Note
Physiological Effects and Tissue Distribution from Large Doses of Tocotrienol in Rats

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Supplementation to an AIN93G-based diet of tocotrienol (T3) for 13 weeks administered to Fischer 344/slc rats showed a safety profile with no side effects. Dose-dependent T3 levels were detected in many tissues. Under the present experimental conditions, a continuous intake of the T3 concentrate would be safe in the rats as long as the T3 content was less than 0.20% of the dietary intake.

Key words: tocotrienol; tocopherol; large dose; tissue distribution; rat

Vitamin E is the generic name for tocopherol (Toc) and tocotrienol (T3) which have respective saturated and unsaturated side chains. Toc occurs in a wide variety of foods, whereas the number of T3-containing foods is limited. Rice bran, palm oil, and annatto seed are rich in T3.1) Among the physiological activities of vitamin E, Toc is known for its well-defined antioxidative activity. T3 has recently gained increased scientific interest due to its anti-antioxidative,2) anti-hypercholesterolemic,3) neuroprotective,4) and antiangiogenic5,6) effects which differ somewhat from those of Toc. These activities are believed to provide health benefits for various medical conditions and diseases.

Regarding the side effects of vitamin E, a relatively large dose of Toc (about 5,500 IU per day) has been reported to have no adverse effects on human health,7) while some studies have suggested that taking more than 400 IU per day for an extended period may increase the risk of death.8) In contrast, to the best of our knowledge, there has been no human study on the side effects of T3. Only two animal studies have found no severe adverse effects from large doses of T3 (less than 120–470 mg/kg of rat/day).9,10) Although these studies were conducted by administering a crude (not purified) T3 diet that also contained significant amounts of Toc (20–30%).

To better ascertain the effect of administering large amounts of T3, we performed an animal study using a T3 concentrate that contained a small amount of Toc, since the use of a crude T3 diet containing Toc would raise concerns about the influence of coadministered Toc on the observed beneficial and/or harmful effects. Indeed, with regard to cholesterol metabolism, dietary Toc is known to attenuate the impact of T3 on the hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in chickens.11) Consequently, in the present study, Fischer 344/slc (F344) rats were supplemented with a large dose of the T3 concentrate for 13 weeks, and the effects on biological parameters in the blood and tissues as well as the tissue distribution of T3 were investigated.

The T3 concentrate composed of 98.7% T3 (2.5% α-T3, 92.0% γ-T3, and 4.2% δ-T3) and 1.0% γ-Toc (wt/wt) was prepared from rice bran kindly provided by Sanwa Yushi Co. (Tendo, Japan). Specific pathogen-free F344 male rats (5 weeks old) were purchased from Japan SLC (Hamamatsu, Japan). After acclimatization for 1 week, the rats were assigned to 4 groups (8 rats in each group): a control group (fed with the AIN93G basal diet12), and 0.02% T3 (fed with the 0.02% T3 concentrate in the basal diet), 0.06% T3 (fed with the 0.06% T3 concentrate in the basal diet), and 0.20% T3 (fed with the 0.20% T3 concentrate in the basal diet) groups.

These rats were housed for 13 weeks in cages kept at 23 ± 2°C with a 12-h light:dark cycle under specific pathogen-free conditions. The rats were allowed free access to water and the experimental diet during the feeding period. The clinical signs and food intake were monitored once a day, and body weight was measured...
The liver, small intestine, and large intestine were then weighed and subjected to a macroscopic examination. Epididymal fat, heart, kidneys, lungs, liver, perirenal fat, and sacrificed by decapitation. The adrenal gland, brain, Tohoku University. In accordance with the Animal Experiment Guidelines of Tohoku University, which was carried out in experiments of Tohoku University reviewed the protocol for this experiment, which was carried out in accordance with the Animal Experiment Guidelines of Tohoku University. After the feeding period, the rats were starved for 6 h and sacrificed by decapitation. The adrenal gland, brain, epididymal fat, heart, kidneys, lungs, liver, perirenal fat, gastrointestinal tract, spleen, testes, and visceral fat were weighed and subjected to a macroscopic examination. The liver, small intestine, and large intestine were then fixed in a formalin solution and embedded in paraffin for a microscopic examination. Five-μm thick sections of each tissue were stained with hematoxylin and eosin. Blood was collected in a tube containing EDTA-2Na as an anticoagulant for blood sampling. A portion of the blood was subjected to a hematological analysis, and red blood cells (RBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), and white blood cells (WBC) were analyzed by Mitsubishi Chemical Medience. T-Cho and HDL-Cho were measured with enzymatic methods as points of reference, T3 did not affect the WBC, RBC, Hb, Ht, MCV, MCH, MCHC, or PLT levels. Regarding the plasma parameters, T3 tended to be lower in the 0.20% T3 group than in the control group (p = 0.1), which may be explained by the inhibitory effect of T3 on the hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. A somewhat higher T3 level was found in the 0.02% T3 group, this being thought to be the result of increased HDL-Cho in the group. There were no differences in the relative weights of any of the tissues, except for liver in all groups, after the 13-week supplementation period (Table 1). The liver weights in the 0.02% T3 group were higher than those in the 0.06% and 0.20% T3 groups, for currently unknown reasons. Interestingly, T3 seemed to decrease the weight of perirenal fat tissues, although this reduction was not statistically significant. The macroscopic examinations indicated no treatment-related changes in any of the organs. No pathological changes were found in the liver, small intestine, or large intestine (data not shown). The hematological and plasma biochemical parameters are summarized in Table 2. It was found that T3 did not affect the WBC, RBC, Hb, Ht, MCV, MCH, MCHC, or PLT levels. Regarding the plasma parameters, T3 tended to be lower in the 0.20% T3 group than in the control group (p = 0.1), which may be explained by the inhibitory effect of T3 on the hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. A somewhat higher T3 level was found in the 0.02% T3 group, this being thought to be the result of increased HDL-Cho in the group. There was thus the possibility that low-dose T3 increased plasma HDL-Cho, and further investigations are needed to evaluate this effect. In respect of the other parameters, T3 seemed to decrease TG, although this reduction was not statistically significant (p = 0.3). There were no differences in the TP, A/G, CRN, BUN, LDL-Cho, T-Bil, AST, or ALT levels for any of the groups. We did not focus on very-low-density lipoprotein (VLDL) in this study. Some studies have reported that VLDL-Cho was decreased by T3 supplementation.

### Table 1. Relative Tissue Weights of the F344 Rats in Each Group

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>0.02% T3</th>
<th>0.06% T3</th>
<th>0.20% T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>0.50 ± 0.01</td>
<td>0.50 ± 0.01</td>
<td>0.49 ± 0.02</td>
<td>0.50 ± 0.01</td>
</tr>
<tr>
<td>Heart</td>
<td>0.25 ± 0.01</td>
<td>0.25 ± 0.01</td>
<td>0.26 ± 0.01</td>
<td>0.27 ± 0.01</td>
</tr>
<tr>
<td>Lung</td>
<td>0.36 ± 0.01</td>
<td>0.34 ± 0.01</td>
<td>0.36 ± 0.02</td>
<td>0.35 ± 0.01</td>
</tr>
<tr>
<td>Liver</td>
<td>3.29 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.42 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.07 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.11 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.20 ± 0.01</td>
<td>0.20 ± 0.00</td>
<td>0.19 ± 0.00</td>
<td>0.19 ± 0.00</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.58 ± 0.01</td>
<td>0.57 ± 0.01</td>
<td>0.56 ± 0.01</td>
<td>0.57 ± 0.01</td>
</tr>
<tr>
<td>Perirenal fat</td>
<td>3.91 ± 0.26</td>
<td>3.74 ± 0.25</td>
<td>3.63 ± 0.12</td>
<td>3.52 ± 0.07</td>
</tr>
<tr>
<td>Testes</td>
<td>0.83 ± 0.01</td>
<td>0.81 ± 0.01</td>
<td>0.85 ± 0.01</td>
<td>0.81 ± 0.04</td>
</tr>
<tr>
<td>Epididymal fat</td>
<td>3.23 ± 0.28</td>
<td>3.47 ± 0.11</td>
<td>3.43 ± 0.11</td>
<td>3.18 ± 0.11</td>
</tr>
<tr>
<td>Gastrointestine</td>
<td>1.48 ± 0.06</td>
<td>1.52 ± 0.07</td>
<td>1.52 ± 0.05</td>
<td>1.47 ± 0.03</td>
</tr>
<tr>
<td>Visceral fat</td>
<td>2.92 ± 0.07</td>
<td>3.26 ± 0.17</td>
<td>3.05 ± 0.07</td>
<td>2.94 ± 0.08</td>
</tr>
</tbody>
</table>

Each value is presented as the mean ± SE (n = 8). The respective body weights of the rats were 361 ± 3, 350 ± 4, 359 ± 6, 349 ± 6 g for the control, 0.02% T3, 0.06% T3, and 0.20% T3 groups.

Means without a common letter differ, p < 0.05.
We have already mentioned two previous rat studies (Nakamura et al.9 and Tasaki et al.10) concerning side effects from large doses of T3. Both of these studies suggested there were no severe adverse effects with a T3 content in the rat diet of less than about 0.20–0.40%. However, those studies used crude (not purified) T3 extracted from palm oil that included Toc. Since T3 and Toc are considered to interact with each other in vivo, the current rat study was performed using a T3 concentrate prepared from rice bran with a low Toc content. Judging from the experimental data (Tables 1 and 2) and histological examination (data not shown), it is highly probable that a continuous intake of the T3 concentrate would be safe if the T3 content in the diet

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was less than 0.20%. The highest dose of T3 used in this study was 170 mg/kg rat-day⁻¹, a dose of T3 that would be considered safe. This dosage was somewhat higher than that used in the Nakamura study (120–130 mg/kg/day for no observed adverse effect level (NOAEL)) and was lower than that in the Tasaki study (300–470 mg/kg/day for NOAEL). These differences may have arisen from differences in the purity and source of T3, the strain of animals, and other experimental conditions between the three studies. Our results suggest that an intake of T3 up to 0.20% of the diet would not induce any side effects and may have beneficial health effects.

The two previous studies also evaluated the effect of extraordinarily high doses of T3. Nakamura et al. reported that the administration of a 3.0% T3 diet to F344 rats for 13 weeks decreased the body weight, food consumption, and blood parameters (MCV, MCH, and PLT) and induced hepatocellular hypertrophy. Tasaki et al. reported that the administration of a 2.0% T3 diet to Wistar Hannover rats for 52 weeks resulted in a decreased body weight gain, prolonged prothrombin time, increased serum ALT, and development of nodular liver lesions. It is therefore conceivable that, although T3 offers various beneficial effects, chronic exposure to very high doses of T3 could induce toxicity in vivo.

T3 predominantly accumulated in white adipose tissue (epididymal fat, perirenal fat, and visceral fat) and skin in this study, after supplementation with the T3 concentrate (Table 3). Comparatively high levels of T3 were found in the ear, adrenal gland, gastrointestinal, heart, lungs, and muscles, while T3 was detected at low levels in brain, kidney, testes, liver, plasma, RBC, spleen, and aorta (data not shown). Increasing the T3 intake increased the T3 concentration in most tissues, although no dose dependence was apparent for the brain. These results are in good agreement with previous reports. Although the mechanism by which T3 selectively accumulated in these tissues is still unclear, based on our previous and present data, we hypothesize that the tissue distribution of T3 depends on the affinity of T3 to vitamin E-binding proteins as well as the cytochrome P450 expression level in each organ. This hypothesis needs further investigation which is currently underway in our laboratory. In contrast, the T3 uptake seemed to reduce the concentration of α-Toc in most tissues such as perirenal fat (Table 3). It may therefore be necessary to monitor Toc levels when relatively large doses of T3 are consumed for nutraceutical purposes.

The physiological activities of T3²⁻⁶ are believed to provide health benefits for certain medical conditions. The use of T3 as a food additive and also as a functional and medicinal food requires the safety and toxicity of T3 to be addressed, but there have been few previous examinations of the side effects of T3. We performed a rat study here, using a T3 concentrate containing approximately 1% Toc, and obtained evidence that a continuous intake of a large amount of the T3 concentrate was safe as long as the T3 content of the diet was less than 0.20%. Consequently, further studies on the safety aspects of T3 in animals as well as human trials are recommended in order to ensure the safe use of T3 for nutraceutical applications.

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References