ELSEVIER

Journal of Nutritional Biochemistry 13 (2002) 96–102

THE JOURNAL OF Nutritional Biochemistry

Green tea flavonoids inhibit the LDL oxidation in osteogenic disordered rats fed a marginal ascorbic acid in diet

Seiichi Kasaoka[a,*], Koji Hase[b], Tatsuya Morita[c], Shuhachi Kiriyama[d]

[a]*Department of Health and Nutrition, Bunkyo University Women's College, 1100 Namegaya, Chigasaki, Kanagawa 253-8550, Japan*
[b]*Laboratory of Mucosal Immunology, Department of Medicine, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0623, USA*
[c]*Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizuoka 422-8529, Japan*
[d]*Laboratory of Nutritional Biochemistry, Otsuma Women's University, Sanbancho 12, Chiyoda-ku, Tokyo 102-8357, Japan*

Received 15 March 2001; received in revised form 25 August 2001; accepted 27 September 2001

Abstract

Osteogenic Disorder Shionogi (ODS) rats can not synthesize ascorbic acid (AA). We have examined the capacity of green tea flavonoids (GTF) to modify low-density lipoprotein (LDL) oxidation in ODS rats with dietary AA restriction. In the first experiment, ODS rats were fed diets containing 300 (AA300 diet) or 0 (AA0 diet) mg AA/kg diets for 20 d. In comparison with the AA300 diet, the AA0 diet significantly decreased the concentrations of plasma AA and α-tocopherol in LDL and significantly shortened the lag time of LDL oxidation *in vitro*. In the second experiment, ODS rats were fed one of the following three diets: the AA300 diet, the diet containing 25 mg AA (AA25, marginal AA)/kg diet (AA25 diet), or the diet containing 25 mg AA + 8 g GTF/kg diet (AA25 + GTF diet) for 20 d. Plasma AA concentration were significantly lower in rats fed AA25 compared with AA300 but not in those fed AA25 + GTF. LDL oxidation lag time was significantly longer in rats fed AA25 + GTF compared with the other two groups. Lag time for LDL oxidation was significantly and positively correlated with LDL α-tocopherol ($r = 0.6885$, $P = 0.0191$). These results suggest that dietary flavonoids suppress the LDL oxidation through the sparing effect on LDL α-tocopherol and/or plasma AA when AA intake is marginal in the ODS rats.

Keywords: Flavonoids; LDL oxidation; ODS rats; Ascorbic acid deficiency; α-tocopherol

1. Introduction

Oxidative modification of low-density lipoprotein (LDL) has been implicated in human atherosclerosis [1]. Lipophilic agents such as vitamin E (mainly α-tocopherol) in LDL are though to be major determinants of their resistance to oxidative damage [2]. Halliwell [3] reported that when isolated LDL is exposed to pro-oxidant conditions *in vitro*, LDL oxidation does not occur to any significant extent until any α-tocopherol present are depleted.

Ascorbic acid (AA) is the most important water soluble antioxidant in extracellular fluids [4]. Its effects on LDL oxidation *in vivo* are inconclusive [5,6]. Samman et al. [5] reported that no change in the oxidisability of LDL was found after 1 g AA supplementation per day in eight male smokers compared to placebo supplementation. On the other hand, Harats et al. [7] reported in human study that a significant positive correlation was observed between plasma AA and the lag time before the initiation of LDL oxidation. They suggested that plasma AA reduced the susceptibility of LDL to oxidation through the sparing effect on α-tocopherol because regeneration of α-tocopherol from tocopheryl radical is mainly due to AA via the donation of a hydrogen atom. Thus, a marginal or severe AA deficiency may compromise antioxidative efficacy of α-tocopherol and promote plasma LDL oxidation.

Recently, flavonoids, a group of plant polyphenolic compounds have attracted much attention for their potential as antioxidants, free radical scavengers, and metal chelators [8]. In addition, epidemiological studies have shown an inverse correlation between dietary flavonoid intake and mortality from coronary heart disease [9]. Increasing evidence from rat and human studies support that dietary flavonoids decrease the susceptibility of isolated LDL to cop-

* Corresponding author. Tel.: +81-467-53-2111 ext. 255; fax: +81-467-54-3803.
E-mail address: kasaoka@shonan.bunkyo.ac.jp (S. Kasaoka).

0955-2863/02/$ – see front matter
PII: S0955-2863(01)00202-9

per-induced peroxidation [10,11]. The mechanisms by which these flavonoids inhibit LDL oxidation have not been clarified. Because of their amphipathic nature, it is not clear whether flavonoids may act within the LDL particles in a manner similar to that of α-tocopherol, or may act in a manner comparable with that of AA in the plasma environment of LDL [11]. Nevertheless, dietary flavonoids may contribute to the protection of LDL oxidation for those populations in particular whose AA intake are marginal or AA requirement are increased by some reasons such as heavy smoking [12].

In this paper, we examined the effects of green tea flavonoids (GTF) on the susceptibility of LDL to oxidative modification in marginal AA deficiency. For this purpose, we used the Osteogenic Disorder Shionogi (ODS) rats that cannot synthesize AA [13], and it is easy to manipulate the plasma AA concentration in ODS rats. Also, plasma concentration of LDL cholesterol [14] and thiobarbituric acid-reactive substances (TBARS) in LDL [15] are higher in ODS rats. These characteristics of ODS rats may be of advantage to get more insight into the interaction of GTF with α-tocopherol and AA related to the plasma LDL oxidation.

2. Methods and materials

2.1. Materials

Green tea flavonoids (GTF) were supplied by Nikken-Food (Tokyo, Japan). GTF were prepared as follows. The water extract from green tea (*Camellia sinensis*) leaves was filtered and concentrated under reduced pressure. The concentrated solution was powdered by spray drying. On analysis, GTF was found to comprise (+)-catechin (6%), (−)-epicatechin (1%), (−)-epicatechin gallate (5%), (+)-gallo catechin (6%), (−)-epigallocatechin (7%) and (−)-epigallocatechin gallate (25%) by weight. GTF also contained 50% dextrin as a bulk, but did not contain any ascorbic acid (AA) (the manufacturer's information). AA was purchased from Wako Pure Chemical Industry (Osaka, Japan).

2.2. Care of animals

Six-wk-old male ODS (od/od) rats were purchased from CLEA Japan (Tokyo, Japan). The animals were housed in individual steel cages with wire screen-bottomed stainless steel cages in a room of controlled temperature (23 ± 2°C) and lighting (lights on from 08:00–20:00 h). After adaptation to a standard diet (Table 1), based on AIN-76 [16], supplemented with 300 mg AA/kg diet for 1 wk, rats were divided into groups on the basis of matched body weight and allowed free access to test diets and water. Body weight and food intake were recorded each morning before replenishing the diets.

The study was approved by the Animal Use Committee of Otsuma Women's University and animals were maintained in accordance with the guidelines for the care and use of laboratory animals, Otsuma Women's University.

Table 1
Composition of standard diet

Ingredient	g/kg
Casein	250
Cornstarch	410
Sucrose	205
Cellulose	40
Corn oil	50
Mineral mixture[a]	35
Vitamin mixture[a]	10

[a] Based on AIN-76 (AIN 1977).

2.3. Feeding studies

2.3.1. Effect of dietary AA on the growth, and plasma and liver variables related to AA deficiency in ODS rats (experiment 1)

Twelve rats weighing 177–203 g were divided into 2 groups ($n = 6$) after dietary acclimation and were allowed free access to the respective test diets containing 300 (AA300) or 0 (AA0) mg AA/kg diets. The basic composition of each test diet was the same as that of the standard diet (Table 1). Ascorbic acid was added to each diet at the expense of an equal amount of cornstarch. On the day of autopsy, diets were withdrawn at 08:00 h. Rats were anesthetized with diethyl ether at 13:00–15:00 h and blood collected from the abdominal aorta with a syringe containing 160 μL of 134 mmol/L EDTA-saline solution as anticoagulant. Plasma was separated from the blood and used for the measurements of AA, α-tocopherol and cholesterol concentrations. Liver was removed, washed with ice-cold 0.15 mol/L of sodium chloride, gently blotted on filter papers and weighed. The liver was frozen in liquid nitrogen and kept at −80°C until analysis.

2.3.2. Effect of GTF on the plasma LDL oxidation in ODS rats fed diet with marginal amounts of AA (experiment 2)

Twenty-three rats weighing 163–183 g were divided into 3 groups ($n = 7$ or 8) after acclimation and were allowed free access to one of the test diets containing 300 (AA300, $n = 8$) or 25 (AA25, $n = 8$) mg AA/kg diets, or 25 mg AA + 8 g GTF/kg diet (AA25 + GTF, $n = 7$), respectively. The basic composition of each test diet was the same as that of the standard diet (Table 1). AA and GTF were added to each diet at the expense of an equal amount of cornstarch. Sampling of plasma and liver were performed in the same manners as described in experiment 1.

2.3.3. Preparation of plasma LDL and measurement of LDL oxidation in vitro

An LDL (density: 1.006 to 1.063 g/mL) fraction was separated by sequential ultracentrifugation [17] with some modifications [18]. This LDL fraction contains some part of IDL at the lower limit and HDL at the upper limit. Two

different plasma samples (2 mL each) in the same group were pooled and used for the isolation of LDL fraction. Four mL of plasma were layered beneath 2 mL of saline containing 0.27 mmol/L EDTA (density: 1.006 g/mL) in an ultracentrifuge tube. The tubes were centrifuged in a Beckman 70.1 Ti rotor in a Beckman L-70 ultracentrifuge at 130,000 × g for 18 h at 12°C. Following the removal of VLDL + chylomicron fraction in the upper layer, the density of bottom layer was adjusted to 1.063 g/mL by the addition of 0.5 volume of saline containing 0.27 mmol/L EDTA and 2.44 mol/L sodium bromide (density: 1.182 g/mL) in another tube. The density-adjusted solution was layered beneath saline containing 0.27 mmol/L EDTA and 0.81 mol/L sodium bromide (density: 1.063 g/mL) in another tube. The tubes were centrifuged in the same rotor at 130,000 × g for 20 h at 12°C. LDL fraction in the upper layer was collected and dialyzed against 10 mmol/L phosphate buffered saline (PBS) (2 L × 4) overnight to remove EDTA. LDL protein concentration was measured by the Lowry method using bovine serum albumin as a standard [19].

The LDL fraction was diluted into 100 μg LDL protein/mL with 10 mmol/L PBS and incubated in a 1.5 mL quartz cuvette (1 cm in diameter) in the presence of 5 (experiment 1) or 8 (experiment 2) μmol/L copper sulfate at 37°C for up to 3 h. The formation of conjugated dienes was determined by measuring the absorbance at 234 nm according to the method of Esterbauer et al [2]. Lag time of LDL oxidation was defined as the time to the intercept of the tangent of the absorbance curve in the propagation phase with that in the lag phase. Propagation ration was determined by the slope of tangent of the curve in the propagation phase and expressed as increased absorbance per min.

2.3.4. Other analytical procedures

Plasma and tissue concentrations of AA were measured by the 2,4-dinitrophenylhydrazine method [20] with a slight modification for the tissue AA. Briefly, livers were homogenized with 10 volumes of an ice-cold aqueous solution of metaphosphoric acid (50 g/L). The homogenate was centrifuged at 3,000 rpm for 15 min, and supernatant obtained was applied to the AA determination. α-Tocopherol in plasma and LDL was extracted with *n*-hexane and measured fluorometrically (Ex 295 nm, Em 320 nm) according to the method of Abe et al [21].

Liver thiobarbituric acid-reactive substances (TBARS) concentration was measured by the method of Uchiyama and Mihara [22] after homogenizing liver with 10 volumes of an ice-cold aqueous solution of potassium chloride (154 mmol/L). Plasma concentrations of total and HDL cholesterols were measured enzymatically using commercial kits (Cholesterol C Test-Wako and HDL Cholesterol Test-Wako, Wako Pure Chemical Industry). Plasma LDL + VLDL cholesterol concentration was calculated as the differences between total and HDL cholesterol.

2.3.5. Statistical analyses

Data were analyzed by Student's *t*-test (experiment 1) or by a one-way ANOVA and significant differences among means were separated by Scheffe's test (experiment 2). When variances were not homogenous by Bartlett test [23], data were transformed logarithmically and the transformed data were analyzed by ANOVA followed by multiple comparison. When variances were not homogenous even after logarithmic transformation, the results were presented as medians with ranges and then analyzed Kruskal-Wallis ANOVA followed by Kolmogorov-Smirnov two-sample test. The correlations among plasma concentrations of AA and α-tocopherol, α-tocopherol concentration in LDL fraction, lag time before the initiation of LDL oxidation, and propagation ratio were analyzed by linear regression [24]. All statements of significant differences showed the 5% level of probability.

3. Results

3.1. Effect of dietary AA on the growth, and plasma and liver variables related to AA deficiency in ODS rats (experiment 1)

Up to d 14, AA300 and AA0 groups showed the same growth rate, but body weight in the AA0 group began to decrease on d 16 (data not shown). The body weight gain and food intake in the AA0 group were significantly lower than those in the AA300 group (Table 2).

Plasma concentrations of total and HDL cholesterol were comparable between the groups (Table 2). Plasma AA concentration was significantly lower in the AA0 group than that in the AA300 group. There was no significant difference in plasma α-tocopherol concentration between the groups. However, the ratio of α-tocopherol to total cholesterol was tended to be lower in the AA0 group than that in the AA300 group.

Liver weight was significantly lower in the AA0 group than that in the AA300 group (Table 2). There was no significant difference in liver lipid concentration between the groups. Liver concentrations of total cholesterol and free cholesterol were significantly higher in the AA0 group than those in the AA300 group, but esterified cholesterol concentration was comparable between the groups. Liver AA concentration was significantly lower in the AA0 group than that in the AA300 group. Liver TBARS concentration was significantly higher in the AA0 group than that in the AA300 group.

Lag time for LDL oxidation was significantly longer in the AA300 group than in the AA0 group. Propagation rate in the AA300 group also tended to be slower than that in the AA0 group ($P < 0.10$, Table 2). α-Tocopherol concentration in LDL fraction was significantly higher in the AA300 group than that in the AA0 group.

Table 2
Food intake, body weight gain, plasma concentrations of cholesterol, ascorbic acid (AA) and α-tocopherol, liver concentrations of lipids, AA and thiobarbituric acid-reactive substances (TBARS), lag time and propagation ratio of LDL oxidation, and α-tocopherol concentration in LDL fraction of ODS rats fed the diets containing 300 (AA300) or 0 (AA0) mg AA/kg diets for 20 d (experiment 1)[a]

	AA300	AA0	*P* value
Food intake, g/20 d	369 ± 9	335 ± 9	0.0223
Body weight gain, g/20 d	107 ± 4	56 ± 3	<0.0001
Plasma			
total cholesterol, mmol/L	2.20 ± 0.07	2.29 ± 0.07	0.3928
HDL cholesterol, mmol/L	1.12 ± 0.04	1.07 ± 0.04	0.4117
VLDL + LDL cholesterol, mmol/L	1.08 ± 0.07	1.22 ± 0.03	0.0893
ascorbic acid, μmol/L	33.7 ± 3.2	19.5 ± 2.2	0.0044
α-tocopherol, μmol/L	28.4 ± 2.5	23.6 ± 2.4	0.1931
α-tocopherol/total cholesterol, × 10^{-3} mol/mol	12.9 ± 1.0	10.2 ± 0.8	0.0686
Liver			
relative weight, g/100 g body weight	4.43 ± 0.08	3.55 ± 0.07	<0.0001
total lipid, mg/g	44.7 ± 1.0	45.3 ± 1.0	0.7001
total cholesterol, μmol/g	6.14 ± 0.20	6.76 ± 0.13	0.0248
free cholesterol, μmol/g	4.79 ± 0.09	5.68 ± 0.05	<0.0001
esterified cholesterol, μmol/g	1.36 ± 0.15	1.08 ± 0.11	0.1569
ascorbic acid, nmol/g	940 ± 38	82 ± 4	<0.0001
TBARS, nmol/g	50.9 ± 0.8	66.7 ± 3.7	0.0019
LDL oxidation[b]			
lag time, min	61.5 ± 6.1	35.5 ± 2.0	0.0155
propagation ratio[c], × 10^{-3} Δ absorbance/min	15.4 ± 0.8	19.4 ± 1.3	0.0600
α-tocopherol, nmol/mg LDL protein	25.9 ± 0.5	21.9 ± 1.0	0.0202

[a] Data are expressed as means ± SEM ($n = 6$). The effects of dietary treatment were examined by Student's *t*-test. Average initial body weight, 187 g (range; 177–203 g).
[b] Data are expressed as means ± SEM ($n = 3$). Two fresh plasma samples (2 mL each) in same group was pooled to isolate LDL fraction.
[c] Propagation ratio was determined by the slope of the tangent of the curve during the propagation phase and expressed as increased absorbance per min.

3.2. Effect of GTF on the plasma LDL oxidation in ODS rats fed diet with marginal amounts of AA (experiment 2)

The AA25 + GTF group showed the lowest food intake among the groups. This reflected the fact that food intake in this group was considerably lower than those in the other two groups for up to d 2 (data not shown). Accordingly, body weight gains for 20 d were lowest in the AA25 + GTF group, highest in the AA300 group and intermediate in the AA25 group (Table 3).

Plasma AA concentration in the AA300 group were significantly higher than that in the AA25 group, but no significant difference was observed between the AA300 and the AA25 + GTF groups (Table 3). Plasma α-tocopherol concentrations did not differ among the groups, but there was a significant and positive correlation between plasma AA and α-tocopherol concentrations ($r = 0.5797$, $P = 0.0037$).

Liver weight was significantly higher in the AA300 group than those in the other groups (Table 3). There was no significant difference in liver weight between the AA25 and the AA25 + GTF groups. Liver AA concentration was also significantly greater in the AA300 group than those in the other two groups. There was no significant difference in liver AA concentration between the AA25 and the AA25 + GTF groups. Liver TBARS concentration was significantly lower in the AA300 group than those in the other two groups. There was no significant difference in liver TBARS concentration between the AA25 and the AA25 + GTF groups.

Kinetics of copper-catalyzed oxidation of LDL in rats fed the AA300, AA25 or AA25 + GTF diets were shown in Fig. 1. Lag time in the AA25 + GTF group was significantly longer than those in the other two groups (Table 3). Propagation ratio in the AA25 + GTF group also tended to be slower than those in the AA300 ($P = 0.0649$) and the AA25 ($P = 0.0649$) groups. α-Tocopherol concentrations in LDL were comparable among the groups, but there was a significant and positive correlation between the α-tocopherol concentration in LDL fraction and the lag time of LDL oxidation ($r = 0.6885$, $P = 0.0191$) (Fig. 2-A). There was no significant difference between the α-tocopherol concentration in LDL fraction and the propagation ratio of LDL oxidation (Fig. 2-B).

4. Discussion

We believe that this is the first time that the antioxidative efficacy of AA on LDL oxidation have been examined in ODS rats. As expected from previous study with ODS rats, feeding the AA0 diet resulted in a decrease in body weight and severe symptoms of scurvy such as bleeding around the nose [25]. ODS rats fed this diet showed depletion [25] of AA and increased TBARS [15] in plasma and liver [25] as compared with those in rats fed AA300 diet. Increasing AA

Table 3
Food intake, body weight gain, plasma concentrations of ascorbic acid (AA) and α-tocopherol, and liver concentrations of AA and thiobarbituric acid-reactive substances (TBARS), lag time and propagation ratio of LDL oxidation, and α-tocopherol concentration in LDL fraction of ODS rats fed the diets containing 300 (AA300) or 25 (AA25) mg AA/kg diets, or 25 mg AA + 8 g green tea flavonoids (GTF)/kg diet (AA25 + GTF) for 20 d (experiment 2)[a]

	AA300	AA25	AA25 + GTF
Food intake, g/20 d	366 ± 5[b]	345 ± 7[a]	327 ± 6[a]
Body weight gain, g/20 d	107 ± 2[c]	85 ± 2[b]	70 ± 3[a]
Plasma			
ascorbic acid, μmol/L	52.6 ± 3.7[b]	35.2 ± 2.5[a]	40.0 ± 4.3[a]
α-tocopherol, μmol/L	22.2 ± 1.1	20.9 ± 1.1	23.8 ± 2.2
Liver			
relative weight[b], g/100 g body weight	4.41 ± 0.06[b]	3.70 ± 0.06[a]	3.74 ± 0.10[a]
ascorbic acid[b], nmol/g	947 ± 48[b]	88 ± 6[a]	75 ± 4[a]
TBARS, nmol/g	55.1 ± 2.2[a]	83.9 ± 4.2[b]	73.2 ± 6.4[b]
LDL oxidation[c]			
lag time, min	44.6 ± 3.0[a]	39.0 ± 2.8[a]	63.6 ± 3.6[b]
propagation ratio[d], $\times 10^{-3}$ Δ absorbance/min	16.3 (15.4–17.2)	19.2 (13.3–26.6)	12.5 (12.5–12.9)
α-tocopherol, nmol/mg LDL protein	22.1 ± 0.5	22.8 ± 0.9	25.9 ± 2.9

[a] Data are expressed as means ± SEM ($n = 8$ for AA300 and AA25, or $n = 7$ for AA25 + GTF); values in a row with no common superscript letters are significantly different ($P < 0.05$) when analyzed by one-way ANOVA, followed by Scheffe's multiple range test. Average initial body weight, 170 g (range; 163–183 g).

[b] Data were transformed logarithmically before analysis.

[c] Data are expressed as means ± SEM ($n = 4$ for AA300 and AA25, or $n = 3$ for AA25 + GTF). Two different plasma samples (2 mL each) in the same group were pooled to isolate LDL fraction.

[d] Propagation ratio was determined in the same manners as described in the footnote to Table 2. Results are medians with ranges in parentheses. The results were statistically analyzed with Kruskal-Wallis one-way ANOVA.

concentration in the diet (AA300 diet) significantly prolonged the lag time and tended to lower the propagation ratio of LDL oxidation compared with those in rats fed the AA0 diet (Table 2). Also, α-tocopherol concentration in LDL fraction was higher in the AA300 diet-fed group than that in the AA0 diet-fed group (Table 2). These results reinforce the previous findings by Harats et al. [7] suggesting that plasma AA reduce the susceptibility of lipoprotein

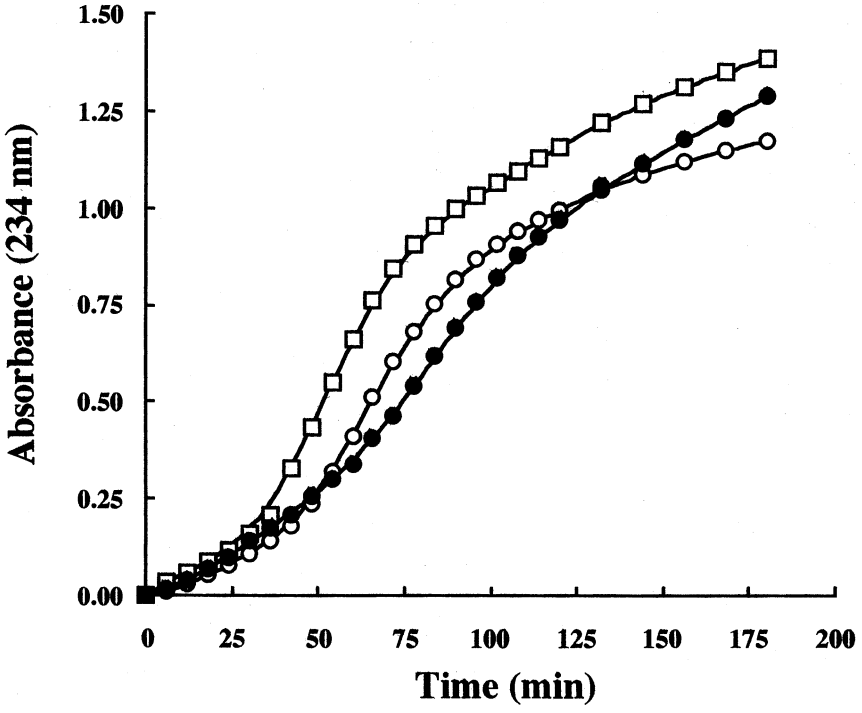

Fig. 1. Kinetics of copper-catalyzed oxidation of LDL fraction in rats fed the diets containing 300 (○) or 25 (□) mg ascorbic acid/kg diets, or 25 mg ascorbic acid + 8 g green tea flavonoids (●)/kg diet for 20 d (experiment 2). Oxidation was induced with the addition of copper sulfate (8 μmol/L). Data represent the mean from 4 (the AA300 and AA25 groups) or 3 (the AA25 + GTF group) determinations.

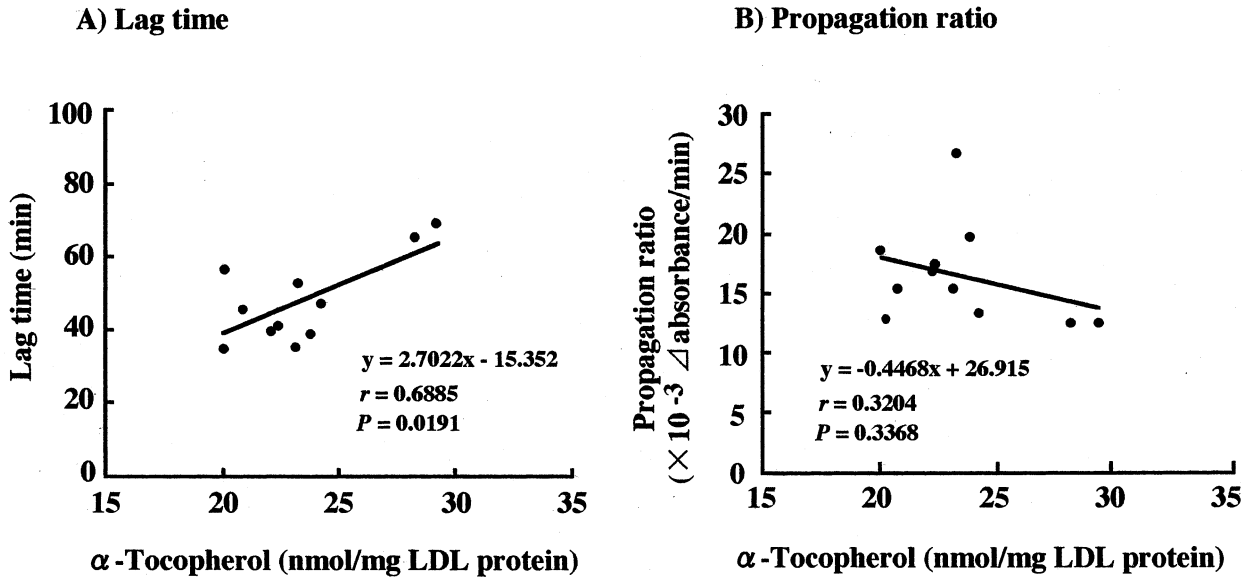

Fig. 2. Linear regression analyses A) between α-tocopherol concentration and the lag time of LDL oxidation or B) between α-tocopherol concentration and propagation ratio of LDL oxidation in LDL fraction of ODS rats fed the diets containing 300 or 25 mg ascorbic acid/kg diets, or 25 mg ascorbic acid + 8 g green tea flavonoids/kg diet for 20 d (experiment 2).

to oxidation through the sparing effect of AA on the consumption of α-tocopherol in LDL particles.

The second experiment was designed to examine the antioxidative efficacy of dietary flavonoids in the ODS rats fed marginal amount of AA in the diet. To meet this purpose, we defined 25 mg AA/kg diet (AA25 diet) as a marginal AA level in a diet. This was based on a previous study by Horio et al. [26] which showed clearly that body weight gain in ODS rats fed a diet containing 50 mg AA/kg diet was comparable to that in ODS rats fed a diet containing 300 mg AA/kg diet. In fact, rats fed the AA25 diet stopped gaining weight on d 16, but thereafter the body weight was maintained up to the end of the experimental period without any symptoms of severe scurvy.

Supplementation of GTF to the AA25 diet (AA25 + GTF diet) significantly prolonged the lag time of LDL oxidation (Table 3), and there was a significant and positive correlation between α-tocopherol concentration in LDL fraction and the lag time of LDL oxidation (*Fig. 2-A*). GTF has an amphipathic nature, it is possible that some parts of GTF might be associated or incorporated into LDL particles. When isolated LDL were depleted of GTF by dialysis the direct influence of GTF in the reaction mixture of LDL oxidation was negligible. Clearly, this dialysis would remove GTF in the aqueous phase (PBS) of the LDL fraction. It is probable also that most of GTF attached to LDL particles might be gradually detached from the particles and passed into the aqueous phase (PBS) during this dialysis repeated dialysis procedure. Thus, the antioxidative effect of GTF remaining in LDL particles appears to be negligible. Taking all of these into consideration, it is possible to assume that GTF reduces the susceptibility of LDL oxidation through the sparing effect on α-tocopherol in LDL particles.

It is still unclear that whether flavonoids scavenge oxygen radical at surface of or within the LDL particles. However, Terao et al. [27] reported the antioxidative effect of quercetin (a flavonoid) and α-tocopherol on phospholipid bilayers. This was done using water-soluble radical initiator, and quercetin was shown to decrease faster than α-tocopherol when quercetin and α-tocopherol were mixed in the liposomes. They suggested that flavonoids served as scavengers of peroxyl radicals near the membrane surface. Therefore, in AA deficiency, some constituents of GTF are also likely to scavenge α-tocopherol radicals near the surface of LDL particles as a substitute for AA. The present study showed that plasma AA concentrations in the AA25 + GTF diet fed-group were comparable to those in the AA300 diet fed-group, while those in the AA25 diet fed-group were significantly lower than those in the AA300 diet fed-group (Table 3). These results suggest that GTF not only serve as radical scavenger, but also spare plasma AA in ODS rats with marginal AA deficiency.

Interestingly, the lag time of LDL oxidation in rats fed the AA25 + GTF diet was significantly longer than that in rats fed the AA300 diet (Table 3). Samman et al. [28] reported in human study that polyphenolic compounds extracted from green tea reduced nonheme-iron absorption. They suggested that chelation of iron might be one of the mechanisms of antioxidant action *in vivo*. However, more research is needed for further elucidation of the mechanisms of antioxidative effect of flavonoids.

The present study clearly shows that dietary flavonoids suppress LDL oxidation through a sparing effect on LDL α-tocopherol when AA intake is marginal in the ODS rats.

Dietary flavonoids may contribute to the protection of LDL oxidation for those populations in particular whose AA intake are marginal or AA requirement are increased by some reasons such as heavy smoking and exposure to xenobiotics, as indicated from previous study with ODS rats fed a diet containing polychlorinated biphenyl [29].

References

[1] J.L. Wiztum, D. Steinberg, Role of oxidized low density lipoprotein in atherogenesis, J. Clin. Invest. 88 (1991) 1785–1792.

[2] H. Esterbauer, H. Striegl, H. Puhl, M. Rotheneder, Continuous monitoring of in vitro oxidation of human low density lipoprotein, Free Radic. Res. Commun. 6 (1989) 67–75.

[3] B. Halliwell, Antioxidants and human disease: a general introduction, Nutr. Rev. 55 (1997) S44–S52.

[4] B. Frei, L. England, B.N. Ames, Ascorbate is an outstanding antioxidant in human blood plasma, Proc. Natl. Acad. Sci. 88 (1991) 11003–11006.

[5] S. Samman, A.J. Brown, C. Beltran, S. Singh, The effect of ascorbic acid on plasma lipids and oxidisability of LDL in male smokers, Eur. J. Clin. Nutr. 51 (1997) 472–477.

[6] J.D. Belcher, J. Balla, G. Balla, D.R. Jacobs, J.M. Gross, H.S. Jacob, G.M. Vercellotti, Vitamin E, LDL, and Endothelium. Brief oral vitamin supplementation prevents oxidized LDL-mediated vascular injury in vitro, Arterioscler. Thromb. 13 (1993) 1779–1789.

[7] D. Harats, S. Chevion, M. Nahir, Y. Norman, O. Sagee, E.M. Berry, Citrus fruit supplementation reduces lipoprotein oxidation in young men ingesting a diet high in saturated fat: presumptive evidence for an interaction between vitamins C and E in vivo, Am. J. Clin. Nutr. 67 (1998) 240–245.

[8] N.C. Cook, S. Samman, Flavonoids—Chemistry, metabolism, cardioprotective effects, and dietary sources, J. Nutr. Biochem. 7 (1996) 66–76.

[9] M.G.L. Hertog, E.J.M. Feskens, P.C.H. Hollman, M.B. Katan, D. Kombout, Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study, Lancet 342 (1993) 1007–1011.

[10] L. Fremont, M.T. Gozzelino, M.P. Franchi, A. Linard, Dietary flavonoids reduce lipid peroxidation in rats fed polyunsaturated or monounsaturated fat diets, J. Nutr. 128 (1998) 1495–1502.

[11] T. Ishikawa, M. Suzukawa, T. Ito, H. Yoshida, M. Ayaori, M. Nishiwaki, A. Yonemura, Y. Hara, H. Nakamura, Effect of tea flavonoid supplementation on the susceptibility of low-density lipoprotein to oxidative modification, Am. J. Clin. Nutr. 66 (1997) 261–266.

[12] A.C. Carr, B. Frei, Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans, Am. J. Clin. Nutr. 69 (1999) 1086–1107.

[13] Y. Mizushima, T. Harauchi, T. Yoshizaki, S. Makino, A rat mutant unable to synthesize vitamin C, Experimentia 40 (1984) 359–361.

[14] F. Horio, N. Takahashi, S. Makino, Y. Hayashi, A. Yoshida, Ascorbic acid deficiency elevates serum level of LDL-cholesterol in a rat mutant unable to synthesize ascorbic acid, J. Nutr. Sci. Vitaminol. 37 (1991) 63–71.

[15] H. Kimura, Y. Yamada, Y. Morita, H. Ikeda, T. Matsuo, Dietary ascorbic acid depresses plasma and low density lipoprotein lipid peroxidation in genetically scorbutic rats, J. Nutr. 122 (1992) 1904–1909.

[16] American Institute of Nutrition, Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies, J. Nutr. 107 (1977) 1340–1348.

[17] R.J. Havel, H.A. Eder, J.H. Bragdon, The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum, J. Clin. Invest. 34 (1955) 1345–1353.

[18] F.T. Hatch, Practical methods for plasma lipoprotein analysis, Adv. Lipid Res. 6 (1968) 1–68.

[19] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–276.

[20] M. Tsujimura, Y. Uchiyama, A. Fujita, On the fractional determination of ascorbic, dehydroascorbic, and diketogulonic acids, Vitamins 43 (1971) 210–220 (in Japanese).

[21] K. Abe, G. Katsui, Fluorometric determination of tocopherol in serum, Eiyo to Shokuryo (J. Jpn. Soc. Food Nutr.) 23 (1975) 277–280 (in Japanese).

[22] M. Uchiyama, M. Mihara, Determination of malonaldehyde precursor in tissues by thiobarbituric acid test, Anal. Biochem. 86 (1978) 271–278.

[23] J.H. Zar, in Biostatistical Analysis, 2nd ed., Prentice-Hall, Englewood Cliffs, NJ, 1984.

[24] G.W. Snedecor, W.G. Cochran, in Statistical Methods, 6th ed., Iowa State University Press, Ames, IA, 1967, pp. 166–190.

[25] F. Horio, K. Ozaki, A. Yoshida, S. Makino, Y. Hayashi, Requirement for ascorbic acid in a rat mutant unable to synthesize ascorbic acid, J. Nutr. 115 (1985) 1630–1640.

[26] F. Horio, K. Ozaki, H. Oda, S. Makino, Y. Hayashi, A. Yoshida, Effect of dietary ascorbic acid, cholesterol and PCB on cholesterol concentrations in serum and liver in a rat mutant unable to synthesize ascorbic acid, J. Nutr. 117 (1987) 1036–1044.

[27] J. Terao, M. Piskula, Q. Yao, Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers, Arch. Biochem. Biophys. 308 (1994) 278–284.

[28] S. Samman, B. Sandstrom, M.B. Toft, K. Bukhave, M. Jensen, S. Sorensen, Green tea or rosemary extract added to foods reduces nonhem-iron absorption, Am. J. Clin. Nutr. 73 (2001) 607–612.

[29] F. Horio, K. Ozaki, H. Oda, S. Makino, Y. Hayashi, A. Yoshida, Effect of dietary ascorbic acid, cholesterol and PCB on cholesterol and bile acid metabolism in a rat mutant unable to synthesize ascorbic acid, J. Nutr. 119 (1989) 409–415.