

Tocotrienol Distribution in Foods: Estimation of Daily Tocotrienol Intake of Japanese Population

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Tocotrienol (T3) is an unsaturated form of natural vitamin E that has been focused on because of its potential health benefits (i.e., antioxidative, antihypercholesterolemic, and antiangiogenic effects). The presence of T3 in some plant sources (e.g., rice bran and palm oil) is known, but its distribution in other edible sources and its daily intake remain unclear. In this study, we aimed at clarifying the distribution of T3 in various food sources and estimating the daily T3 intake of Japanese population. T3 contents of 242 food items and 64 meal items were measured by using normal-phase HPLC with fluorescence detection. As for the results, T3 contents were nondetectable to 12 mg T3/kg wet wt of food items, and nondetectable to 1.3 mg T3/item of processed (cooked) meal. Accordingly, the daily intake of T3 was estimated as 1.9–2.1 mg T3/day/person. The estimated daily intake of T3 appears rather low compared with the intake of tocopherol (8–10 mg/day/person as reported in the Japanese National Nutrition Survey), and additional T3 is important for its therapeutic aspects.

KEYWORDS: FLD-HPLC; tocotrienol; tocopherol

INTRODUCTION

Tocotrienol (T3, **Figure 1**) is the generic term of unsaturated vitamin E, which is distinguished from tocopherol (Toc, well-known vitamin E) by the degree of saturation of its side chain: T3 has an isoprenoid side chain with three double bonds, whereas Toc contains a fully saturated phytyl tail. T3 has recently been receiving considerable attention for its several biological properties (1). T3 shows antioxidative (2), antihypercholesterolemic (3), anticancer (4), and neuroprotective activities (5) better than those of Toc. In addition, some of our previous studies demonstrated that T3 suppressed abnormal angiogenesis, which is the important stage in the progression of some disorders (i.e., diabetic retinopathy, rheumatoid arthritis, and cancers) (6–8). These findings (1–8) suggest that T3 has a wide variety of health benefits.

T3 has been known to be present abundantly in some plants such as cereals, rice bran, and palm oil (9). A study by Adrian et al. (10) reported T3 and Toc contents in consumed foods, suggesting that humans regularly receive T3 from their diet. However, daily intake of T3 from foods remains unclear, and it is still controversial whether the amount of T3 intake is sufficient enough for its physiological effects. Moreover, since each isomer of T3 demonstrates a different degree of physiological effects (e.g., δ -T3 and α -T3 show the strongest and weakest antiangiogenic action, respectively (11)), it is important to have data on the isomeric diversity of T3 in foods. The knowledge (daily intake and isomeric composition) will be useful in application and development of T3 nutraceutical products for nutritional and clinical purposes.

In the present work, we utilized normal-phase high-performance liquid chromatography with fluorescence detection (FLD-HPLC) for the determination of T3 contents in 306 dietary items. This was because in our previous studies (12, 13) FLD-HPLC provided individual separation of 4 isomers of T3 and 4 isomers of Toc with good sensitivity and selectivity, whereas reverse-phase HPLC is unable to separate the β - and γ -forms of both T3 and Toc (14). Accordingly, on the basis of the quantitative data, the T3 daily intake of Japanese population was estimated by using two estimation methods: the weighted sum of T3 contents in 17 categories of foods (242 food items being analyzed) and the average sum of T3 contents in meal items (64 meal items being analyzed).

MATERIALS AND METHODS

Chemicals. Four T3 standards (α -, β -, γ -, and δ -T3) were obtained from Eisai (Tokyo, Japan). Four Toc standards (α -, β -, γ -, and δ -Toc), ethanol, 2,2,5,7,8-pentamethyl-6-hydroxychromane (PMC), methanol, hexane, 1,4-dioxane, and 2-propanol were purchased from Wako Pure Chemical Industries (Osaka, Japan). All reagents used were of analytical grade.

Food Samples. Food items were from a local market (Sendai, Japan), and sets of accompanying dishes were from Fundely Co. Ltd. (Tokyo, Japan). All food samples were lyophilized, ground, and stored at 4 °C or below until vitamin E analysis.

Extraction of T3 and Toc. For vitamin E extraction, 0.5 g of sample (or 0.5 mL of liquid food) was suspended in 0.5 mL of 1% (w/v) NaCl aqueous solution. The suspension was mixed with 9 mL of 3% ethanolic pyrogallol and 1 mL of 50 μ M ethanolic PMC. The mixture was saponified with 0.5 mL of 60% KOH aqueous solution at 70 °C for 30 min. After that, the saponified solution was cooled by ice, 22.5 mL of 0.9% NaCl aqueous solution was added, and the mixture was extracted with 15 mL of hexane/

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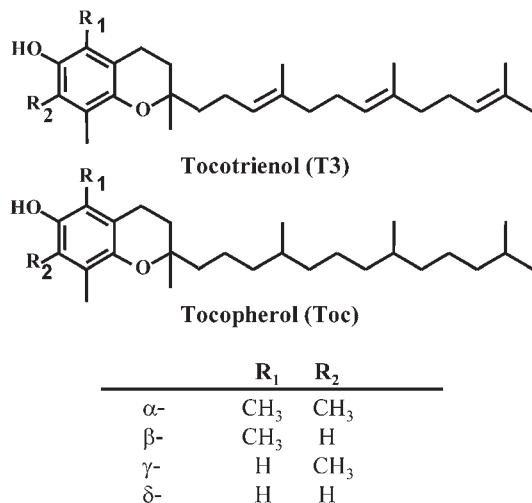


Figure 1. Chemical structure of tocotrienol (T3). T3 has an unsaturated isoprenoid tail, which differs from the saturated phytol side chain of tocopherol (Toc).

ethylacetate (9:1, v/v). After centrifugation at $1,000 \times g$ for 5 min, the upper layer was collected, and the extraction was then repeated. The collected upper layers were combined, dried, and redissolved in hexane (1–10 mL). The solution was passed through a GL ChromaDex 13N filter before vitamin E analysis by HPLC.

Determination of T3 and Toc in Food Samples. T3 and Toc levels in food samples were determined using FLD-HPLC. The HPLC system consisted of a JASCO PU-980 pump (Japan Spectroscopic Co., Tokyo, Japan), a JASCO CO-860 column oven, and a Reodyne 7125 injector (Cotati, CA, USA). An Inertsil SIL 100A-5 (4.6 mm \times 250 mm; GL Science, Tokyo, Japan) was used as a HPLC column. A mixture of hexane/1,4-dioxane/2-propanol (1000:40:5, v/v/v) was used as the mobile phase. The flow rate was adjusted to 1.0 mL/min, and the column temperature was maintained at 35 °C. T3 and Toc were detected by a RF-10AXL FLD detector (excitation 294 nm, emission 326 nm; Shimadzu, Kyoto, Japan). All peak areas were registered using a SIC Chromatocorder 21J integrator (System Instruments, Tokyo, Japan). The concentrations of T3 and Toc in the samples were calculated with calibration curves of standard T3 and Toc and then were corrected using the peak area ratios of the vitamin E isomers to PMC (internal standard). The determination was made three times in each sample, and the quantitative data were presented as mg/kg sample (wet wt) for food items or mg/item for processed food meals.

Estimation of T3 Daily Intake of Japanese Population. Two methods for estimating daily intake of T3 were used. With the first method, an intake of T3 was calculated from the weighted sum of T3 contents in different foods consumed. In Japan, food is classified into 17 categories (cereals, potatoes, sugars and sweeteners, grains, nuts and seeds, vegetables, fruits, mushrooms, algae, seafoods, meats, eggs, milks and dairy products, fats and oils, confectionery, beverages, and flavoring and seasoning materials) according to the Ministry of Health, Labour and Welfare. The intake amounts of food from those 17 categories are annually reported in the Japanese National Nutrition Survey, as the sum of intake amounts of food from their subcategories. For instance, according to the survey of year 2006, the intake amount of the category of fats and oils (10.2 g) was from the sum amounts of four subcategories: butter (1.0 g), margarine (1.0 g), plant lipids (8.0 g), and animal fats (0.1 g). In the present study, T3 contents in 242 food samples (from the 17 food categories) were quantified, and the daily intake of T3 was calculated as T3 daily intake = the sum of (average T3 content of each subcategory \times daily intake amount of the subcategory). On the other hand, with the second method, an intake of T3 was estimated on the basis of three meals being taken per day (as breakfast, lunch, and dinner), under the assumption that a meal consisted of a bowl of rice (160–200 g), a set of accompanying dishes (200–260 g), and a small bowl of soup (150–200 mL). Therefore, the daily intake of T3 was calculated as: T3 daily intake = 3 \times the sum of (average T3 contents in rice, accompanying dish, and soup). The daily intakes of T3 calculated from the two methods were expressed as mg T3/day/person.

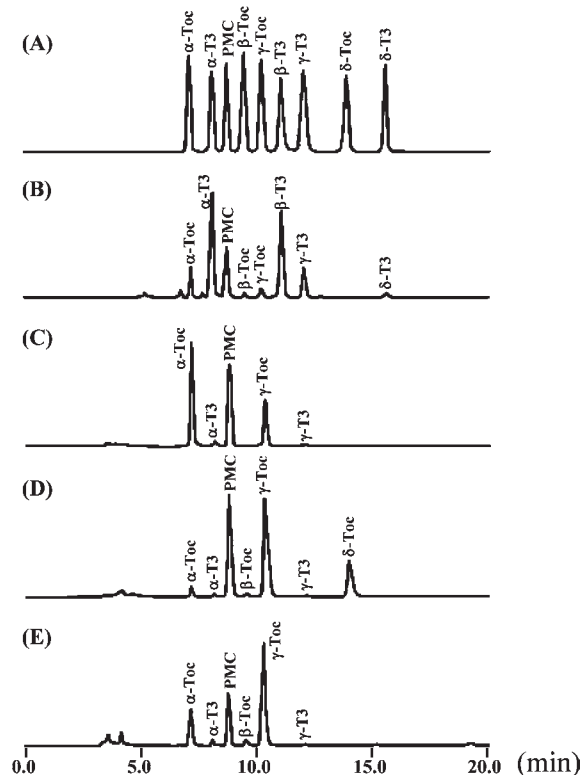


Figure 2. Typical HPLC chromatograms of standard vitamin E and PMC (internal standard) (A), barley (pressed grain) (B), chicken breast (C), soybean miso (D), and egg drop soup (E).

Statistical Analysis. The data were expressed as mean \pm SD ($n = 3$). Statistical comparisons were made with Student's t test.

RESULTS

Distribution of T3 in Food Samples. Chromatograms of a mixture of T3 and Toc standards as well as those of some foods analyzed are shown in Figure 2. The normal-phase FLD-HPLC enabled separation of all vitamin E peaks within 20 min. Table 1A in Supporting Information provides quantitative data of the T3 and Toc contents in 242 ingredient items. A variation of T3 content among the food samples was found: the sum of T3 isomers was nondetectable to 12.12 mg T3/kg sample. The average contents of T3 in each food category are presented in Figure 3.

In the cereal category, T3 levels were in the range of 0.17–10.97 mg/kg; items high in T3 were brown rice and wheat flour. In the potato group, taros and long sweet potatoes contained T3 (0.10 and 0.12 mg T3/kg), whereas sweet potatoes did not. In the grain category, T3 contents were 0.49–12.12 mg/kg, and the category of nuts and seeds contained 0.12–3.26 mg T3/kg. All vegetables contained Toc (1.27–80.75 mg/kg), but less than half of the vegetable samples had T3 contents (nondetectable to 2.72 mg/kg). The categories of fruits and algae contained small amounts of T3 as low as 0.03–0.78 mg/kg and nondetectable to 0.43 mg/kg, respectively. In the seafood category, T3 was detected in only half of the samples (nondetectable to 8.80 mg/kg). T3 presence in the categories of meats and eggs was nondetectable to 0.89 mg/kg and nondetectable to 1.50 mg/kg, respectively. Some items from the category of milks and dairy products contained T3 (nondetectable to 2.84 mg/kg). T3 contents in the categories of fats and oils, confectionaries, and flavoring and seasoning materials were nondetectable to 1.44, 0.20–3.60, and nondetectable to 5.49 mg/kg, respectively.

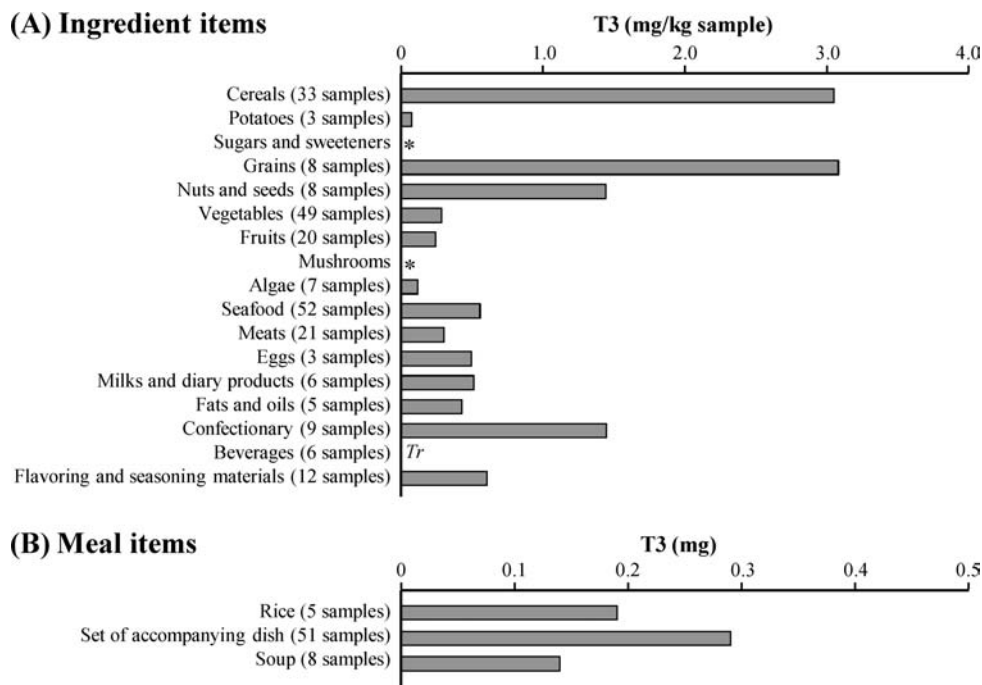


Figure 3. Average T3 contents in analyzed foods by mean of categories of food as classified in the Japanese National Nutrition Survey (**A**) and by processed meal items (**B**). One meal consists of a dish of rice, a set of accompanying dishes (2–3 submenus), and a small bowl of soup. Asterisk (*) denotes no quantitation determined in categories of sugars and sweeteners and of mushrooms because they have been reported to contain no Toc, implying a lack of T3. *Tr* = trace.

Table 1. Isomeric Ratios (%) of T3 in Samples from the Categories of Cereals, Grains, and Nuts and Seeds

food item	α -T3	β -T3	γ -T3	δ -T3	food item	α -T3	β -T3	γ -T3	δ -T3
rice products					soba products				
brown rice	15	11	63	11	buckwheat flour	36		64	
well-milled rice	10		74	16	corn products				
well-milled rice with embryo	28		63	9	corn (Cuzco)	48		46	6
Kiritanpo	22		78		popcorn	14	12	74	
rice cake		100			cornflakes	17		58	25
wheat products					barley products				
soft flour	7	93			barley (pressed grain)	8	63	23	6
hard flour	8	85		7	barley flour	8	10	82	
premixed flour for hot cake	12	70	14	4	soybean products				
premixed flour for tempura	6	94			soybeans		100		
white table bread	16	61	18	5	Kinugoshi-tofu	100			
raisin bread	20	62	18		Abura-age	100			
soft roll	20	61	19		Itohiki-natto		100		
bean jam bun	22	57	21		Okara		100		
custard cream bun	22	37	41		other grains				
jam bun	21	56	23		adzuki beans				100
udon		100			kidney beans	10		32	58
somen	100				nuts and seeds				
spaghetti		100			almonds	100			
Kuruma-fu		100			cashew nuts	28	31	41	
bread crumbs	55	45			sweet chestnuts	100			
Chiao tzu pastry	55	29	16		sesame seeds	100			
Shao mai pastry		100			pistachio nuts			100	
instant ramens/noodles					pine nuts	72		28	
instant ramen cup (1)	25	30	45		peanuts				100
instant ramen cup (2)	5	32	63		peanut butter				100
instant chinese noodle cup	38		62						
instant yaki-soba cup	31	26	43						

The quantitative data of T3 in processed meal items (160–200 g of rice, 200–260 g of accompanying dish, and a small bowl of soup) are reported in **Table 1B** in Supporting Information. Ranges of T3 were 0.16–0.25 mg in the group of rice, nondetectable to 1.26 mg in the group of accompanying dish, and 0.06–0.29 mg in the group of soup. The average T3 contents in

rice, accompanying dish, and soup were 0.19, 0.29, and 0.14 mg, respectively.

Isomeric Diversity of T3 in Food Categories Rich in T3. The isomeric ratios of T3 in analyzed samples from the categories of cereals, grains, and nuts and seeds (the categories high in T3 content) are reported in **Table 1**. The predominant T3 isomer

Table 2. Daily Intake of T3 of Japanese Population As Classified by Food Categories

food category	food intake ^a (g/day)	T3 content (mg/kg)	T3 daily intake ^b (mg/day/person)	food category	food intake ^a (g/day)	T3 content (mg/kg)	T3 daily intake ^b (mg/day/person)
1. cereals				8.1.2. redfish and masu salmon	4.3	0.622	0.003
1.1. rice products				8.1.3. sea beam and marbled sole	6.5	0.240	0.002
1.1.1. rices	340.2	4.244	1.444	8.1.4. tunas and marlins	5.3	0.309	0.002
1.1.2. processed rices	4.6	1.657	0.008	8.1.5. other fishes	9.5	0.306	0.003
1.2. wheat products				8.1.6. shells	3.6	0.530	0.002
1.2.1. wheat powders	4.2	3.757	0.016	8.1.7. squids and octopus	5.0	1.498	0.007
1.2.2. breads	30.0	3.982	0.119	8.1.8. shrimps and crabs	5.8	0.430	0.002
1.2.3. sweet bun	6.0	4.421	0.027	8.2. processed seafoods			
1.2.4. udon, noodles	38.4	0.096	0.004	8.2.1. salted dry seafoods	15.9	1.271	0.020
1.2.5. instant noodles	4.3	2.333	0.010	8.2.2. canned seafoods	1.9		
1.2.6. pasta	8.0	1.1477	0.011	8.2.3. seafoods in soy sauce	0.3		
1.2.7. other wheat products	4.9	2.263	0.011	8.2.4. frozen seafoods	9.8		
1.3. other				8.2.5. fish hams and sausages	0.5		
1.3.1. soba products	5.8	2.705	0.016	9. meats			
1.3.2. corn products	0.5	3.896	0.002	9.1. pork and beef			
1.3.3. other	3.0	3.748	0.011	9.1.1. beef	13.8	0.351	0.005
2. potatoes	62.1	0.072	0.004	9.1.2. pork	30.8	0.236	0.007
3. grains				9.1.3. ham and sausages	12.4	0.395	0.005
3.1. soybeans products				9.2. chicken	21.2	0.278	0.006
3.1.1. soybeans	1.5	3.502	0.005	10. eggs	36.0	0.500	0.018
3.1.2. tofu	35.2	0.244	0.009	11. milk and dairy products			
3.1.3. deep-fried tofu	8.2	12.120	0.099	11.1. cow's milk	93.3		
3.1.4. natto	6.7	1.654	0.011	11.2. cheeses	2.3	0.113	~0
3.1.5. other	3.5	0.551	0.002	11.3. other dairy products	8.2	0.947	0.008
3.2. other	1.3	3.155	0.004	12. fats and oils			
4. nuts and seeds	2.1	1.438	0.003	12.1. butters	1.0	0.697	0.001
5. vegetables				12.2. margarines	1.0		
5.1. vegetables with green or yellow leaves	95.6	0.403	0.039	12.3. plants lipids	8.0		
5.2. other vegetables	168.5	0.264	0.044	12.4. animal fats	0.1	1.441	~0
5.3. vegetable juices	9.0			13. confectionery			
5.4. pickled vegetables				13.1. Japanese-style confectionary	10.8	2.210	0.024
5.4.1. pickled vegetable leaves	5.1	0.234	0.001	13.2. cakes and pastries	7.2	1.138	0.008
5.4.2. pickled radish and other pickled products	9.5	0.248	0.002	13.3. biscuits	1.7	1.262	0.002
6. fruits				13.4. candies	0.3	0.455	~0
6.1. fresh fruits				13.5. other	6.0	0.202	0.001
6.1.1. strawberries	0.1	0.360	~0	14. beverages			
6.1.2. citrus fruits	24.1	0.148	0.004	14.1. tea drinks	310.1	Tr	~0
6.1.3. bananas	11.9	0.28	0.003	14.2. coffee and cocoa drinks	118.1	Tr	~0
6.1.4. apples	25.6	0.652	0.017	15. flavoring and seasoning materials			
6.1.5. other	32.2	0.268	0.009	15.1. seasoning materials			
6.2. yams	1.1	0.105	~0	15.1.1. sauces	2.1	0.249	0.001
6.3. fruit juices	12.5	0.154	0.002	15.1.2. shoyu	17.5		
7. algae	12.8	0.119	0.002	15.1.3. mayonnaise	3.2		
8. seafoods				15.1.4. miso	12.4	5.492	0.068
8.1. raw seafoods				15.1.4. other	56.9	0.301	0.017
8.1.1. pompanoes and sardines	11.8	0.380	0.004	15.2. flavoring materials	0.3		

^a Food intake (g/day) referred to results of the Japanese National Nutrition Survey (2006). ^b T3 daily intake (mg/day/person) was calculated as; (food intake) × (T3 content).

in rice products is γ -T3, whereas β -T3 is the major T3 constituent in wheat products. The groups of instant ramens/noodles, soba products, and corn products contain a large portion of γ -T3. In barley products, pressed barley has β but barley flour has γ as the main T3 form, respectively. Soybean products contain only α - or β -isomer, whereas adzuki beans and kidney beans contain δ -T3. In the group of nuts and seeds, α -T3, γ -T3, and δ -T3 are the predominant T3 isomers in almonds, chestnuts, and sesame seeds, respectively.

Estimation of T3 Daily Intake of Japanese Population. On the basis of the quantitative results (Table 1 in Supporting Information), the T3 daily intake of Japanese population was calculated. Table 2 shows the estimation of T3 intake based on the weighted sum of T3 contents in foods consumed. According to Table 2, people obtain a large portion of T3 from cereals and grains (1.68 and 0.13 mg T3/day/person, respectively), and

therefore T3 daily intake was calculated as 2.15 mg T3/day/person. On the other hand, on the basis of T3 contents present in processed meal items (Table 1B in Supporting Information), the intake of T3 was estimated as 1.86 mg T3/day/person (as calculated from 3 × (the sum of T3 contents in rice (0.19 mg T3), accompanying dish (0.29 mg T3), and soup (0.14 mg T3)).

DISCUSSION

Vitamin E represents a family of four T3 and four Toc, with four different isomers as α , β , γ , and δ based on the position and number of methyl groups on the hydrophilic chromanol ring. Because of the presence of a unique Toc-like structure with three double bonds on its hydrophobic isoprenoid tail, T3 can enter the cell more freely (15) and perform functional potency more efficiently compared with Toc (16). To date, nonantioxidative properties of T3 (such as anticancer, neuroprotective, and

hypocholesterolemic functions) have become better known, and much attention has been paid to the pathophysiological roles of T3. Recently, it has become clear that different T3 isomers demonstrate different levels of biological effects. For instance, a study by Yu et al. found that δ -T3 was very effective in promoting apoptosis and killing breast cancer cells (17). Das et al. reported that γ -T3 and, to some extent, α -T3 but not δ -T3 exhibited cardioprotective effect (18). Our previous study on antiangiogenic function showed that the inhibitory potency of T3 isomers was δ - > β - > γ - > α -T3 (11). Therefore, isomeric form of T3 should be considered for the best result of specific therapeutic use.

Since it is important to have quantitative data of each vitamin E separately, in this study, normal-phase FLD-HPLC was used as the quantitative tool for analysis of T3 and Toc in food samples. The HPLC enables separation of all eight vitamin E isomers (four T3 and four Toc) as well as their internal standard (Figure 2), whereas reverse-phase HPLC does not (14). In this study we could successfully measure T3 and Toc in food samples within 20 min (Figure 2).

T3 contents (as well as Toc) in more than 200 kinds of food items from the 17 categories of foods were determined (Table 1A in Supporting Information). The richest sources of T3 were cereals (3.05 mg T3/kg as an average), grains (3.08 mg T3/kg), nuts and seeds (1.44 mg T3/kg), and confectionary (1.45 mg T3/kg) (Figure 3). The foods that contained the least T3 content were from the categories of potatoes (0.07 mg T3/kg) and algae (0.12 mg T3/kg). However, we did not determine T3 contents in foods from the categories of sugars and sweeteners and of mushrooms, because their contents of Toc is estimated to be zero according to the standard table of food composition in Japan (the fifth revised and enlarged edition), implying the absence of T3 since Toc is cobiosynthesized together with T3 in eukaryotic chloroplasts. In addition, T3 and Toc in beverages (green tea, Oolong tea, coffee, and milk cocoa) were trace and were not included into Table 1A in Supporting Information.

Because the samples from categories of cereals, grains, and nuts and seeds were high in T3 content (Figure 3), the items should be considered as good sources of natural T3, and their isomeric ratios of T3 are shown in Table 1. It is clear that γ -T3 is the main isomer in rice products and β -T3 is the major T3 constituent in wheat products, which are in agreement with other reports (19, 20). A high amount and ratio of α -T3 was found in almonds. Although a number of studies report palm oil as a good source of δ -T3 (21, 22), in this study, adzuki beans appeared as another source of δ -T3.

On the basis of the quantitative data, T3 intake was calculated as 2.15 mg T3/day/person by mean of the weighted sum of T3 in ingredient items (Table 2). On the other hand, since thermal processing (cooking) may induce oxidative reactions and degrade some nutrients in foods, we did another estimation of T3 daily intake based on processed meals (64 meal items were analyzed (Table 1B in Supporting Information)). The estimation was done under the assumption that a meal consisted of rice, an accompanying dish, and soup, which is the most popular pattern of meals in Japan. For rice samples, we analyzed Gomoku rice and sprouted brown rice, because there is an increasing trend in consuming the two kinds of rice, especially by persons who are concerned about their health. Interestingly, despite many studies reporting the accumulation of T3 in rice bran (12), we could detect T3 in cooked white rice (0.16–0.17 mg T3/a small bowl of rice). Under the assumption that three meals are taken a day, T3 intake was calculated as 1.86 mg T3/day/person. The intake amounts of T3 from the two estimation methods might be somewhat different from the actual intake value. However, by using the two estimation methods, daily intake of Toc is

calculated as 10.70 and 8.82 mg α -Toc/day/person, which are quite similar to 8.9 mg α -Toc/day/person as reported by the Japanese National Nutrition Survey (2006). Thus, we believe that daily intake of T3 of the Japanese population is around 1.86–2.15 mg T3/day/person, which appears relatively low compared with that of Toc (2 mg T3/day/person compared with 9 mg Toc/day/person). Therefore, additional amounts of T3 might be necessary for therapeutic performance of T3.

In summary, we measured T3 and Toc contents in food ingredients and meal items and found diversity in distribution of T3 in the samples. On the basis of the quantitative data, T3 daily intake of Japanese population was estimated around 1.86–2.15 mg/day/person. The data would be useful for nutritional and clinical applications of T3.

ABBREVIATIONS USED

FLD, fluorescence detection; HPLC, high-performance liquid chromatography; PMC, 2,2,5,7,8-pentamethyl-6-hydroxychromane; Toc, tocopherol; T3, tocotrienol.

Supporting Information Available: Tocotrienol (T3) and tocopherol (Toc) contents in food samples. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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