed among any of the experimental groups (Fig. 4). As shown in Table 2, the WAT weights also remained the same among mice on different VE diets. The effects of the HT3 and T3 mixture on blood glucose levels tended to be lower than those in the control group (Fig. 4). While dietary HT3 did not change serum TG levels. Further, mice fed with HT3 tended to show decreased total cholesterol (P value, 0.181) and increased HDL-C levels (P value, 0.054). These changes resulted in a significant increase in the ratio of HDL-C to total cholesterol in serum. The trend of serum cholesterol profile was also observed in other VE diet groups.

Dietary VE supplements have been shown to improve insulin action and lipid metabolism accompanied with a reduction in inflammation via the downregulation of pro-inflammatory factors as well as hydroxyl radical and scavenging peroxides\(^{17}\). Although the direct effect of VE homologs on obesity is still unclear, these anti-inflammatory and antioxidative properties suggest the potential of VE in metabolic diseases. Moreover, some studies have reported bene-
ficial effects of VE on metabolic parameters without changes in obesity. Our results also suggest protective effects of HT3 against metabolic dysfunctions without changes in WAT weight. Given that hypertrophic adipocytes initiate metabolic disorders through dysregulation of inflammatory adipokines in WAT, the effect of HT3 on adipocyte size and proinflammatory adipokine production such as monocyte chemotactic protein-1 and interleukin 6 should be investigated in future studies.

We also observed an increase in the liver weight in mice fed with the HT3 and T3 mixture. To investigate the cause of this phenomenon, we asked whether HT3 affected fat accumulation in liver, and analysis revealed that there was no significant difference in hepatic lipid content. Some histopathological changes, such as hepatocyte enlargement, were observed in rats fed with a high dose (more than 450 mg/kg body weight/day) of T3 diet. While dietary T3 (85–100 mg/kg body weight/day) was also found to improve the liver profile in obesity model by inhibiting steatosis and inflammation and reducing liver injury. Thus, hepatic hypertrophy observed in this study is considered a consequence of high-dose dietary intake of HT3, an equivalent amount of 320 mg/kg body weight/day and reflects a biological response to HT3 in KK-Ay mice. However, further study on the molecular mechanism underlying liver hypertrophy is required for safety utilizing VE isomers as a functional food component.

We next measured the individual tocopheranols in the liver and epididymal WAT in mice fed with HT3. In the liver (Fig. 5), although α-Toc was found to be the most abun-

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**Table 2** Liver and white adipose tissue (WAT) weights of mice fed with the experimental diet.

|                      | Control | α-Toc | T3 mixture | HT3 |
|----------------------|---------|-------|------------|-----|
| **Liver**            |         |       |            |     |
| Weight (g)           | 3.8 ± 0.1 | 4.4 ± 0.1 | 5.0 ± 0.2* | 5.0 ± 0.4* |
| Lipid content (mg/g liver) | 389.7 ± 11.7 | 401.1 ± 13.2 | 351.2 ± 15.5 | 401.1 ± 19.0 |
| **WAT weight**       |         |       |            |     |
| Epididymal WAT (g)   | 1.9 ± 0.1 | 1.6 ± 0.1 | 1.7 ± 0.1 | 1.7 ± 0.1 |
| Mesenteric WAT (g)   | 0.9 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.9 ± 0.1 |

Data are represented as the mean ± standard error (SE; n = 6). Statistical analysis was performed using one-way ANOVA followed by Dunnett’s multiple comparisons test (*p < 0.05 versus the control group).
dant among tocopherolins, low levels of α-type T1, T2 and T3 were observed. No β/γ- or δ-type homologs were detected, except for β/γ-T2, in the liver. In contrast, not only α-type but also β/γ- and δ-tocopherolins accumulated in WAT. Interestingly, α-T3 and β/γ-T3 accumulated as much as α-Toc. Overall, although no δ-T1 or δ-Toc was detected, the accumulated levels of minor homologs appeared to be influenced by content in the diet. Biased accumulation of α-Toc in the liver could be explained by the ability of α-Toc transfer protein (α-TTP), which selectively binds to α-Toc and preferentially transfers it to all body tissues via circulation. Other homologs with a lower affinity to α-TTP are catabolized by cytochrome P450-catalyzed ω-hydroxylation followed by excretion with bile acid. On the other hand, when compared among α-tocopherolins, we found that the amount of α-T1 accumulated in the liver was similar to that of α-T3, although the amount of α-T3 in the experimental diet was higher. Since binding to α-TTP is associated with stability in the liver, high affinity for α-TTP in comparison to α-T3. Comparison among α-tocopherolins revealed that the presence of double bonds in the side-chain does affect the α-TTP affinity.

A contradiction to the fact that α-T3 and β/γ-T3 have low affinity to α-TTP was speculated as a result of the metabolic property of WAT. Machlin et al.²³ showed that the clearance speed of dietary α-Toc in the WAT is much slower than in the liver. In addition, Uchida et al.²⁴ found that the concentrations of α-Toc, α-T3 and γ-T3 in the livers of rats fed with VE-deficient diets decreased in one week, whereas those in WAT did not change for four weeks. Such a metabolic property of WAT has also been observed in our previous study that showed MDT accumulation in the tissues throughout the whole body, with higher accumulations in the WAT. In the present study, we found that in addition to α-T1, other chromanol types of T1 and T2 also accumulated in the WAT, whereas little amount was observed in the liver. This tissue specificity is probably due to the metabol-
ic properties rather than the α-TTP-dependent influx. Our results indicate that WAT serves as an accumulation site for various VE homologs. Thus, WAT is of interest as a target tissue for investigating the physiological function of minor homologs.

Recent studies on the health benefits of the VE family have attracted a great deal of attention to the higher activities of T3, such as glucose and lipid metabolism, in comparison to Toc. The molecular analysis performed by Fang et al. showed that α-, γ- and δ-T3 but not α-Toc act as agonists for peroxisome proliferator-activated receptor (PPARs), with a preference for PPARα. Moreover, γ-T3 was found to inhibit the activation of nuclear factor kappa B, which leads to an inflammatory action, whereas γ-Toc does not. Furthermore, we previously reported that MDT induces adipogenesis in 3T3-L1 cells with high activity relative to α-Toc, suggesting that the side-chain structure affects not only the potency but also the functional properties of Toc. These lines of evidence suggest that the number and position of double bonds in the side-chain affect the VE activity. However, we did not observe any differences in the activities between the HT3 and T3 mixture groups in the present study. This could be partly due to the low concentrations of T1 and T2 in HT3. Shibata et al. reported that α-Toc attenuates the beneficial effects of T3 on lipid metabolism by inhibiting the distribution of T3 in the tissues. These compounds may interfere with the tissue accumulation of T1 and T2 isoforms. Thus, further experiments are necessary to uncover the active properties of T1 and T2 and their mechanisms using purer T1 and T2 and eliminating the interference by Toc and T3.

One limitation of the present study is that we did not determine the configuration and position of the double bonds in the produced minor homologs. Considering the stability of Toc during hydrogenation, the contamination of decomposed products in HT3 would be small. To identify the properties on bioactivity of individual homologs, their accurate structures should be determined in future studies.

4 Conclusions

In summary, we herein proposed a new method to generate minor homologs of VE. We showed that hydrogenation could partially saturate α-T3 and confirmed the time-dependent generation of α-T1 and T2 using LC-MS/MS analysis. We also demonstrated that the generated HT3 affected the glucose and lipid metabolism in KK-Ay mice. VE analysis in the liver and epididymal WAT revealed tissue-specific accumulation of dietary T1 and T2 homologs. Various minor homologs accumulated in WAT, suggesting that WAT can be a target for investigating the physiological functions of T1 and T2 homologs in future studies. Our results show that hydrogenation is a useful method for generating T1 and T2 homologs, which can be applied to explore the relationship between the structure and functionality of tocochromanols. The results of this study lay the foundation for further studies on minor homologs of VE as a novel substance in dietary supplements and pharmaceuticals.

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Supporting Information

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