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Dietary Effect of α-Tocopherol and Tocotrienols on Lipid Metabolism and Immune Function of Aged Sprague-Dawley Rats

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Eight-month-old male Sprague Dawley rats were fed diets containing α-tocopherol (Toc) or tocotrienol (T3) mixture (composed of 20.5% α-Toc, 21.4% α-T3, 36.5% γ-T3 and other analogs) at the 0.1 or 0.5% level for 3 weeks to examine their dietary effects on lipid metabolism and immune indices. Feeding of α-Toc and T3 significantly decreased liver phosphatidylcholine peroxide and serum phospholipid levels. In the regulation of immunoglobulin level, significant increase in serum IgA level was observed in the rats fed α-Toc or T3, but the effect on immunoglobulin productivity from spleen and mesenteric lymph node (MLN) was not as marked. Feeding of α-Toc and T3 significantly decreased LTB_4-releasing activity of peritoneal exudate cells without decreasing arachidonic acid level. The suppression of LTB_4 release was more marked in the rats fed 0.1% α-Toc or T3 than in these fed them at the 0.5% level. When animals were killed after 10 h of fasting, T3 was detected only in MLN and epidydimal adipose tissue. These results suggest that T3 modulates lipid metabolism and immune functions as well as α-Toc, and that MLN and adipose tissue are the main target tissues of T3.

Keywords: tocotrienol, tocopherol, lipid metabolism, immunoglobulin production, LTB_4 release

Foods contain various types of antioxidants which express a wide variety of biological effects, such as anticancer (Kinnick *et al*., 1997; Aliva *et al*., 1994), hypocholesterolemic (Quershi *et al*., 1991, Formica & Regelson, 1995), and immunoregulatory activities (Pearce *et al*., 1984; Yamada *et al*., 1999; Matsuo *et al*., 2000). Tocopherols (Toc) are the most popular natural antioxidants ubiquitously present in both the plant and animal kingdom, and tocotrienols (T3) are structural analogs of Toc with 3 double bonds in the side chain. These Toc and T3 derivatives have also been reported to express various biological effects, such as antioxidative (Kamat *et al*., 1997), anticancer (Rahmat *et al*., 1993, Makpol *et al*., 1997), hypocholesterolemic (Qureshi *et al*., 1991) and immunoregulatory activities (Gu *et al*., 1999; Kaku *et al*., 1999).

Among the above biological effects, immunoregulatory activity is important for the maintenance of our health. Allergic reactions are usually classified into 4 types and type I allergy plays an important role in the induction of allergies against food and environmental antigens (Metcalfe, 1991). In this type of allergy, the induction of allergen-specific IgE and the release of chemical mediators such as histamine and leukotrienes (LT) are critical. On the other hand, allergen-specific IgA suppresses the allergic reaction through the inhibition of allergen absorption, and allergen-specific IgG through the competition with IgE. It has been reported that various food components such as unsaturated fatty acids, antioxidants and dietary fibers affect the above immune functions (for review, see Yamada *et al*., 1999). It has also been reported that oral administration of Toc derivatives induces class-specific regulation of immunoglobulin (Ig) production, for instance, down-regulation of IgE production and up-regulation of IgA and IgG production (Gu *et al*., 1994, 1999; Kaku *et al*., 1999). In addition, Toc derivatives exert an anti-allergic effect through the suppression of 4-series LT production (Gu *et al*., 1994; 1995). The above studies showed that T3 exerts similar, but somewhat different immunoregulatory effects as Toc. Though these observations were obtained from feeding experiments using young rats, it has been reported that various immune functions are changed with the progression of aging (Paganelli *et al*., 1994). In the present study, we compared the dietary effect of Toc and T3 on aged rats to learn the difference and age-dependency of their biological effects.

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Materials and Methods

Materials α-Toc, T3 mixture and tocol were provided by Eisai Co. Ltd. (Tokyo). The T3 mixture contained 20.5% α-Toc, 0.7% β-Toc, 1.0% γ-Toc, 0.5% δ-Toc, 21.4% α-T3, 3.5% β-T3, 36.5% γ-T3 and 8.6% δ-T3. Fetal bovine serum (FBS) was purchased from Intergen Co. Ltd. (Purchase, NY). Tyrode buffer (pH 7.2) was composed of 137 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1.1 mM $MgCl_2$, 11.9 mM $NaHCO_3$, 0.4 mM NaH_2PO_4, and 5.6 mM glucose.

Animal experiments Animal experiments were conducted in accordance with the guidelines on Animal Experiments in Faculty of Agriculture and the Graduate Course, Kyushu University, and the Law (No. 105) and Notification (No. 6) of the Government. Eight-month-old male Sprague-Dawley (SD) rats were obtained from Seac Yoshitomi (Yoshitomi) and housed individually in a room controlled at 20°C and a light cycle of 08:00 to 20:00. After acclimation for a week, rats were divided into 5 groups (10 animals each) and allowed free access to experimen-

tal diets and water. Diets were prepared according to the recommendation of the American Institute of Nutrition, AIN-93G and contained 10.0% safflower oil, 10.0% sucrose, 13.2% α-cornstarch, 20.0% casein, 36.7% cornstarch, 5.0% cellulose, 1.0% vitamin mix, 3.5% mineral mix, 0.25% choline bitartrate, 0.3% L-cystine and 0.0014% *t*-buthylhydroquinone. Part of the cornstarch in each diet was changed to 0.1% α-Toc, 0.5% α-Toc, 0.1% T3 mixture, or 0.5% T3 mixture. After 3 weeks of feeding, rats were fasted for 10 h and 5 in each group were killed by withdrawing blood from the aorta under diethyl ether anesthesia. Liver, lung, heart, kidney, spleen, mesenteric lymph node (MLN) and epididymal adipose tissue were immediately excised and weighed. The other 5 rats of each dietary group were used for isolation of peritoneal exudate cells (PEC). In a short-term feeding experiment, two 8-week-old SD rats were fed the diet containing 1% T3 for 24 h and killed without fasting to determine the levels of T3 homologues in various tissues.

Determination of lipid components Tissue lipids were extracted and purified by the method of Folch *et al*. (1957), to determine total cholesterol, triglyceride and phospholipid levels using the commercial kits Cholesterol C Test, TG-G Test or PL-B Test (all from Wako Pure Chemicals, Osaka). Phosphatidylcholine (PC) was separated and its fatty acid content was analyzed by gas chromatography using SUPELCO WAX-10 (0.53 mm×30 m) column (SUPELCO, Tokyo), according to the method of Ikeda *et al*. (1989). The levels of lipid oxidation products in serum and liver were measured as the levels of PC hydroperoxides (PCOOH) according to the method of Miyazawa *et al*. (1987) and tribarbituric acid reactive substances (TBARS) according to the method of Yagi (1976). The levels of α-Toc and T3 homologues were determined using the method of Burton *et al*. (1985). In the case of serum, 1 ml of serum was mixed with 1 ml of 0.05 M sodium dodecylsulfate, 2 ml of ethanol and 1 ml of hexane using a vortex mixer for 1 min and centrifuged for 1 min at 4000×*g*. Then, 0.5 ml of supernatant was collected, dried under N_2 gas flow and the residue was dissolved in 100 μl of mobile phase solution (a 985:10:5 (v/v/v) mixture of hexane, 1,4-dioxiane and 2-propanol) supplemented with 10 mg/ml of tocol. In the case of tissue samples, 0.2 g of each tissue was homogenized with 10 ml of 0.05 M sodium dodecylsulfate solution and 1 ml of the homogenate was treated as serum, as described above. Twenty microliters samples were applied to a high performance liquid chromatograph equipped with an absorptiometer at 292 nm (SPD-10AV, Shimadzu, Kyoto) and a Wakosil 5sil 4.6 mm×250 mm ODS column (Wako, Osaka). The levels of α-Toc and T3 homologues were determined by comparing their peaks with the peak of tocol, which was added as an internal standard.

Determination of immunoglobulin levels Serum Ig levels were measured by enzyme-linked immunosorbent assay (ELISA), as described previously (Lim *et al*., 1994). Spleen and MLN lymphocytes were isolated by the method of Lim *et al*. (1994), cultured for 24 h in RPMI 1640 medium supplemented with 10% FBS in a 24-well plastic culture plate (Becton Dickinson, Franklin Lake, N.J.), and Ig levels in the culture supernatant were measured by ELISA.

Determination of LTB_4-releasing activity of peritoneal exudate cells PEC were isolated from the rats fed the above diets and stimulated with 5 μM calcium ionophore A23187 to measure LTB_4-releasing activity, according to the method of Matsuo *et al*. (1996). Tyrode buffer containing 0.1% bovine serum albumin (BSA) was injected into the rat peritoneal cavity. After the abdomen was gently massaged, the cavity was opened to collect the fluid containing PEC with a Pasteur pipette. Cells were washed with Tyrode buffer and centrifuged at 200×*g* and at 4°C. To remove erythrocytes, cells were suspended in a ammonium chloride buffer (pH 7.4) containing 150 mM NH_4Cl, 10 mM $KHCO_3$, and 10 mM EDTA-2Na and incubated for 5 min at 4°C. The remaining cells were resuspended in Tyrode buffer.

Then, PEC (2×10^6 cells) were incubated with 5 μM calcium ionophore A23187 for 20 min at 37°C. The amount of LTB_4 released into the medium was measured using a high performance liquid chromatography method reported by Matsuo *et al*. (1996).

Statistics Data were analyzed by Duncan's new multiple range test to evaluate the significance of differences (Duncan, 1955).

Results

Effect on growth and tissue weight of aged rats Eight-month-old SD rats were fed diets containing α-Toc or T3 for 3 weeks and killed after a 10 h fast to analyze the dietary effect on lipid metabolism. When fed the diets containing α-Toc or T3 at either the 0.1 or 0.5% level, there was no significant difference in food intake, body weight, or food efficiency. Nor was there any significant difference in the weights of heart, lung, liver, kidney, spleen, or epididymal adipose tissue.

Dietary effect on lipid parameters and oxidation in aged rats Table 1 shows the effect of dietary α-Toc and T3 on the lipid oxidation products in serum and liver. Significant increase in PCOOH level in serum was observed only in the rats fed 0.5% α-Toc, while in liver, significant decrease of PCOOH level was observed in rats fed both α-Toc and T3. In the animals fed α-Toc, the decreasing effect was more marked at the 0.5% level than the 0.1% level, while there was no dose-dependent effect in T3. In the case of serum TBARS, significant decrease was observed

Table 1. Effect of dietary α-tocopherol and tocotrienol on lipid oxidation product levels in serum and liver of Sprague-Dawley rats.

	Serum PCOOH/PC (ppm)	Liver PCOOH/PC (ppb)	Serum TBARS (nM MDA/ml)
None	0.51 ± 0.03^{a}	42 ± 6^{a}	3.24 ± 0.30^{ac}
0.1% Toc	0.46 ± 0.08^{a}	27 ± 4^{b}	2.76 ± 0.35^{ac}
0.5% Toc	0.89 ± 0.15^{b}	12 ± 3^{b}	1.47 ± 0.05^{b}
0.1% T3	0.30 ± 0.10^{a}	24 ± 4^{b}	3.73 ± 0.63^{c}
0.5% T3	0.41 ± 0.08^{a}	24 ± 5^{b}	2.44 ± 0.21^{ab}

Data are means±SE (n=4 or 5) and values without a common superscript letter are significantly different at $p<0.05$.

Table 2. Effect of dietary α-tocopherol and tocotrienol on serum lipid levels of Sprague-Dawley rats.

	Total cholesterol (mg/dl)	Phospholipids (mg/dl)	Triglycerides (mg/dl)
None	85.3 ± 3.2	153 ± 12^{a}	135 ± 26
0.1% Toc	72.5 ± 6.9	145 ± 6^{a}	142 ± 12
0.5% Toc	70.0 ± 7.6	100 ± 12^{b}	88 ± 17
0.1% T3	75.8 ± 7.8	110 ± 13^{b}	97 ± 14
0.5% T3	73.6 ± 9.7	104 ± 10^{b}	96 ± 12

Data are means±SE (n=4 or 5) and values without a common superscript letter are significantly different at $p<0.05$.

only in rats fed the diet with 0.5% α-Toc.

Table 2 shows the effect on serum lipid levels. A decreasing tendency in total cholesterol level was observed in rats fed both α-Toc and T3, but the difference was not significant, while phospholipid level significantly decreased in those fed α-Toc at the 0.5% level and those fed T3 at the 0.1 or 0.5% level. A similar tendency was observed for triglycerides, but the difference was not significant.

Table 3. Effect of dietary α-tocopherol and tocotrienol on serum immunoglobulin levels of Sprague-Dawley rats.

	IgA (μg/ml)	IgG (mg/ml)	IgM (mg/ml)
None	128±23[a]	10.7±2.4[a]	9.1±4.7
0.1% Toc	155±7[ab]	14.3±1.4[ab]	11.2±2.0
0.5% Toc	218±14[b]	17.2±2.4[b]	12.3±3.9
0.1% T3	211±24[b]	14.5±1.6[ab]	6.9±1.4
0.5% T3	214±29[b]	17.2±0.5[b]	15.2±1.8

Data are means±SE (n=4 or 5) and values without a common superscript letter are significantly different at p<0.05.

Table 4. Effect of dietary α-tocopherol and tocotrienol on immunoglobulin productivity of spleen and mesenteric lymph node lymphocytes of Sprague-Dawley rats.

	IgA (ng/ml)	IgG (ng/ml)	IgM (ng/ml)
Spleen			
None	17.1±0.9	53.9±4.9[ab]	22.3±3.2[ab]
0.1% Toc	25.4±5.1	66.9±6.8[a]	25.2±2.5[a]
0.5% Toc	18.4±3.2	63.0±9.8[a]	24.9±2.3[a]
0.1% T3	16.9±5.5	45.5±8.7[ab]	16.9±1.8[b]
0.5% T3	18.4±3.0	36.7±3.0[b]	15.0±1.8[b]
MLN			
None	32.3±5.1	46.6±12.8	5.8±2.2
0.1% Toc	25.7±6.9	45.3±15.8	6.1±2.8
0.5% Toc	27.2±3.4	33.1±2.1	4.6±1.2
0.1% T3	30.6±4.5	42.8±9.4	7.4±1.0
0.5% T3	34.7±2.3	36.4±3.6	5.4±0.6

Data are means±SE (n=4 or 5) and values without a common superscript letter are significantly different at p<0.05.

Effect on immune function in aged rats Table 3 shows the effect of dietary α-Toc and T3 on serum Ig levels. Significant increase in serum IgA level was observed in the rats fed 0.5% α-Toc, and those fed 0.1 or 0.5% T3. In the case of IgG, also, significant increase was observed in those fed 0.5% α-Toc or T3. A tendency similar to IgG was seen for IgM, but the difference was not significant.

IgG productivity of spleen lymphocytes showed an increasing tendency in those fed α-Toc and a suppressing tendency in those fed T3, though the difference was not marked (Table 4). Dietary effect of α-Toc and T3 was negligible on MLN lymphocytes.

Table 5 shows the effect on fatty acid content and LTB_4-releasing activity of PEC isolated from rats fed the above diets. In PEC, arachidonic acid level was around 3%, while that of liver phosphatidylcholine was around 30%. A significant increase in arachidonic acid level was observed in the rats fed 0.5% α-Toc or T3, though the increase was not marked. On the other hand, a significant decrease in LTB_4-releasing activity was observed in those fed α-Toc and T3, and this effect was more marked in rats fed at the 0.1% level than at the 0.5% level. These results suggest that the inhibition of LTB_4 release by dietary α-Toc and T3 is independent of arachidonic acid level.

Tocopherol and tocotorienol distribution in aged rats killed

Table 5. Effect of dietary α-tocopherol and tocotrienol on arachidonic acid content and chemical mediator releasing activity of peritoneal exudate cells of Sprague-Dawley rats.

	Arachidonic acid content (%)		PEC LTB_4 releasing activity ($ng/10^6$ cells)
	Liver PC	PEC total	
None	31.8±1.0[ab]	2.5±0.0[a]	15.3±0.6[a]
0.1% Toc	30.5±0.3[ab]	2.5±0.1[a]	7.9±0.4[b]
0.5% Toc	29.3±1.4[a]	3.1±0.0[b]	11.6±0.4[c]
0.1% T3	32.9±0.7[b]	2.6±0.0[a]	9.0±0.6[b]
0.5% T3	32.9±0.5[b]	3.5±0.1[c]	13.2±1.0[c]

Data are means±SE (n=4 or 5) and values without a common superscript letter are significantly different at p<0.05. PC: phosphatidylcholine, PEC: peritoneal exudate cells; LTB_4: leukotriene B_4.

Table 6. Effect of dietary α-tocopherol and tocotrienol with fasting on their levels in various tissues of Sprague-Dawley rats.

		None	0.1% Toc	0.5% Toc	0.1% T3	0.5% T3
Serum (μg/ml)	α-Toc	7.0±0.9[a]	14.9±2.3[bc]	20.2±2.8[b]	7.6±1.3[a]	10.4±0.9[ac]
	α-T3	ND	ND	ND	ND	ND
	γ-T3	ND	ND	ND	ND	ND
Liver (mg/g)	α-Toc	0.03±0.01[a]	0.43±0.05[b]	1.32±0.13[c]	0.04±0.02[a]	0.18±0.02[a]
	α-T3	ND	ND	ND	ND	ND
	γ-T3	ND	ND	ND	ND	ND
Spleen (μg/g)	α-Toc	14.4±3.2[a]	28.8±4.4[a]	67.7±10.1[b]	17.9±4.0[a]	25.2±2.3[a]
	α-T3	ND	ND	ND	ND	ND
	γ-T3	ND	ND	ND	ND	ND
MLN (μg/g)	α-Toc	97±6[a]	123±9[a]	215±24[b]	102±17[a]	111±16[a]
	α-T3	0.1±0.1[a]	0.8±0.4[a]	3.6±0.6[a]	18.9±3.2[b]	63.7±8.4[c]
	γ-T3	ND	ND	ND	16.3±3.7[a]	77.7±13.5[b]
Heart (μg/g)	α-Toc	106±9[ac]	106±1[ac]	116±3[a]	96±4[bc]	88±2[b]
	α-T3	ND	ND	ND	ND	ND
	γ-T3	ND	ND	ND	ND	ND
Adipose (μg/g)	α-Toc	214±20	237±41	235±28	194±19	207±12
	α-T3	1.6±1.6[a]	9.4±4.5[a]	7.8±3.8[a]	18.2±2.3[a]	103.2±9.7[b]
	γ-T3	ND	ND	5.5±5.5[a]	26.1±2.7[a]	149.7±23.2[b]
Kidney (μg/g)	α-Toc	10.0±4.1[a]	13.4±1.3[a]	22.7±1.6[b]	4.5±2.9[a]	12.0±3.2[a]
	α-T3	ND	ND	ND	ND	ND
	γ-T3	ND	ND	ND	ND	ND
Lung (μg/g)	α-Toc	30.4±5.1[a]	33.8±9.4[a]	56.1±3.6[b]	20.2±2.2[a]	19.8±2.2[a]
	α-T3	ND	ND	ND	ND	ND
	γ-T3	ND	ND	ND	ND	ND

Data are means±SE (n=4 or 5) and values without a common superscript letter are significantly different at p<0.05. ND: not detected.

Table 7. Effect of dietary tocopherol and tocotrienol without fasting on their levels in various tissues of Sprague-Dawley rats.

	α-Toc	α-T3	γ- T3	δ- T3
Serum (μg/ml)	10.9±1.8	4.2±1.5	3.9±2.4	1.1±0.7
Blood clot (μg/g)	64.6±41.6	13.0±1.2	12.8±12.8	ND
MLN (μg/g)	104.0±7.1	117.9±7.3	136.1±61.3	47.0±18.3
Liver (μg/g)	87.9±24.3	31.8±4.5	37.7±6.0	ND
Epididymal adipose (μg/g)	94.8±9.0	19.5±2.4	35.6±0.2	ND
Renal adipose (μg/g)	86.4±3.4	17.8±2.2	34.6±1.5	ND
Subcutaneous adipose (μg/g)	95.9±10.9	17.0±3.2	30.7±6.0	6.0±6.0
Brown adipose (μg/g)	184.1±11.3	57.2±6.5	90.3±20.6	40.7±9.2
Brain (μg/g)	18.6±0.7	0.6±0.6	ND	ND
Heart (μg/g)	48.1±14.0	28.0±9.2	34.7±6.4	8.2±3.1
Lung (μg/g)	36.7±13.4	11.7±2.6	11.2±1.5	ND
Spleen (μg/g)	69.6±12.1	30.1±18.5	30.8±27.2	8.6±8.6
Kidney (μg/g)	23.0±2.4	2.8±0.9	0.7±0.6	ND
Muscle (μg/g)	10.7±3.3	1.1±1.1	ND	ND
Testis (μg/g)	21.8±5.5	ND	ND	ND
Vice-testis (μg/g)	29.3±2.3	4.6±0.3	4.1±1.0	ND

Data are means±SE (*n*=2).

after fasting Table 6 shows the α-Toc and T3 levels in various tissues of SD rats fed the above diets. α-Toc was detected in all groups of rats and in all tissues, independent of the Toc supplementation in experimental diets.

T3, on the other hand, was not detected in most tissues even in rats fed the diet containing 0.1 or 0.5% T3, except in MLN and epididymal adipose tissue. In MLN, a dose-dependent increase of α- and γ-T3 was observed, and this was also true in epididymal adipose tissue. In this tissue, α- and γ-T3 were detected in rats fed the diet containing 0.5% α-Toc, but not in those fed the diet containing 0 or 0.1% α-Toc.

Tocopherol and tocotorienol distribution in young rats killed without fasting To determine the route of T3 transportation to MLN and adipose tissue, 8-week-old SD rats were fed diet containing 1% T3 for 24 h and killed without fasting. Table 7 shows the distribution of α-Toc and T3 homologues in various tissues. In this experiment, α-T3 was detected in various tissues except in testis, but the level varied with the tissue: it was high in MLN, brown adipose, liver and spleen, and low in brain, muscle, kidney and vice-testis. The level of γ-T3 was not detected in brain, muscle or testis, was low in kidney and vice-testis, and high in MLN and brown adipose tissue. δ-T3 was also detected in MLN and brown adipose tissue, though the level was much lower than the above T3 homologues.

Discussion

T3 are Toc homologues with 3 double bonds in their side chain and both composed of 4 homologues (α-, β-, γ-, and δ-), according to the methylation pattern on the chroman ring. Toc and T3 have been reported to exert similar biological effects in some cases, but to exert different effects in other cases. When 4-week-old SD rats were fed diets containing α-Toc or T3 at the 0.1% level, T3 decreased serum triglyceride level more strongly than α-Toc (Kaku *et al*., 1999). On the other hand, a significant increase in serum IgA level was observed in rats fed α-Toc, but not in rats fed T3 (Kaku *et al*., 1999). In the present study, we examined the dose-dependent effect of α-Toc and T3, to learn the difference in the biological effect between these two homologues. Since the inhibition of lipid peroxidation by lipophilic antioxidants is believed to suppress aging, we examined their dietary effect on 8-month-old, aged rats.

T3 seemed to be more efficient than α-Toc in it Bantioxidative activity, judging from the serum PCOOH level, while α-Toc seemed more efficient than T3, judging from the serum TBARS level. T3 decreased phospholipid level more strongly than α-Toc at the 0.1% level, but the decrease in total cholesterol or triglyceride level was not significant in the aged rats. The decrease of phospholipid level by T3 feeding was not dose-dependent and there was no significant difference between Toc and T3 groups at the 0.5% level. These results suggest that there is an upper limit in the regulatory activity of tocopherol derivatives on lipid metabolism. Kaku *et al*. (1999) found that T3 significantly decreased serum triglyceride level, but the decrease in total cholesterol or triglyceride level was not significant in young rats. In addition, they reported that T3 exerted stronger effect on serum lipid levels than Toc in young SD rats, as observed here in aged rats. These results suggest that T3 modifies lipid metabolism more strongly than α-Toc and that this effect is age-dependent.

In immune functions, significant increases of IgA, IgG, and IgM productivity and a significant decrease of IgE productivity have been reported in spleen lymphocytes of young Brown Norway rats fed α-Toc or T3 at the 0.2% level by Gu *et al*. (1999). They also reported that significant increases in IgA and IgG productivity were observed in MLN lymphocytes of rats fed T3, but not in those of rats fed α-Toc. Kaku *et al*. (1999) found that in young SD rats fed α-Toc or T3 at the 0.1% level, a significant increase in serum IgA level was observed in the former animals, but the increase was not significant in the latter. They also reported that a significant increase of IgM productivity was observed in spleen lymphocytes and significant increases of IgA, IgG and IgM productivity in MLN lymphocytes isolated from rats fed T3, but the increase of Ig productivity in those fed α-Toc was detected only in IgA productivity of MLN. In aged SD rats, serum IgA and IgG levels increased in either rats fed either substance, but the effect on Ig productivity was not marked in either spleen or MLN lymphocytes. These findings suggest that oral administration of α-Toc or T3 increases serum Ig levels in a dose-dependent manner.

Oral administration of antioxidants has also been reported to modify chemical mediator-releasing activity of rat PEC. Matsuo *et al*. (2000) reported a significant decrease of LTB_4-releasing activity of PEC isolated from young Wistar rats fed diet containing 1% tea polyphenols, independent of the level of arachidonic acid the substrate of lipoxygenase for 4-series LT production. It

has also been reported that oral administration of the n-3 unsaturated fatty acids α-linolenic, eicosapentaenoic and docosahexaenoic acids decreases LTB_4-releasing activity of rat PEC by decreasing arachidonic acid level in young Wistar (Matsuo *et al*., 2000) and SD rats (Hung *et al*., 1999, 2000). In the present study, we showed that a significant decrease of LTB_4-releasing activity of PEC isolated from aged SD rats fed α-Toc or T3 was induced without decreasing arachidonic acid level. These results suggest that α-Toc and T3 suppress LTB_4 release from PEC by inhibiting lipoxygenase activity, as reported in tea polyphenols (Yamada *et al*., 1999). Such inhibition of 4-series LT production by α-Toc and T3 feeding may suppress the expression of type I allergy, as well as suppression of the IgE production reported by Gu *et al*. (1999). In addition, enhancement of IgA and IgG production may protect our bodies from invasion by allergens and infectious agents. These results suggest that α-Toc and T3 are useful for maintenance of immune function in both young and aged rats.

Tissue distribution of T3 showed that no T3 derivative was detectable in serum or most tissues when the rats were killed after a 10 h fasting, except in MLN and epidydimal adipose tissue. On the absorption of T3 derivatives, Ikeda *et al*. (1996) reported that α-T3 is absorbed into the lymph more rapidly than α-Toc and γ-T3 in young SD rats. In addition, Hayes *et al*. (1993) reported that T3 was detected in serum in hamster killed without fasting. Thus, T3 is apparently rapidly metabolized or transported to specific tissues. We therefore killed young SD rats without fasting after a 24 h feeding of the T3 diets. In this case, α- and γ-T3 were detected in various tissues including serum and the T3 content was highly tissue-dependent. Among them, MLN and brown adipose tissue showed high T3 levels, but very low level in brain, muscle and testis, suggesting that T3 derivatives are quickly absorbed into lymph and transferred to blood via liver, then incorporated into various tissues in a tissue-dependent manner. T3 derivatives may thus quickly disappear from most tissues except some MLN and adipose tissues. The high T3 content in these tissues suggests they are major targets of T3.

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