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Journal of Nutritional Biochemistry 15 (2004) 142–148

THE JOURNAL OF Nutritional Biochemistry

Effect of genistein supplementation on tissue genistein and lipid peroxidation of serum, liver and low-density lipoprotein in hamsters

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Received 1 October 2002; received in revised form 20 May 2003; accepted 23 June 2003

Abstract

The aim of this study was to investigate the effects of genistein supplementation in a vitamin E-deficient diet on the genistein concentrations and the lipid oxidation of serum, liver and low-density lipoprotein (LDL) of hamsters. Thirty-six male hamsters were randomly divided into three groups and fed a vitamin E-deficient semisynthetic diet (AIN-76) containing different levels of genistein, i.e., G0 (control group, genistein-free diet), G50 (50 mg genistein/kg diet) and G200 (200 mg genistein/kg diet) for 5 weeks. The concentrations of genistein in serum and liver significantly increased with the increase of genistein supplementation. The vitamin E contents in LDL were higher in hamsters fed G50 or G200 diets than in hamsters fed genistein-free diet. Genistein supplementation to hamsters significantly reduced the propagation rate during conjugated diene formation of LDL oxidation, and the lag time of LDL oxidation in hamsters fed G200 diets was significantly lower than that of G0 diets. In addition, genistein supplementation significantly raised serum total antioxidant capacity and decreased the thiobarbituric acid-reactive substances (TBARS) of LDL and liver in hamsters. However, no significant differences in TBARS were found in serum, irrespective of genistein addition. On the other hand, the relative contents of polyunsaturated fatty acids in LDL were decreased after genistein supplementation. There was a negative correlation between lag time and P/S ratio, and a positive correlation between lag time and vitamin E contents. These data demonstrate that genistein supplementation markedly increased its concentrations in body tissues and reduced oxidative stress of lipid oxidation of serum, liver and LDL.

Keywords: Genistein; Vitamin E; Conjugated diene; Lipid oxidation; LDL; TBARS

1. Introduction

The oxidative hypothesis of atherosclerosis suggests that circulating low-density lipoprotein (LDL) is oxidized in vivo which leads to its enhanced uptake by macrophages inside the arterial system, and is believed to subsequently result in foam cell formation, one of the first stages of atherogenesis [1–3]. Therefore, retardation of LDL oxidation would be helpful in lowering the incidence of cardiovascular disease. On the other hand, LDL oxidizability is dependent on the amount of available related substrates, such as antioxidant contents, [4,5] its fatty acid compositions [6,7] and cholesterol contents, [8] in which antioxidants play an important role in preventing lipid oxidation and slowing the progression of atherosclerotic lesions [9,10]. This work has attracted increasing interest in the related research during the last decades. Nevertheless, it is conceivable that some factors in relation to LDL oxidation still remain unclear.

According to the results of epidemiological studies, the consumption of soy and soy products containing isoflavones is associated with a reduced risk of coronary heart disease and is inversely correlated to cancer-caused human mortality [11–14]. Many of these human and animal studies suggest that such health benefits of isoflavones are dependent on their antioxidative activity. Soybean contains many physiologically active materials for which genistein, one of the major isoflavones, has recently attracted much attention, having a multiple functions including inhibition of tyrosine kinase, estrogenic activity and antioxidant activity [14–17]. However, little is known about soybean protective mechanisms. Reports have pointed out that genistein exhibits antioxidant properties by protecting from microsomal lipid peroxidation induced by an Fe^{2+}-ADP complex, [18] pre-

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0955-2863/04/$ – see front matter
doi:10.1016/j.jnutbio.2003.06.001

venting LDL oxidation in vitro as mediated by copper and peroxyl radical, [16] and protecting endothelial cells from damage by atherogenic LDL [19].

Although genistein may contribute to antioxidant properties in vivo and in vitro models, the effect of dietary administration of genistein on LDL oxidation remains still unknown. In the present study, we examine the effect of genistein supplementation on genistein contents and lipid oxidation of LDL, serum and liver in hamsters fed vitamin E-deficient diets as an experimental model of oxidative stress.

2. Materials and methods

2.1. Animals and diets

Five-week-old male hamsters were obtained from Animal Center of the National Taiwan University Hospital (Taipei, Taiwan). Each two animals were housed in individual stainless steel cages in a room maintained at 23 to 25°C and with controlled lighting in a 12-hr light/dark cycle (0700-1900). Water and food were supplied *ad libitum*. After one week adaptation to a laboratory rodent diet (#5001, PM Feeds, Inc., St. Louis, MO), thirty six hamsters were divided into three equal groups and switched to a control diet (vitamin E-deficient), control diet containing 50 ppm (mg genistein/kg diet) and 200 ppm genistein (purity: >98%, MD Bio Co., UK)(called as G0, G50 and G200 group, respectively) for 5 weeks. The composition of the control diet (in g per kg diet) was made based on the AIN-76 experimental diet: [20] casein, 200 (ICN Biomedical Inc., Aurora, OH); corn oil 100; α-cellulose, 50; mineral mixture (AIN-76, ICN), 35; vitamin mixture, 10; choline bitartrate, 2; DL-methione, 3 (Sigma) and a mixture of sucrose and corn starch (1:3, in weight) to make a 1-kg diet mixture.

All the diets were prepared using vitamin E-reduced corn oil and vitamin E-free vitamin mixture. The vitamin E-free vitamin mixture was the same as AIN-76 vitamin mixture except that it lacked vitamin E acetate. The vitamin E-reduced corn oil was made from commercial corn oil (Fong Leng, Tainan, Taiwan) using activated charcoal according to the method of Mohri et al. [21] Compared with the original corn oil, the charcoal-treated corn oil had 53% reduction in vitamin E content (62% and 41% reduction of α- and γ-tocopherol concentrations, respectively). The vitamin E-deficient diet contained less than 3.0 mg/kg α-tocopherol. Food intake and body weight were monitored daily and weekly, respectively. All animal experimental procedures were approved by the Animal Committee of Fu Jen University, following *the Guide for the Care and Use of Laboratory Animals*, National Science Council, Taiwan.

At the end of the experiment, animals were food-deprived overnight (14 hr) and killed under carbon dioxide anesthesia by withdrawing blood using a vacuum tube from the heart between 9:00 AM and 11:00 AM Serum and liver handling and storage were performed as previously described [22]. Liver homogenate was prepared with 10 volumes of ice-cold 10 mM phosphate buffer saline (PBS, pH 7.4) containing 1.15% KCl using a Potter-Elvehjem type homogenizer. Portions of the homogenates were measured immediately for thiobarbituric acid-reactive substances (TBARS). The serum of each two hamsters with equal volume (about 1.5 mL) were pooled to one sample for the following analysis.

2.2. LDL isolation

LDL (1.019 to 1.063 g/mL) was isolated by sequential ultracentrifugation as previously described, [23] using a 70.1 Ti rotor in a Beckman model L8-70M ultracentrifuge (Palo Alto, CA). In brief, serum was adjusted for density with KBr to 1.019 and centrifuged at 40000 rpm for 16 h at 4°C. After removing the top layer, the density of the remaining fraction was adjusted to 1.063 and centrifuged at 40000 rpm for 18 h. The top of the LDL fraction was collected, then dialyzed in the dark for 24 h at 4°C against 2 L 10 mM PBS (pH 7.4) to eliminate KBr. The protein content of LDL was determined by the method of Lowry et al. [24] using bovine serum albumin as the standard. The dialyzed LDL was diluted before the start of the oxidation with EDTA-free PBS buffer (pH 7.4) to a final concentration of 0.2 mg protein/mL. The dialyzed and diluted LDL was stored under nitrogen gas in the dark at 4°C for a maximum of 1 week.

2.3. LDL oxidation

For conjugated diene assay, oxidation was initiated by addition of a freshly prepared $CuCl_2$ solution (final concentration 10 μM) and monitored continuously at 234 nm absorbance at 37°C [25], and recorded every 5 min for at least 3 hr on a Jasco V-530 specrophotometer (Jasco International Co., Ltd., Tokyo, Japan) equipped with a six-position automatic cell changer (NCP-508). Lag times were defined as the time interval between initiation and the intercept of the tangent of the slope of the absorbance curve. The propagation rate of LDL oxidation can be calculated from the slope of the absorbance curve during the propagation phase, expressed as nmoles of dienes produced per minute per mg of LDL protein, using the molar coefficient of conjugated dienes at 234 nm of 2.95×10^4. The maximum diene formed was calculated by the absorbance difference between the maximal and minimum amount of dienes formed during LDL oxidation and used the same molar extinction coefficient of conjugated dienes, expressed as μmol/mg LDL protein.

2.4. Measurement of antioxidant status

The serum TAS (total antioxidant status) was measured by enzymatic kits (Randox diagnostics Cat No NX 2332, County Antrim, UK). The thiobarbituric acid-reactive substances (TBARS) of serum, LDL and liver homogenates were assayed as previously described [26].

2.5. Fatty acid analysis

LDL lipids were extracted [27] and fatty acids were analyzed after saponification and methylation as previously described [22].

2.6. Determination of antioxidant substances

Vitamin E contents of liver, serum, LDL and sample corn oil were determined by reverse-phase high-performance liquid chromatography (HPLC) as reported by Hatam and Kayden [28]. One gram of liver was first extracted by the method of Folch et al. [27] for lipids, then saponified in saturated KOH in a water bath (70°C), extracted with hexane, dried under nitrogen gas and resuspended in methanol/ascorbic acid before injection onto the HPLC. For analysis of sample corn oil, 0.5 g oil was directly saponified and extracted as described above. For analysis of serum and LDL, 0.5 mL were extracted with hexane as described above, dried under nitrogen gas and directly injected onto HPLC. A Shimazu LC-10AD HPLC (Tokyo, Japan) was used with a 4 × 125 mm LiChrospher RP-18 column containing 5 μm particles (E Merck, Darmstadt, Germany). Methanol was used as an eluting solvent at a flow rate of 1.0 mL/min. The α-tocopherol was detected at UV 292 nm (SPD-10AV UV-VIS Detector, Shimazu). A pure standard material of α-tocopherol (Sigma Chemical Co., St. Louis, MO, USA) was used to construct a standard curve, and the retention time of a typical α-tocopherol peak was 5.5 ± 0.2 min. Chemlab software (Scientific Information Service Co., Taipei, Taiwan) and a personal computer were used to integrate and run the data. The genistein contents of serum and liver were analyzed according to the method of Franke et al. [29] using flavone as an internal standard. The samples were incubated with β-glucuronidase and sulfatase (Sigma); the genistein was extracted with 80% acetonitrile-0.1% HCl. After centrifugation, the supernatant was dried by nitrogen gas and analyzed by HPLC.

2.7. Cholesterol analysis

Serum and LDL-cholesterol concentrations were determined enzymatically using commercial kits (Merck Co., Darmstadt, Germany).

Table 1
Effect of genistein supplementation on total antioxidant status (TAS) of serum and thiobarbituric acid-reactive substances (TBARS) of serum, LDL and liver in hamsters[1]

	G0	G50	G200
Serum TAS (nM) (n = 6)[2]	1.00 ± 0.12[b]	1.98 ± 0.34[a]	2.07 ± 0.51[a]
Serum TBARS (μM) (n = 6)[2]	16.5 ± 1.6	14.8 ± 2.3	14.1 ± 2.8
Liver TBARS (nmol/g) (n = 12)	194 ± 64[a]	141 ± 44[b]	151 ± 41[b]
LDL TBARS (nmol/mg protein)(n = 6)[2]	53.4 ± 15.9[a]	40.2 ± 12.8[a,b]	29.6 ± 11.7[b]

[1] All values are means ± SD. TAC; total antioxidant status G0; control diet, G50 and G200 were control diet containing 50 and 200 ppm genistein, respectively.

[2] Serum from two hamsters with equal volumes were pooled to one sample.

[a] Values in the same row with different superscripts are significantly different at $P < 0.05$.

[b] Values in the same row with different superscripts are significantly different at $P < 0.05$.

2.8. Statistical analysis

The results were expressed as means ± SD. Data were analyzed by a one-way analysis of variance followed by inspection of all differences between pairs of means by Duncan's multiple-range test. Pearson correlation coefficients were used to measure the relationship between individual parameters of LDL and its oxidation indexes. $P < 0.05$ was regarded to be statistically significant.

3. Results

3.1. Growth performance

Hamsters weighing an initial average of 119 to 120 g, consumed 6.5 to 6.8 g/day of diets during 5-week feeding, and final body weight was 121 to 125 g. Feed efficiency and liver weight of hamsters fed control diet or control diet with different levels of genistein were comparable among the three groups (data not shown).

3.2. Antioxidant status of serum and liver

Serum total antioxidant status (TAS) of hamsters fed genistein-containing diets (G50 and G200) was significantly higher than that of hamsters fed the control diet (G0), whereas there was no significant difference between G50 and G200 groups (Table 1). On the other hand, liver TBARS of hamsters fed genistein-containing diets (G50 and G200) were markedly lower than that of hamsters fed the control diet (G0). However, the TBARS values of serum were comparable among the three groups.

Table 2
Effect of genistein supplementation on the conjugated dienes formation of LDL isolated from hamsters[1,2]

	G0	G50	G200
Lag time (min)	75.7 ± 8.0[b]	77.3 ± 7.5[a,b]	89.0 ± 8.2[a]
Propagation rate (nmol/ min/mg protein)	47.1 ± 7.5[a]	30.1 ± 13.2[b]	26.3 ± 3.8[b]
Maximum diene formed (μmol/mg protein)	1.02 ± 0.17[a]	0.65 ± 0.36[b]	0.61 ± 0.09[b]

[1] All values are means ± SD (n = 6).
[2] Serum from two hamsters with equal volumes were pooled to one sample, then isolated LDL fraction by ultracentrifugation.
[a] Values in the same row not sharing common letters are significantly different at $P < 0.05$.
[b] Values in the same row not sharing common letters are significantly different at $P < 0.05$.

3.3. LDL oxidation

Supplementation of genistein to the diets reduced the TBARS of LDL in hamsters, and significant difference was found between G200 and G0 diets (Table 2). In vitro tests showed that hamsters fed G200 diets exhibited marked retardation in LDL oxidation under copper-induced oxidation. As shown in Fig. 1, the conjugated diene formation of LDL in hamsters was higher in G0 diet than in those of G50 and G200 diets. The lag phase of LDL oxidation in hamsters fed G200 diets was longer than for those fed G0 diets (Table 2). In addition, hamsters fed either G50 or G200 diets had significantly lower conjugated diene formation rates and less conjugated diene formation during the propagation phase of LDL oxidation compared with those fed genistein-free diets.

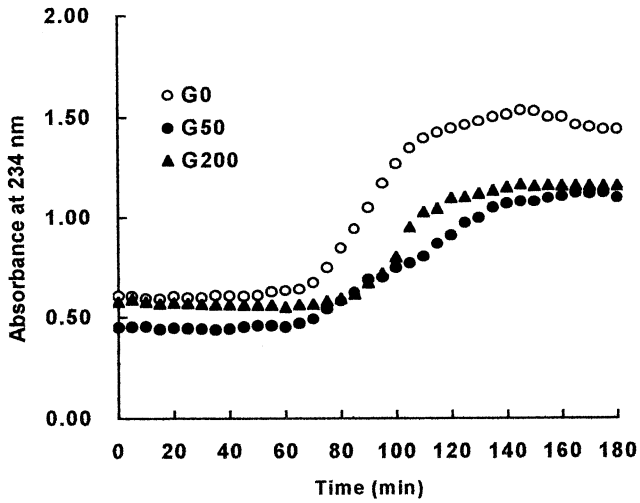

Fig. 1. Effect of genistein supplementation on the conjugated dienes formation of LDL isolated from serum of hamsters. All values are means of 6 samples. ○: G0, control; ●: G50, G0 containing 50 ppm genistein; ▲: G200, G0 containing 200 ppm genistein.

Table 3
Effect of genistein supplementation α-tocopherol and genistein contents of serum, LDL and liver in hamsters[1,2]

	G0	G50	G200
Serum (μM)			
α-Tocopherol	32.3 ± 12.±	29.7 ± 12.±	21.4 ± 11.±
Genistein	ND[c]	2.81 ± 0.1±[b]	6.00 ± 0.3±[a]
LDL (nmol/ mg protein)			
α-Tocopherol	7.91 ± 1.39[b]	9.77 ± 2.33[a,b]	12.1 ± 2.8[a]
Liver (nmol/g)			
α-Tocopherol	11.6 ± 4.3	10.9 ± 5.1	10.9 ± 4.4
Genistein	ND[c]	22.9 ± 5.5[b]	103.3 ± 14.7[a]

[1] All values are means ± SD of six samples for serum and LDL, and 12 samples for liver. ND: no detectable.
[2] Serum from two hamsters with equal volumes were pooled to one sample.
[a] Values in the same row with different superscripts are significantly different at $P < 0.05$.
[b] Values in the same row with different superscripts are significantly different at $P < 0.05$.
[c] Values in the same row with different superscripts are significantly different at $P < 0.05$.

3.4. Genistein and vitamin E status

The contents of genistein in serum and liver of hamsters were markedly increased with the increase of genistein in diets after five weeks of feeding (Table 3). Moreover, the α-tocopherol contents of LDL in hamster fed G200 diets were higher than those fed genistein-free (G0) diets. However the concentrations of α-tocopherol in serum and liver of hamsters were similar among the three groups whether genistein was added to the diets or not.

3.5. Fatty acid compositions of LDL

The relative fatty acid contents of LDL in hamsters fed the three diets were shown in Table 4. Hamsters fed genistein-containing diets (G50 and G200) exhibited less unsaturated fatty acids, especially linoleic acid, and more saturated fatty acids in LDL, and thus had lower P/S ratio when compared with genistein-free diets.

3.6. Serum and LDL cholesterol

Genistein supplementation to hamsters did not affect the concentration of serum and LDL. The average levels of serum total- and LDL-cholesterol in G0, G50 and G200 were 3.89, 3.69 and 3.80, and 1.34, 1.28 and 1.24 mM, respectively.

3.7. Correlations between LDL components and LDL oxidation

Table 5 summarized the correlation coefficients for the relationship between LDL components and LDL oxidation. Significant correlation was found between LDL oxidation

Table 4
Effect of genistein supplementation on fatty acid composition of LDL in hamsters[1,2]

Fatty acids[3]	G0	G50	G200
		(g/100 g fatty acid)	
14:0	2.9 ± 1.±[b]	4.2 ± 2.±[a]	3.2 ± 1.±[b]
16:0	32.1 ± 4.4[b]	37.6 ± 5.1[a]	38.8 ± 4.8[a]
18:0	28.6 ± 6.0[b]	33.4 ± 7.4[b]	37.7 ± 6.4[a]
18:1 (n-9)	21.5 ± 5.4[a]	12.1 ± 4.8[b]	10.8 ± 2.6[b]
18:2 (n-6)	10.4 ± 3.5[a]	6.0 ± 2.±[b]	5.0 ± 3.±[b]
P/S ratio[4]	0.16 ± 0.04[a]	0.08 ± 0.02[b]	0.06 ± 0.02[b]

[1] All values are means ± SD of six samples.
[2] Serum from two hamsters with equal volumes were pooled to one sample, then isolated LDL fraction by ultracentrifugation.
[3] Fatty acids comprising less than 1% were omitted.
[4] P/S ratio: polyunsaturated fatty acids/saturated fatty acid.
[a] Values in the same row not sharing common letters are significantly different at $P < 0.05$.
[b] Values in the same row not sharing common letters are significantly different at $P < 0.05$.

and its components. A strongly negative correlation was observed between lag time of LDL oxidation and PUFA/SFA ratio ($r = -0.75$, $P = 0.004$), and a positive one between LDL oxidation and α-tocopherol contents ($r = 0.73$, $P = 0.004$). Although the α-tocopherol contents were not correlated with TBARS of LDL, a correlation was found between TBARS of LDL and PUFA/SFA ($r = 0.56$, $P = 0.016$).

4. Discussion

Dietary administration of 50 and 200 ppm genistein for 35 days appears to have no effect on hamster growth rate on the basis of the observation that body weights were not significantly different between control and experimental groups. A similar dose of genistein used in mice neither affects animal growth nor causes apparent toxicity [30].

It has been previously assessed that the bioavailabitity of soymilk daidzein is superior to that of genistein in adult females, [31] whereas recently published reports by Izumi et al. [32] show that soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides, and genistein is absorbed more efficiently than daidzein in humans. Therefore, the genistein was used in this study, but fed for only five weeks in our experiment because of the expense of genistein. This relatively short-term genistein supplementation may not achieve a full enhancement of antioxidant capacity in the case of oxidative stress vs. long-term supplementation.

Table 5
Correlation coefficients for relationships between LDL compositions and oxidation indexes in LDL of hamsters fed diets varying in genistein for 5 wk[1]

	Lag time	Propagation rate	TBARS
		r(P)	
PUFA/SFA[2]	−0.75 (0.004)	0.28 (0.332)	0.56 (0.016)
α-Tocopherol	0.73 (0.004)	0.36 (0.200)	−0.44 (0.094)

[1] Data of 18 samples as presented in Table 1-4 were used.
[2] PUFA/SFA: polyunsaturated fatty acids/saturated fatty acid.

A unique feature of this study is that, to our knowledge, this is the first report of plasma genistein in hamsters fed a genistein-containing diet. The increase in genistein levels of serum and liver might indicate that the genistein provided by genistein-containing diets was well incorporated. Goodderham et al. [33] reported a dramatic increase in plasma concentrations of isoflavones after a period of regular soy supplementation (60 g/day for 28day) in normal man, and genistein levels reaching 0.9 μM, the value of one third of G50 diets in our study. It has also been reported that genistein reaches a maximum concentration (12 μM) at 2 h after single oral dosing, and after 10 to 48 h washout, plasma concentration was 1 to 6 μM [34]. Both food diaries and food-frequency questionnaires estimate that plasma isofalvone concentrations positively correlate with estimated dietary soya intake at British women [35].

In this study, genistein supplementation affected α-tocopherol metabolism in hamster LDL, but not in serum and liver in the case of vitamin E deficiency (Table 3). However, TBARS production as a consequence of vitamin E deficiency was reduced by intake of genistein in a dose-dependent manner in LDL. Genistein inhibited the oxidative damage, especially in the liver such that TBARS of both G50 and G200 diets were significantly lower than that of the G0 diet, considering that the antioxidative component of genistein was absorbed and distributed to the liver more than serum. The mechanisms of absorption and metabolism of polyphenols remain obscure, but recent studies focusing on the antioxidant have reported that foodstuffs including sesame seed, [36,37] green tea [38] and grape [39] decrease oxidative stress induced by vitamin E deficiency in rats.

Because the sample volumes of LDL in this experiment were not enough to determine genistein levels of LDL, the effect of dietary administration of genistein on LDL genistein contents and direct correlation with lipid peroxidation of LDL in animals remains unknown, although genistein *per se* exhibits antioxidant properties in vitro in LDL, [16,19] However, our results demonstrate that dietary supplementation of 200 ppm genistein significantly enhances the resistance of LDL oxidation in hamsters. Cai and Wei [30] have shown that dietary administration of 250 ppm genistein to mice markedly enhances the activities of antioxidant enzymes in tissues. Further investigation is required to determine why TBARS in serum does not respond to a high dose of dietary genistein.

Much interest has been focused on the lag phase of LDL, during which there is almost no diene production, and which is considered to be one of major measure of the susceptibility of LDL to oxidation. In general, it is believed that the susceptibility to oxidation for a large part is determined by

its endogenous antioxidant content. Since α-tocopherol (the major form of vitamin E) is a primary antioxidant in LDL, [4, 10] it is conceivable to assume that the length of the lag phase highly correlates with the α-tocopherol content of LDL. However, several studies showed that there is no simple linear correlation between the two [5,40,41]. Although it has been shown that in vivo and in vitro supplementation with α-tocopherol does increase its oxidation resistance, [25,42] it is not possible to predict the length of lag phase from the α-tocopherol content of an individual LDL and thus its oxidation resistance. According to an extensive review of Esterbauer et al., [5] the oxidation resistance (lag time) of an individual LDL depends not only on its vitamin E content but also on other, vitamin E independent factors which could to a great extent determine the lag time and thus the susceptibility of LDL to oxidation. They also suggest several other factors among them the ratio of polyunsaturated fatty (PUFA) content to saturated fatty acid content of LDL, which might contribute to oxidative susceptibility. Here we showed the important role, in this context, of PUFA and SFA content of LDL. A high PUFA to SFA ratio was shown to strongly correlate with the susceptibility of LDL to oxidation.

Among the experimental oxidative stress models, vitamin E-deficient diets have been well investigated in animals. In these animals, there is a deficiency of vitamin E in the cell membranes, the antioxidative status in the lipid bilayers is decreased, and severe membrane damage occurs [43]. Yamashita et al. [36, 37] have shown that the effects of antioxidative lignans in sesame seeds on vitamin E-deficient rats involve maintenance of vitamin E levels in the organs. However, recent reports by Yamagishi et al. [44] show that oral intake of polyphenols extracted from cacao liquor significantly reduce oxidative stress induced by vitamin E deficiency in rats without maintaining vitamin E levels in the plasma and tissues. It has been demonstrated that the length of the lag time in LDL oxidation seems highly correlated with the α-tocopherol content of LDL [25] and heavy oxidation of LDL occurs only when the endogenous vitamin E contained in LDL is exhausted [45]. However, other studies showed that the lag time of LDL is not linearly correlated with the α-tocopherol [4, 46]. Although the vitamin E content of diets in the present study was restricted, the lag time of LDL oxidation might mainly increase with increase in genistein supplementation through retaining vitamin E in LDL indicating that dietary administration of 200 ppm genistein significantly retained α-tocopherol in LDL particles.

Because LDL contains more complex lipids like the cholesterol esters, phospholipids and triacylglycerols, it might produce fatty acid hydroperoxides from the PUFA or cholesterol oxidation products. Although LDL oxidation is strongly dependent upon its PUFA and α-tocopherol content as reviewed by Esterbauer et al., [5] cholesterol oxidation products are probably a reflection of lipoprotein peroxidation and could account for some of the effects of oxidized LDL [47]. Lowering the cholesterol content of LDL could clearly reduce the amount of oxysterols produced and quantitatively reduce its oxidizability potential as Kleinveld et al. [8] (1993) have shown. In the present study, the cholesterol concentrations of LDL and serum in individual groups were comparable. The oxysterol contents of LDL, if any differences exist in individual groups, might be contributed to the status of antioxidant enzymes or substances in the serum or LDL. Therefore, the major products at the earliest stages of LDL peroxidation, in vitro, might result from the PUFA hydroperoxides, and conjugated diene formation. In this situation, the linoleic acid content in LDL might be one of the main factors influencing LDL peroxidation. Our study seemed to consistently show that the peroxidation rate of LDL in vitro correlated with the extent of PUFA contents in LDL, suggesting that hamsters fed a diet containing more genistein would be more effective in preventing serum, liver and LDL oxidation than hamsters fed corresponding genistein-free diets. The ratio of PUFA/SFA in LDL appears to correlate with the rate of lipid peroxidation,

In conclusion, whatever the protective effect of genistein, the present study has demonstrated that orally-ingested gensitein markedly reduces oxidative stress induced by vitamin E deficiency in hamsters. This capacity to lower LDL oxidative stress might be attributed to it maintaining vitamin E levels in LDL. Further studies are necessary to investigate gensitein-enhanced antioxidant capacities and inhibition of lipid peroxidation.

Acknowledgments

The study was supported by a grant, NSC 89 to 2320-B-030 to 009, from the National Science Council of Taiwan.

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