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Supplementation of Naringenin and Its Synthetic Derivative Alters Antioxidant Enzyme Activities of Erythrocyte and Liver in High Cholesterol-Fed Rats

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Abstract—The antioxidative effects of naringenin (**1**) and its synthetic derivative, naringenin 7-*O*-cetyl ether (**2**), were tested. Male rats were fed a 1 g/100 g high-cholesterol diet for 6 weeks with supplements of either **1** or **2** (0.073 mmol/100 g diet) to study the effects on the antioxidant enzyme activities in the erythrocyte and liver. The erythrocyte catalase (CAT) and superoxide dismutase (SOD) activities were significantly higher in the compounds **1** or **2** supplemented groups than in the control group, whereas the hepatic SOD and CAT activities were significantly lower in the compound **2** supplemented group. The compounds **1** and **2** supplements to a high cholesterol diet lowered or tended to lower the plasma TBARS levels, that is, lipid peroxide products, while enhancing the plasma paraoxonase activity. These results indicate that the supplementation of **1** and **2** was effective in improving the antioxidant capacity of the erythrocyte and liver, plus the synthetic functional compound **2** appeared to be as potent as **1** in enhancing the antioxidant defense system.

Introduction

Antioxidants are important aspect of health maintenance based on their modulation of the oxidative process in the body. As such, there has been considerable recent interest in the possibility that an increased dietary intake of antioxidants may protect against cardiovascular disease. This can also be attributed to the knowledge that oxidative events in vivo appear to play a role in the pathogenesis of atherosclerosis.[1] Antioxidants attenuate the atherogenic process in animal models, mainly due to their free radical scavenging capabilities.[2]

Flavonoids are widely recognized as a naturally occurring antioxidant that can inhibit lipid oxidation in a biological membrane. They usually contain one or more aromatic hydroxyl groups and it is this moiety that is responsible for the antioxidant activity of the flavonoid.[3] Among naturally occurring citrus flavonoids, naringin and hesperidin have already been pharmacologically evaluated as potential anticancer agents[4] and anti-atherogenic compounds.[5] In addition, van Acker et al.[3] provided evidence that naringenin, aglycone of naringin, can assume the role of α-tocopherol as a chain-breaking antioxidant in liver microsomal membranes.

Numerous studies in vitro have shown a close relationship between the chemical structure and biologic activity of flavonoids,[6,7] whereby their basic structure can be modified to increase or decrease their biologic activity.[8] The purpose of this study was to examine the influence of naringenin (**1**) and its synthetic derivative, naringenin-7-*O*-cetyl ether (**2**), on the activities of antioxidant enzymes in the erythrocyte and liver, as well as their influence on the level of plasma and hepatic lipid peroxidation in high cholesterol-fed rats (Fig. 1).

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Results

The erythrocyte SOD activity was significantly higher in compound **2** supplemented group than in the control group (Fig. 2A). The supplementation of compounds **1** and **2** significantly elevated the erythrocyte CAT activity compared to the control group (Fig. 2B). No significant differences were observed in the erythrocyte GSH-Px activity and GSH level between the groups (Fig. 2C and D). Accordingly, compounds **1** and **2** supplementation did not seem to affect the erythrocyte GSH-Px activity and GSH level in high cholesterol-fed rats. However, in contrast, the hepatic GSH-reductase activity and GSH level were significantly higher in compounds **1** and **2** supplemented groups compared to the control group (Table 2). In addition, the hepatic SOD and CAT activities were significantly lower in compound 2 supplemented group than in the control group (Table 2). Compounds **1** and **2** supplement lowered the hepatic GSH-Px activity. The glucose-6-phosphate dehydrogenase (G6PD) activity did not differ between

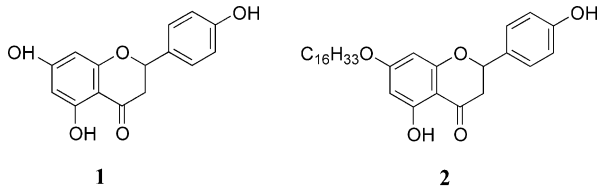

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Figure 1. Structures of Naringenin **1** and naringenin 7-*O*-cetyl ether **2**.

Table 1. Composition of experimental diets (g/100 g diet)

Component	Control	1	2
	$n=10$	$n=10$	$n=10$
Casein	20.0	20.0	20.0
D,L-methionine	0.3	0.3	0.3
Corn starch	15.0	15.0	15.0
Sucrose	49.0	48.98	48.964
Cellulose powder	5.0	5.0	5.0
Corn oil	5.0	5.0	5.0
Mineral mixture[a]	3.5	3.5	3.5
Vitamin mixture[b]	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2
Cholesterol	1.0	1.0	1.0
Compound 1	—	0.02[c]	—
Compound 2	—	—	0.036[d]
Total	100	100	100

[a]AIN-76 mineral mixture (Harlan Teklad Co., USA).
[b]AIN-76 vitamin mixture (Harlan Teklad Co., USA).
[c]Supplemented with 0.073 mmol/100 g diet (M_A 272.26).
[d]Supplemented with 0.073 mmol/100 g diet (M_A 496.8).

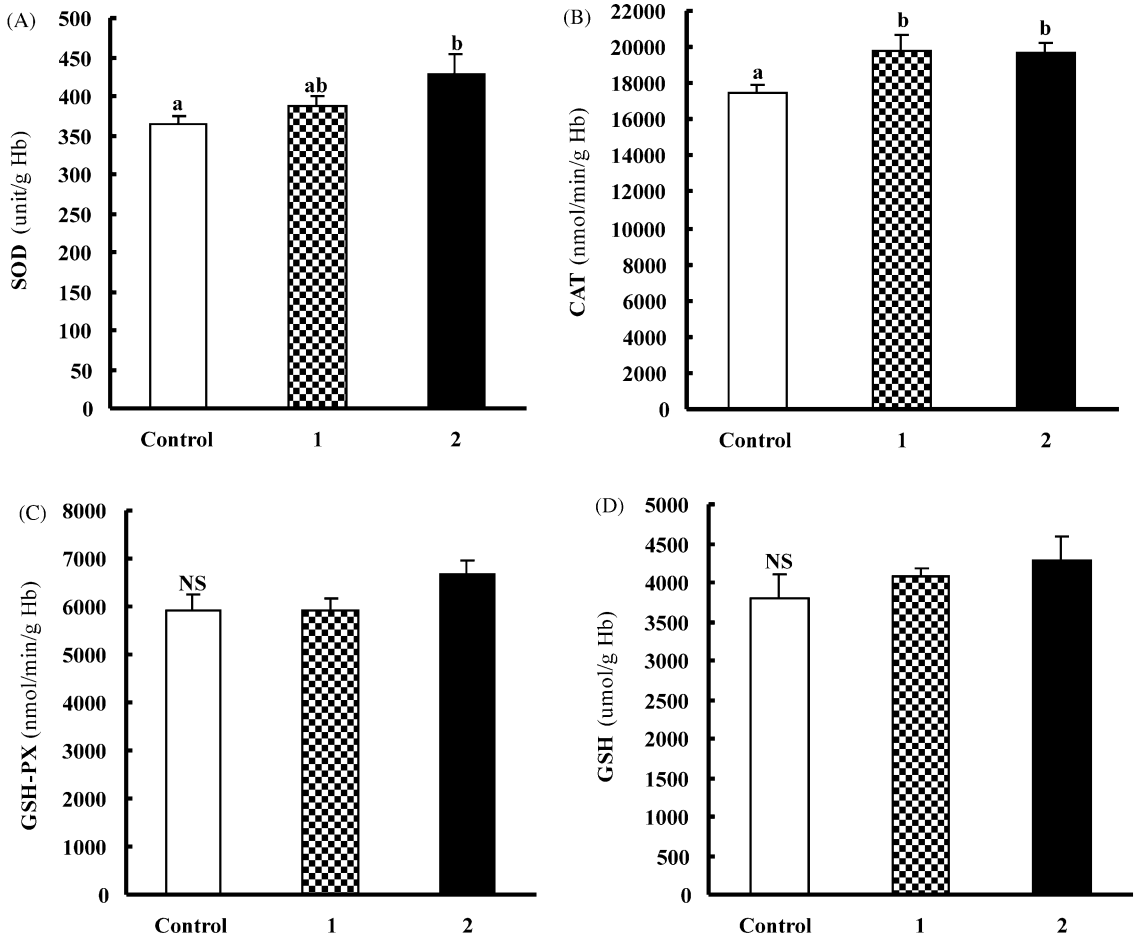

Figure 2. Effects of **1** and **2** supplementation on erythrocyte antioxidant enzyme activities: (A) superoxide dismutase; (B) catalase; (C) glutathione peroxidase; (D) glutathione concentration) in high cholesterol-fed rats. Mean±SE. ^{NS}Not significantly different ($p<0.05$) between groups. The means not sharing a common letter are significantly different between groups ($p<0.05$).

Table 2. Effects of **1** and **2** supplementation on hepatic antioxidant enzyme activities and glutathione levels in high cholesterol-fed rats[a]

	Control	1	2
SOD (unit/mg protein)	85.20±4.55[a]	71.79±3.60[ab]	70.66±4.97[b]
CAT (umol/min/mg protein)	177.18±8.13[a]	160.27±8.91[ab]	138.95±5.56[b]
GSH-Px (nmol/min/mg protein)	2.13±0.36[a]	1.05±0.30[b]	0.93±0.14[b]
GR (nmol/min/mg protein)	23.38±2.50[a]	31.89±2.48[b]	31.31±1.27[b]
G6PD (nmol/min/mg protein)	23.98±0.75[NS]	22.84±1.46	23.04±0.88
GSH (nmol/g of tissue)	10.28±0.34[a]	11.69±0.30[b]	12.03±0.130[b]

NS, Not significantly different ($p<0.05$) between groups. The means not sharing a common letter are significantly different between groups ($p<0.05$).
[a]Mean±SE.

Table 3. Effects of **1** and **2** supplementation on TBARS levels in plasma and hepatic tissue in high cholesterol-fed rats[a]

	Control	1	2
Plasma (nmol/mL)	3.64±0.21[a]	3.49±0.09[ab]	3.14±0.15[b]
Liver (nmol/g)	31.83±0.65[NS]	30.93±1.78	31.12±1.02

NS, Not significantly different ($p<0.05$) between groups. The means not sharing a common letter are significantly different between groups ($p<0.05$).
[a]Mean±SE.

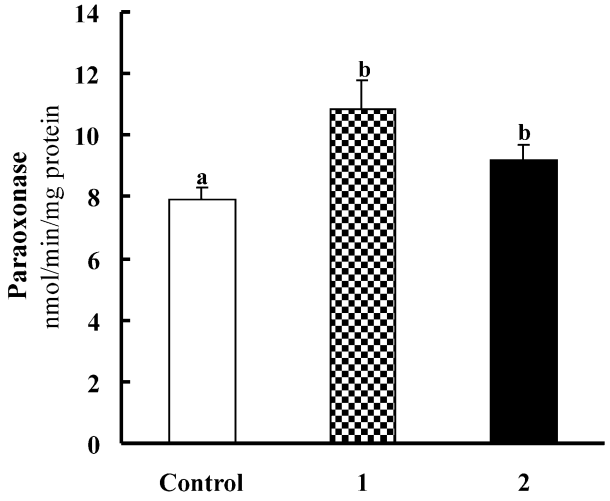

Figure 3. Effects of **1** and **2** supplementation on plasma paraoxonase activity in high cholesterol-fed rats. Mean±SE. The means not sharing a common letter are significantly different between groups ($p<0.05$).

the groups. When compound 2 was supplemented, the plasma TBARS level was significantly lower compared to the control group, whereas the hepatic TBARS level was no different between the groups (Table 3).

The plasma paraoxonase (PON) activity, which is recognized as an antioxidant enzyme that can hydrolyze lipid peroxide in lipoproteins, was significantly higher in the compounds **1** and **2** supplemented groups by 37 and 16% compared to the control group, respectively (Fig. 3). The enzyme biomaker activities related to liver damage, that is, serum alanine aminotransferase and aspartate aminotransferase, were not significantly different between the groups (data not shown).

Discussion

Flavonoids are diphenylpropanes that commonly occur in plants and are a frequent component of the human diet with various biological activities.[9] In addition, flavonoids have been shown to be very potent antioxidants, because they have a high scavenging activity.[10,11] Recently, the role of 1 and the related citrus falvonoid, hesperetin, in the prevention and treatment of disease has received considerable attention.[12] A previous study by the current authors demonstrated that the supplementation compound **1** (0.1% wt/wt) results in a significant reduction in the plasma and hepatic total cholesterol concentrations in hyperlipidemic rats.[13] The present study examined the effect of a low dose of **1** and **2** (0.073 mmol/100 g diet) on the antioxidant defense system in high cholesterol-fed rats. As a result, certain differences were identified in the erythrocyte and hepatic antioxidant enzyme activities in the compounds **1** and **2** supplemented rats compared to the control group. The structural difference between the two compounds is the presence of a C_{16} ether group instead of a hydroxyl group in naringenin structure.

Erythrocyte and hepatic tissue contain enzymes that contribute to the antioxidant defense mechanism. Oxidative stress is one of the causative factors that link hypercholesterolemia with the pathogenesis of atherosclerosis. This stress results from an imbalance between the production of free radicals and the effectiveness of the antioxidant defense system.[14] Erythrocyte is especially susceptible to oxidative damage resulting from a high concentration of oxygen and hemoglobin, and the latter would appear to be a particularly powerful promoter of oxidative processes.[15] Disorders in erythrocyte antioxidant parameters have also been reported in subjects with cardiovascular disease.[16]

In the present study, the erythrocyte CAT activity was elevated by both **1** and **2** supplements, yet the effect of **2** on elevating the SOD activity was more potent than that of naringenin. The increased SOD and CAT activities in the erythrocyte may have been due to the activation of these enzymes by **1** and **2**, thereby resulting in a lower superoxide anion level. CAT is one of the most important means by which the erythrocyte disposes of H_2O_2, plus CAT protects the erythrocyte from reactive oxygen species (ROS).[17] As shown in the results, **1** and its derivative supplement significantly increased the erythrocyte CAT activity, while compound **2** supplement significantly lowered the plasma TBARS level compared to the control group. If the erythrocyte CAT activity is

not sufficiently enhanced to metabolize H_2O_2, this can lead to an increased TBARS level in the plasma. The compound **1** supplement also tended to lower the plasma TBARS level. However, the GSH-Px activity and GSH content in the erythrocyte were unaffected by **1** or **2** supplementation. Therefore, in the current study, the higher CAT and/or SOD activity appeared to contribute to a reduced ROS level in the erythrocyte of the compounds **1** or **2** supplemented groups.

PON, which is exclusively bound to high-density lipoproteins, is recognized as an antioxidant enzyme because it hydrolyzes lipid peroxides.[18] In the present study, the plasma PON activity was significantly higher in compounds **1** and **2** supplemented groups compared to the control group. Since PON is inactivated by oxidized low- density lipoproteins and preserved by antioxidants,[19] it is feasible that the high cholesterol diet caused an increase in the lipid peroxide level resulting in the inactivation of PON.[20] Alternatively, PON can hydrolyze a specific lipid peroxide or serve as a target for peroxides, and the latter effect can also result in PON autoinactivation.[21] In addition, as PON activity is inversely related to the development of atherosclerosis, PON would appear to be an important marker for both cardiovascular disease and the oxidative status.[22]

The present study indicated that **1** and **2** supplements to a high cholesterol diet lowered or tended to lower the plasma TBARS level, while enhancing the plasma PON activity. It would appear that the compounds **1** and **2** supplements mediated to enhance this enzyme activity, although the exact mechanism is still unclear. There was also an inverse association observed between the plasma TBARS level and plasma PON activity. In addition, PON has been shown to use both lipid peroxides and H_2O_2 as substrates for its enzyme reaction.[22] H_2O_2 is a major ROS produced by the arterial wall cells during atherogenesis, and is converted into a more potent ROS under oxidative stress leading to LDL oxidation.[23,24] The ability of PON to hydrolyze H_2O_2 may thus play an important role in eliminating potent oxidants involved in atherosclerosis.

In contrast to the erythrocyte, the hepatic SOD and CAT activities were significantly lower in the compound **2** supplemented group than in the control group. The supplementation of **1** and **2** to high cholesterol fed-rats lowered the activity of hepatic GSH-Px, which is a key enzyme in the GSH system, whereas it increased both the hepatic GR activity and GSH level. GSH plays an unique role in the cellular defense system against toxic chemicals of endogenous and exogenous origin, as such the depletion of GSH increases the vulnerability to free radical damage.[25,26] Accordingly, **1** and **2** appeared to prevent the lowering of the hepatic GSH content and GR activity. The hepatic TBARS content did not differ between the groups. When comparing the antioxidative parameters in the erythrocyte and liver between the groups, the results suggested that the overall antioxidative system of the liver was more stabilized than that of the erythrocyte.

Conclusion

The results of the current study indicate that **1** and **2** were able to reduce the oxidative stress intensity in high cholesterol-fed rats. The erythrocyte also appeared to be the first line of defense for an antioxidative reaction. The erythrocyte antioxidant enzymes responded continuously to the oxidative stress induced by high cholesterol feeding, whereas the hepatic antioxidant system was stabilized or even enhanced by **1** or **2**. Of the two compounds tested, **2** appeared to be slightly more effective than *1* in enhancing the antioxidant capacity of the erythrocyte and liver in the high cholesterol-fed rats. Whether this metabolic difference resulted from changes in the absorption process or another metabolic process due to the chemical structure remains to be elucidated.

Experimental

Synthesis of naringenin 7-*O*-cetyl ether (2)

Ten grams (36.7 mmol) of naringenin was dissolved in a mixture of 100 mL of acetone and 100 mL of dimethylformamide. Hexadecanyl bromide [13.5 mL (44.2 mmol)] and 4.70 g (44.3 mmol) of sodium carbonate were added to the mixture and stirred in a water bath at 80 °C for 12 h. The resulting solution was cooled, then 100 mL of water and 800 mL of ethyl acetate were added and the mixture extracted with ethyl acetate. The extract was then washed with water and concentrated under reduced pressure. The solid formed was filtered using a glass filter and dried under reduced pressure to give 10.1 g of compound **2**. In addition, the residue was concentrated and subjected to silica gel column chromatography [45×150 mm, 70–230 mesh, eluent: hexane/ethyl acetate (8:2)] to obtain an additional 3 g of naringenin 7-*O*-cetyl ether as a pale yellow solid (yield: 72%).

Spectral data of naringenin 7-*O*-cetyl ether (2)

The structure of **2** was confirmed by NMR spectra. The ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury 400 NMR spectrometer using the solvent peak as reference (^1H 7.25 ppm and ^{13}C 77.0 ppm of $CDCl_3$, respectively). Low- and high- resolution- FABMS were recorded on a high-resolution Tandem Mass (JMS-HX 110/110A, Jeol Ltd.) spectrometer. The structure was confirmed as shown in Figure 1. Compound **2** (naringenin 7-*O*-cetyl ether): white solid; mp 114–117 °C; ^1H NMR ($CDCl_3$) δ 12.0 (s,–OH), 7.32 (d, J=8.4 Hz, 2H), 6.87 (d, J=8.4 Hz, 2H), 6.04 (d, J=2.0 Hz, 1H), 6.02 (d, J=2.0 Hz, 1H), 5.33 (dd, J=13.2, 2.8 Hz, 1H), 5.09 (s,–OH), 3.95 (t, J=6.8 Hz, 2H), 3.07 (dd, J=17.2, 13.2 Hz, 1H), 2.77 (dd, J=17.2, 2.8 Hz, 1H), 1.75 (quin, J=6.8 Hz, 2H), 1.44–1.36 (m, 2H), 1.34–1.22 (m, 24H), 0.87 (t, J=6.8 Hz, 3H) ppm; ^{13}C NMR ($CDCl_3$) δ 195.8, 167.6, 164.0, 162.8, 156.0, 130.6, 127.9 (2C), 115.6 (2C), 103.0, 95.6, 94.6, 78.9, 68.6, 43.2, 32.0, 29.75–29.69 [six carbons,–(CH2)6–], 29.62, 29.57, 29.4, 29.3, 28.9, 25.9, 22.7, 14.2 ppm; 1D NOESY NOE contacts were observed between H (6.04 and 6.02 ppm) and H

(3.95 ppm); FABMS m/z 497 [M + H]+ (100), 495 (44), 153 (85), 147 (39); HRFABMS m/z found for 497.3267 (calcd for $C_{31}H_{45}O_5$ 497.3267).

Animals and diets

Thirty male Sprague–Dawley rats weighing between 75 ± 5 g were purchased from the Bio Genomics, Inc. (Seoul, Korea). The animals were individually housed in stainless steel cages in a room with controlled temperature (24 °C) and lighting (alternating 12-h periods of light and dark). All the rats were fed a pelletized commercial chow diet for 10 days after arrival. Next, the rats were randomly divided into three groups ($n = 10$) and fed a high-cholesterol diet (1%, wt/wt) for 6 weeks, with two of the groups receiving a compound **1** (Sigma Chemical Co.) and **2** supplement, respectively. The structures of the experimental materials are shown in Figure 1. The amount of compound **1** mixed with the diet was 0.02% (wt/wt) **1**, which was equivalent to 0.073 mmol/100 g diet. Compound **2** was added to the diet based on the equivalent mmol to **1**, that is 0.073 mmol **2**/100 g diet or 0.036 g 2/100 g diet. The two flavonoids were expected to undergo similar metabolism after ingestion. The composition of the experimental diet, as shown in Table 1, was based on the AIN-76 semi-synthetic diet.[27,28] The animals were given food and distilled water freely during the experimental period.

Preparation of samples

Blood samples were collected from the inferior vena cava into heparin-coated tubes. After centrifugation at 1000*g* for 15 min at 4 °C, the plasma and buffy coat were carefully removed. The separated cells were then washed three times by resuspending in a 0.9% NaCl solution and repeating the centrifugation. The washed cells were lysed in an equal volume of water and mixed thoroughly. The hemoglobin concentration was estimated in an aliquot of the hemolysate, using a commercial assay kit (No. 525-A, Sigma, Chemical Co.). An appropriate dilution of the hemolysate was then prepared from the erythrocyte suspension by the addition of distilled water to estimate the catalase (CAT) and glutathione peroxidase (GSH-Px) activities and glutathione (GSH) levels.

In addition, to remove the hemoglobin by precipitation with chloroform ethanol,[29] 0.4 mL of an ethanol choloroform (3:5, v/v) mixture was added to an aliquiot (1 mL) of the hemolysate cooled in ice. This mixture was stirred constantly for 15 min and then diluted with 0.2 mL of water. After centrifugation for 10 min at 1600 *g*, the pale yellow supernatant was separated from the protein precipitate and used to assay the superoxide dismutase (SOD).

The preparation of the enzyme source fraction in the hepatic tissue was as follows. One gram of hepatic tissue was homogenized in a 5-fold weight of a 0.25 M sucrose buffer, centrifuged at 600*g* for 10 min to discard any cell debris, then the supernatant was centrifuged at 10,000*g* for 20 min to remove the mitochondria pellet. Finally, the supernatant was further ultracentrifuged at 105,000*g* for 60 min to obtain the cytosol supernatant. The amount of protein in the mitochondrial and cytosolic fractions was measured according to the method of Bradford[30] using bovine serum albumin as the standard.

Paraoxonase activity

The plasma paraoxonase (PON) activity was measured using the method developed by Mackness et al.[31] with a slight modification. The hydrolysis rate of paraoxon was assessed by measuring the liberation of *p*-nitrophenol at 405 nm at 25 °C for 90 s. The basal reaction mixture included 5.5 mM paraoxon (*O*,*O*-diethyl-*O*-*p*-nitrophenylphosphate, Sigma Chemical Co.) and 2 mM $CaCl_2$ in a 0.1 M Tris–HCl buffer (pH 8.0). A molar extinction coefficient of 17,000 M^{-1} cm^{-1} was used to determine the activity, which was expressed as *p*-nitrophenol nmol produced/min/mg protein.

Antioxidant enzyme activities

The SOD activitiy was spectrophotometrically measured using a modified version of the method developed by Marklund and Marklund.[32] Briefly, SOD was detected on the basis of its ability to inhibit superoxide-mediated reduction. One unit was determined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50%. The activity was expressed as unit/g Hb and that of the tissue as unit/mg protein. The CAT activity was measured using Aebi's[33] method with a slight modification, in which the disappearance of hydrogen peroxide was monitored spectrophotometrically at 240 nm for 5 min. A molar extinction coefficient of 0.041 mM^{-1} cm^{-1} was used to determine the CAT activity. The activity was defined as the decrease in H_2O_2 nmol/min/g Hb and that of the tissue as μmol/min/mg protein.

Glutathione and related enzyme activities

The GSH-Px activity was measured using Paglia and Valentine's[34] method with a slight modification. The reaction mixture contained 1 mM glutathione, 0.2 mM NADPH, and 0.24 units of glutathione reductase in a 0.1 M Tris–HCl (pH 7.2) buffer. The reaction was initiated by adding 0.25 mM H_2O_2 and the absorbance was measured at 340 nm for 5 min. A molar extinction coefficient of 6.22 mM^{-1} cm^{-1} was used to determine the activity. The activity was expressed as the oxidized NADPH nmol/min/g Hb and that of the tissue as nmol/min/mg protein. The hepatic glutathione reductase (GR) activity was determined using the method of Pinto and Bartley[35] by monitoring the oxidation of NADPH at 340 nm. The reaction mixture contained 1 mM EDTA and 1 mM GSSG in a 0.1 M potassium phosphate buffer (pH 7.4). The activity was expressed as the oxidized NADPH nmol/min/mg protein. The hepatic glucose-6-phosphate dehydrogenase (G6PD) activity was determined using the method of Pitkanen et al.[36] The reaction mixture contained 55 mM Tris–HCl (pH 7.8), a 3.3 mM $MgCl_2$ buffer, and 6 mM G6P. The activity was expressed as the reduced NADPH nmol/min/mg

protein. The total GSH content was determined using the method of Ellman[37] based on its reaction with 5,5′-dithiobis-(2-nitrobenzoic acid) to yield the yellow chromophore, 5-thio-2-nitrobenzoic acid at 412 nm.

Lipid peroxidation

As a marker of the lipid peroxidation production, the plasma or hepatic thiobarbituric acid reactive substances (TBARS) concentrations were measured using the method of Ohkawa et al.[38] Two hundred microliters of the plasma and hepatic homogenate (20%, w/v) was mixed with 200 μL of 8.1% (w/v) sodium dodecyl sulfate, 1.5 mL of 20% (w/v) acetic acid (pH 3.5), and 1.5 mL of 0.8% (w/v) TBA. The reaction mixture was heated at 95 °C for 60 min. After cooling, the hepatic mixture was added to 1.0 mL of distilled H_2O and 5.0 mL of a butanol:pyridine (15:1) solution. The reaction mixture was then centrifuged at 800*g* for 15 min and the resulting colored layer was measured at 532 nm using 1,1,3,3- tetraethoxypropane (Sigma Chemical Co.) as the standard.

Statistical analysis

All data is presented as the mean ± SE. The data was evaluated by one-way ANOVA using an SPSS program, and the differences between the means assessed using Duncan's multiple-range test. Statistical significance was considered at $p < 0.05$.

Acknowledgements

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References and Notes

1. Morton, L. W. R.; Caccta, A. A.; Puddey, I. B.; Croft, K. D. *Clin. Exp. Pharmacol. Physiol.* **2000**, *27*, 152.
2. Paul, A.; Calleja, L.; Joven, J.; Viella, E.; Ferré, N.; Camps, J.; Girona, J.; Osada, J. *Int. J. Vit. Nutr. Res.* **2001**, *71*, 45.
3. van Acker, F. A. A.; Schouten, O.; Haenen, G. R. M. M.; van der Vijgh, W. J. F.; Bast, A. *FEBS Lett.* **2000**, *473*, 145.
4. Guthrie, N.; Carroll, K. K. Inhibition of Mammary Cancer by Citrus Bioflavonoids. In *Flavonoids in the Living System*; Manthey, J. A., Buslig, B. S., Eds.; Plenum: New York, 1998; p 227.
5. Samman, S.; Wall, P. M. L.; Cook, N. C. Flavonoids and Coronary Heart Disease: Dietary Perspectives; In *Flavonoids in the Living System*; Manthey, J. A., Buslig, B. S., Eds.; Plenum: New York, 1999; p 469.
6. Krol, W.; Czuba, Z.; Scheller, S.; Paradowski, Z.; Shani, J. *J. Ethnopamarcol.* **1994**, *41*, 121.
7. Ferriola, P. C.; Cody, V.; Middleton, E., Jr. *Biochem. Pharmacol.* **1989**, *38*, 1617.
8. Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. *Lancet* **1993**, *342*, 1007.
9. Peterson, J.; Dwyer, J. *Nutr. Res.* **1998**, 18, 1995.
10. Bors, W.; Heller, W.; Michel, C.; Saran, M. *Methods Enzymol.* **1990**, *186*, 343.
11. Cao, G.; Sofic, E.; Prior, R. L. *Free Radic. Biol. Med.* **1997**, *22*, 749.
12. Montanari, A.; Chen, J.; Widmer, W. Citrus Flavonoids: A Review of Past Biological Activity Against Disease. In *Flavonoids in the Living System*; Manthey, J. A., Buslig, B. S., Eds.; Plenum: New York, 1998; pp 103–113.
13. Lee, S. H.; Park, Y. B.; Bae, K. H.; Bok, S. H.; Kwon, Y. K.; Lee, E. S.; Choi, M. S. *Ann. Nutr. Metab.* **1999**, *43*, 173.
14. Halliwell, B. *Pathol. Biol.* **1996**, *44*, 6.
15. Clemens, M. R.; Waller, H. D. *Chem. Phys. Lipids* **1987**, *45*, 251.
16. Akkus, I.; Saglam, N. I.; Caglayam, O.; Vural, H.; Kalak, S.; Saglam, M. *Clin. Chimi. Acta.* **1996**, *244*, 173.
17. Agar, N. S.; Sadrsadeh, S. M. H.; Hallaway, P. E.; Eaton, J. W. *J. Clin. Invest.* **1986**, *77*, 319.
18. Mackness, M. I.; Mackness, B.; Durrington, P. N.; Connelly, P. W.; Hegele, R. A. *Curr. Opin. Lipidol.* **1996**, *7*, 69.
19. Aviram, M.; Rosenblat, M.; Billecke, S.; Erogul, J.; Sorenson, R.; Bisgaier, C. L.; Newton, R. S.; La Du, B. N. *Free Radic. Biol. Med.* **1999**, *26*, 892.
20. Mackness, M.; Bouiller, A.; Hennuyer, N.; Mackness, B.; Hall, M.; Tailleux, A.; Duriez, P.; Delfly, B.; Durrigton, P.; Fruchart, J. C.; Duverger, N.; Caollaud, J. C.; Castro, G. *Biochem. Biophys. Res. Commun.* **2000**, *269*, 232.
21. Aviran, M.; Rosenblat, M.; Bisgaier, C. L.; Newton, R. S.; Primo-Parmo, S. L.; La Du, B. N. *J. Clin. Invest.* **1998**, *101*, 1581.
22. Aviran, M. *Molec. Med. Today* **1999**, 5, 381.
23. Hazen, S. L.; Hau, F. F.; Duffin, K.; Heinecke, J. W. *J. Biol. Chem.* **1996**, *271*, 23080.
24. Wilkins, G. M.; Leae, D. S. *Biochim. Biophys. Acta.* **1994**, *1215*, 250.
25. Reed, D. J. *Ann. Rev. Pharmacol. Toxicol.* **1990**, *30*, 603.
26. Shan, X. O.; Aw, T. Y.; Jone, D. P. *Pharmacol. Ther.* **1990**, *47*, 61.
27. American Institute of Nutrition *J. Nutr.* **1977**, *107*, 1340.
28. American Institute of Nutrition *J. Nutr.* **1980**, *110*, 1717.
29. McCord, J. M.; Fridovich, I. J. *Biol. Chem.* **1969**, *244*, 6049.
30. Bradford, M. M. *Ann. Biochem.* **1976**, *72*, 248.
31. Mackness, M. I.; Harty, D.; Bhatnagar, D.; Winocour, P. H.; Arrol, S.; Ishola, M.; Durrington, P. N. *Atherosclerosis* **1991**, *86*, 193.
32. Marklund, S.; Marklund, G. *Eur. J. Biochem.* **1974**, *47*, 469.
33. Aebi, H. (1974) Catalase. In *Method of Enzymatic Analysis* Academic: New York, 1974; Vol. 2, p 673.
34. Paglia, E. D.; Valentine, W. N. *J. Lab. Clin. Med.* **1967**, *70*, 158.
35. Pinto, R. E.; Bartley, W. *Biochem. J.* **1969**, *112*, 109.
36. Pitkanen, E.; Pitkanen, O.; Uotil, L. *Eur. J. Clin. Chem. Clin. Biochem.* **1997**, *35*, 761.
37. Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, 82, 70.
38. Ohkawa, H.; Ohishi, N.; Yake, K. *Ann. Biochem.* **1979**, *95*, 351.