

Effects of Different Grape Formulations on Antioxidative Capacity, Lipid Peroxidation and Oxidative DNA Damage in Aged Rats

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Summary In this study, the freeze-dried powders from whole grapes, pomace and juice of Campbell Early (*Vitis labruscana* Bailey) were prepared to determine the amount of total flavonoids, vitamins A, C, and E, and dietary fiber. Effects of whole grape, pomace, or juice intakes on their antioxidative capacity and DNA damage were investigated in Sprague-Dawley male rats. A total of 120 rats at 13 mo old and weighing 549 ± 4 g were blocked into 8 groups according to body weight and raised for 3, 5, or 7 mo with diets containing 2% (w/w) dry powder of three different parts of grapes and 0.02% (w/w) CdCl₂. The contents of flavonoids, antioxidant vitamins A and E, and dietary fiber in freeze-dried powder were the highest in grape pomace, but the vitamin C contents were similar among the three powders. In all the 16, 18, and 20-mo-old animals, plasma and liver thiobarbituric acid reactive substances levels of grape-ingesting groups were lower than those of the controls and that of the grape pomace group was the lowest among the groups. Cd administration increased plasma and liver thiobarbituric acid reactive substances levels remarkably; however, Cd+grape groups were lower than the Cd-control group. Red blood cell superoxide dismutase activity of 18- and 20-mo-old rats was higher than that of 16-mo-olds, showing an age-related increase; however, red blood cell catalase and glutathione peroxidase activities decreased with age. Grape diets promoted superoxide dismutase, catalase, glutathione peroxidase activities, and the grape pomace increased the activities most significantly among three different parts of the grape. Cd decreased superoxide dismutase, catalase, glutathione peroxidase activities; however Cd+grape groups showed similar activities to the non-Cd control group. Liver superoxide dismutase activity was decreased with age but catalase activity of 18-mo-old rats was higher than those of 16- and 20-mo-old groups, and glutathione peroxidase activities of 16- and 18-mo-old groups were similar but that of 20-mo-old groups decreased markedly. Grape intake increased these three antioxidative enzyme activities while Cd administration decreased catalase and glutathione peroxidase activities except superoxide dismutase activity. The concentration in the kidney of 8-hydroxy-2'-deoxyguanosine in the 18- and 20-mo-old rats was higher than that in the 16-mo-old groups, and grape intake showed a protecting effect on DNA from age-related or Cd-induced oxidative damage. In conclusion, grape intakes, especially grape pomace with the highest content of flavonoids, β -carotene, tocopherols and dietary fiber among the three parts, showed the prominent antioxidative capacity of inhibiting age-related or Cd-induced increase of lipid peroxidation and DNA damage effectively, promoting liver and red blood cell antioxidant enzyme activities.

Key Words grape, cadmium (Cd), aging, DNA damage, antioxidative capacity

The polyphenolic content of grapes is in the range of 50–490 mg per 100 g of fresh matter, which is a higher value than that of other fruits such as grapefruit, oranges, peaches, pears and so on (1). Naturally red wine has much higher values of polyphenolic compounds than white wine (1, 2), because the breakdown of grape solids, including the skin and seeds following the crushing of the grapes, facilitates the liberation of phenolic compounds in red wine. Red wines and grape

juices contain a wide variety of the same biologically active flavonoids, primarily of the flavonol type. Keevil et al. (3) reported that drinking grape juice for 1 wk reduced the whole blood platelet aggregation but orange and grapefruit juice did not. Grape juice had almost three times the total polyphenolic concentration of orange or grapefruit juice and there were large differences in the classes of flavonoids present in the juices. Grape juice contained flavonols, anthocyanidins, and proanthocyanidins, and there was no evidence that these polyphenolic compounds were present in the orange and grapefruit juice. Resveratrol and quercetin,

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which are abundant in grape skins and seeds, inhibited the platelet aggregation and the oxidation of LDL (low-density lipoprotein); therefore, they may provide cardiovascular protection (4).

Cd that is accumulated in the body stimulates the generation of free radicals, acts itself as an electrophile, impairs various organs, especially the kidneys, and moreover accelerates aging (5). Cd also strongly antagonizes microminerals such as Fe, Cu, Zn, and Se in their absorption and bioavailability and is thought to affect the activity and biosynthesis of antioxidative enzymes that contain microminerals. In a study of Cd-induced oxidative stress and antioxidative agents, when rats were administered vitamin E, Cd-induced lipid peroxides were decreased significantly and the activity of free radical scavenging enzymes was increased (6).

If supplementing dietary antioxidants diminishes molecular DNA damage, it will be expected to have a preventive effect on cancer and degenerative diseases of aging. Indeed, in a controlled smoking cessation study (7, 8) the decrease in 8-hydroxy-2'-deoxyguanosine (8OHdG) excretion was a mirror of the increase in plasma vitamin C concentration; however, so far intervention studies have not provided support for the notion of a beneficial effect of antioxidants. In smokers, daily administration of β -carotene, vitamin C, vitamin E or coenzyme Q had no effect on the excretion rate of 8OHdG (9, 10). Upon depletion of ascorbic acid in healthy men, the level of 8OHdG in sperm DNA was increased; upon the replenishment of ascorbic acid, the 8OHdG level in sperm was returned to the initial values (11, 12).

Many Korean scientists have been interested in and studied constantly the effects of Korean native plants or herbs in terms of their antioxidative capacity, lipid metabolism, and Cd detoxification (6, 13); however, experimental animals in most studies were raised only for a month and the data related to DNA damage and aging were not abundant. Moreover, in the studies of pharmacologically active substances such as ginseng (*Panax Ginseng* C.A. Meyer) and *Opuntia ficus-indica* fruit powder (14), senescence accelerated mice (SAM) were used as an aging model. Because the life span of SAM is shorter than that of normal mice, SAM have been used frequently in aging-related experiments, but it is not thought to be normal that senescence is accelerated genetically.

Therefore, the freeze-dried powders from whole grape, pomace, and the juice of Campbell were prepared to determine the amount of flavonoids, antioxidative vitamins A, C, and E, and dietary fiber. We also performed a long-term nutritional intervention study to see the effects of different grape formulations on antioxidative capacity, lipid peroxidation, and DNA damage in the Cd-administered and aged rats.

MATERIALS AND METHODS

Animal husbandry and feeding regimens. A total of 120 Sprague-Dawley male rats (inbred, originating from Samtaco Bio-Korea) of 13 mo of age weighing

548.8 ± 4.3 g were blocked into 8 groups according to body weight and raised for 3, 5, or 7 mo with diets containing 2% (w/w) dry powders of three different parts of the grape and 0.02% (w/w) CdCl₂. Animals were maintained under a barrier system which regulated temperature (22–24°C), humidity (45±5%), and a lighting cycle (light on 07:00–19:00 h) with free access to experimental diets and deionized water. All experiments were performed in compliance with the guidelines of the Guide for the Care and Use of Laboratory Animals, routinely used in our laboratory. At the end of each period, animals were deprived of feed overnight and harvested after anesthetization with ether, and the organs and tissues including the kidneys, liver, spleen, and epididymal fat pads were removed and frozen until analysis.

Eight experimental diets were fed to the experimental animal groups, varying in composition with respect to the part of grape powder and Cd administration. The grapes used in the experimental diets were Campbell Early (*Vitis labruscana* Bailey, Young-dong, Kyoung-Buk, Korea) which had been the most purchased and consumed in Korea in August 2000. Before freeze-drying, all grapes were washed well with tap water 3 times, crushed completely with a grinder (a single phase motor, Myoung-Jin Co.), and sieved with a 40-mesh sieve in order to partition the crushed whole grapes into juice and pomace parts. The partitioned grapes as whole grape, juice and pomace were individually freeze-dried, pulverized with a fitz mil (The Fitz Patrick Co., No. DASO6), sieved with a 40-mesh sieve, and added to the experimental diets. Cd was added as CdCl₂ to experimental diets in 0.02% (200 ppm, 122.5 ppm of Cd) of total diet weight.

The composition of the diet (15) which was used for experimentation was the same as shown in Table 1. The diet intake was regularly measured three times a week, and the body weight of the animals was measured twice a month. The whole blood was put into a polystyrene tube which contained heparin (25,000 IU/5 ML), centrifuged at 2,800 rpm at 4°C, the lower layer red blood cell (RBC) and separated from the plasma of the upper layer, and kept in a deep-freezer at -70°C in order to measure lipid peroxides and antioxidative enzyme activity. The RBC layer was mixed with ice cold saline of equivalent volume, centrifuged for 10 min at 2,800 rpm and 4°C repeatedly, and finally mixed with a 0.9% NaCl solution of equivalent volume for 50% hematocrit suspension. Liver and kidney weights were measured on ice baths immediately after rinsing with ice cold saline and removing the moisture, and kept in a deep-freezer at -70°C until analysis of peroxide contents, antioxidant enzyme activity and 8OHdG. In addition, the spleen and epididymal fat pad (EFP) weights were also measured.

Assay of antioxidative substance and dietary fiber contents in the grape powders. Total flavonoid content in grape powders was measured by using a spectrophotometer (420 nm) according to the method of Kang (16). Beta-carotene was analyzed by using a High Per-

Table 1. Composition of experimental diets.

(g/kg diet)

Ingredients	Groups ¹							
	C(-)	W(-)	P(-)	J(-)	C(+)	W(+)	P(+)	J(+)
Cornstarch	700.7	680.7	680.7	680.7	700.5	680.5	680.5	680.5
Casein	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Corn oil ²	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Soybean oil ³	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Mineral mix ⁴	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0
Vitamin mix ⁵	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Choline chloride	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
L-Cystine	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Grape powder	—	20	20	20	—	20	20	20
Cadmium chloride	—	—	—	—	0.2	0.2	0.2	0.2
Total	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

¹ C(-): Control diet (Grape-free diet), W(-): Experimental diet containing 2% whole grape powder, P(-): Experimental diet containing 2% grape pomace powder, J(-): Experimental diet containing 2% grape juice powder, C(+): Control diet containing 0.02% cadmium chloride (CdCl₂) (Grape-free diet), W(+): Experimental diet containing 2% whole grape powder and 0.02% CdCl₂, P(+): Experimental diet containing 2% grape pomace powder and 0.02% CdCl₂, J(+): Experimental diet containing 2% grape juice powder and 0.02% CdCl₂.

² Fatty acids per 100 g total fatty acids of corn oil (%): 16:0 10.36, 18:0 1.80, 18:1 26.59, 18:2 60.43, 18:3 0.82.

³ Fatty acids per 100 g total fatty acids of soybean oil (%): 16:0 10.45, 18:0 4.11, 18:1 23.17, 18:2 55.18, 18:3 7.08.

⁴ Mineral mix (AIN-93M-MX) (g/kg mixture): calcium carbonate, anhydrous 357, potassium phosphate, monobasic 250, sodium chloride 74, potassium sulfate 46.6, potassium citrate, tri-potassium, monohydrate 28, magnesium oxide 24, ferric citrate 6.06, zinc carbonate 1.65, manganous carbonate 0.63, cupric carbonate 0.3, potassium iodate 0.01, sodium selenate, anhydrous 0.01025, ammonium paramolybdate 0.00795, sodium meta-silicate 9, hydrate 1.45, chromium potassium sulfate 12, hydrate 0.275, boric acid 0.0815, sodium fluoride 0.0635, nickel carbonate 0.0318, lithium chloride 0.0174, ammonium vanadate 0.0066, powdered sucrose 209.806.

⁵ Vitamin mix (AIN-93-VX) (mg/kg mixture): nicotinic acid 3,000, Ca pantothenate 1,600, pyridoxine-HCl 700, thiamin-HCl 600, riboflavin 600, folic acid 200, D-biotin 20, vitamin B-12 (cyanocobalamin) 2,500, Vitamin E (all-*rac*- α -tocopheryl acetate) (500 IU/g) 800, Vitamin A (all-*trans*-retinyl palmitate) (500,000 IU/g) 800, vitamin D3 (cholecalciferol) (400,000 IU/g) 250, vitamin K (phylloquinone) 75, powdered sucrose 974.655 g.

formance Liquid Chromatography (HPLC, Waters 2690 separations module) equipped with a UV detector at 450 nm according to Nelis's method (17). A reverse-phase LUNA 5u C18(2) (250×4.6 mm micron.) column was used, operated at room temperature, with acetonitrile : dichloromethane : methanol (7 : 2 : 1, v/v) as the mobile phase at a flow rate of 1 ML/min. The content of total vitamin C was measured by using a spectrophotometer (Spectronic 301, Milton Roy) at the wavelength of 520 nm (18). Vitamin E was measured by using HPLC (Waters 2690 separations module) equipped with a UV detector of 295 nm according to the AOAC authorized method (19). A normal-phase μ -Porasil™ column (125 Å, 10 μ m, 3.9×300 mm, Waters) was used, operated at room temperature, with hexane : isopropanol : acetic acid = 1,000 : 5 : 5 (v/v) as the mobile phase at a flow rate of 0.8 ML/min.

For the quantitative analysis of dietary fibers, we used the kit (total dietary fiber assay kit, Technical Bulletin No. TDFAB-3, Sigma) based on the method published in the 16th Edition of the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) (20).

TBARS content and antioxidative enzyme activities in plasma and liver. Plasma thiobarbituric acid reactive

substances (TBARS) were measured by using a luminescence spectrometer (Perkin Elmer, LS50, excitation 515 nm, emission 553 nm) according to the fluorometry method described by Yagi (21). And the TBARS contents of liver were determined by spectrophotometric measurement (HP 8453, Hewlett Packard) at 532 nm according to Buckingham (22).

The superoxide dismutase (SOD) activities of RBC and liver were measured by spectrophotometer at 550 nm according to the method of Flohé (23) and protein contents of all enzyme samples were measured by Lowry's method (24). RBC and liver catalase activity also were assayed by spectrophotometer at 550 nm according to the method described by Johansson and Borg (25). RBC and liver glutathione peroxidase (GSH-Px) activities were measured by using a spectrophotometer at 365 nm according to the method described by Flohé and Gunzler (26).

Concentration of 8-hydroxy-2'-deoxyguanosine in kidney tissue. The concentration of 8OHdG in the kidney was measured so that it could be used as the index of oxidative DNA damage. From kidney tissues, cellular DNA was extracted by using a DNA Extractor kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and isolated DNA was hydrolyzed into nucleosides to be used for the

analysis of HPLC (27). The HPLC system was comprised of a Waters 2690 Separation Module, a Waters 464 pulsed Electrochemical Detector (ECD, +750 mV), and a UV detector (UVD, 254 nm). Samples of DNA hydrolysate were injected into a LC-18-DB precolumn cartridge and Supelcosil™ LC-18-S column (5 μ m, 4.6 mm \times 25 cm; Supelco, Bellefonte, PA, USA) and isocratically eluted (mobile phase 10% methanol, 50 mM NaH₂PO₄ buffer solution; flow rate 0.5 mL/min). Nucleosides were monitored at 254 nm by the UV detector and 8OHdG by electrochemical detector. Concentrations of 8OHdG in cellular DNA were expressed as the number per 10⁵ of deoxyguanosine.

Statistical analysis. The results were expressed as means \pm standard error of the mean. First of all, the data classified by age factor (16, 18 and 20 mo) were analyzed by a one-way analysis of variance (1-way ANOVA) and mean differences among groups were evaluated by the Duncan's multiple range test at $p < 0.05$. The resultant data were classified by two factors—the types of grape powder and Cd administration. These were analyzed using a two-way analysis of variance (2-way ANOVA) at $\alpha = 0.05$ level. In the end, the data were classified by age factor (16, 18 and 20 mo), the types of grape powder and Cd administration and were analyzed using a three-way analysis of variance (3-way ANOVA) at $\alpha = 0.05$ level.

RESULTS

Antioxidative contents in grape powders

The yields of freeze-drying and contents of total flavonoids, antioxidative vitamin, β -carotene, vitamin C and vitamin E and dietary fibers in grape sample powders were determined as shown in Table 2. The yields of freeze-drying in grape samples were as follows: whole grape 13.33%, grape pomace 20.34% and grape juice

10.07%. Yield of grape pomace was higher by about 1.5–2 times than that of whole grape or grape juice. The contents of total flavonoids in 1 g grape powder samples are the highest in grape pomace (5.52 mg/g) and the lowest in grape juice (2.61 mg/g). The contents of β -carotene were 7.64 μ g/g in whole grape powder, 13.29 μ g/g in grape pomace powder and 1.72 μ g/g in grape juice powder. The contents of vitamin C in grape samples were similar among all three parts of grapes: whole grape 0.60 mg/g, grape pomace 0.50 mg/g and grape juice 0.59 mg/g. The contents of vitamin E were the highest in grape pomace powder (138.34 μ g/g) and the lowest in grape juice powder (17.92 μ g/g). Total dietary fibers were determined as follows: whole grape 193.78 mg/g, grape pomace 323.73 mg/g and grape juice 43.23 mg/g. About 90% of the dietary fiber in grape pomace and whole grape powder was insoluble but in the case of grape juice, about 70% of the fiber was water-soluble. Except for vitamin C, the contents of flavonoids, β -carotene, vitamin E and total dietary fiber were the highest in grape pomace powder and the lowest in grape juice powder. We suggested that the reason why the contents of antioxidative components were the highest in grape pomace might have been that the larger quantities of grape seeds and skins than whole grape or grape juice powder, particularly that of grape seeds, contributed to the increase of fat-soluble vitamin contents.

Body weight, food intake, and epididymal fat pad weight of experimental animals

The changes of body weight (BW), daily food intake, and epididymal fat pad (EFP) weight per BW of experimental animals are shown in Table 3. The rate of BW change of 16-mo-old animals was the highest in the Cd (–) control group and was a significantly higher rate than in the other Cd (–) groups except the Cd (–) juice group. All 16-mo-old Cd groups showed negative rates except the grape pomace group. In 18-mo-old animals, the rate of BW change was not affected by grape intake but was significantly influenced by Cd administration; therefore, Cd (–) groups showed positive rates except the grape juice group and all Cd groups showed negative rates. The rate of BW change in 20-mo-old animals was significantly affected by Cd administration but not by grape intake. Therefore, BWs of Cd (–) groups increased but those of Cd groups decreased.

In 16-mo-old animals, food intake of the Cd (–)-control group and Cd-grape juice [J(+)] were significantly higher than that of the Cd whole grape [W(+)] group, but food intake itself was not influenced by either grape intake or Cd administration. However, food intake of 18-mo-old animals was affected by grape intake but not by Cd administration. Therefore, grape groups showed higher intakes than their control groups. In 20-mo-old animals, there was no significant difference among groups and food intake was not affected by both grape intake and Cd administration. Generally, food intake slightly decreased with age; therefore, 20-mo-old groups showed lower intakes than 16- and 18-mo-old animals, but food intake itself was not influenced by ei-

Table 2. Yields of different parts of grapes and contents of total flavonoids, β -carotene, vitamin C, vitamin E and total dietary fiber in grape powders.

Constituents	Types of powder		
	Whole grape	Grape pomace	Grape juice
Yields (%)	13.33	20.34	10.07
Total flavonoids (mg/g powder)	3.63	5.52	2.61
β -Carotene (μ g/g powder)	7.64	13.29	1.72
Vitamin C (mg/g powder)	0.60	0.50	0.59
Vitamin E (α -TE ¹ μ g/g powder)	72.59	138.34	17.92
α -Tocopherol (μ g/g powder)	59.44	115.23	14.40
β -Tocopherol (μ g/g powder)	12.10	20.55	3.59
γ -Tocopherol (μ g/g powder)	71.01	128.38	17.27
δ -Tocopherol (μ g/g powder)	6.29	6.17	1.12
Total dietary fibers (mg/g powder)	193.78	323.72	43.23
IDF (mg/g powder)	173.22	289.58	11.71
SDF (mg/g powder)	20.56	34.14	31.52

¹ α -Tocopherol equivalent = $1 \times \alpha$ -tocopherol + $0.5 \times \beta$ -tocopherol + $0.1 \times \gamma$ -tocopherol.

Table 3. Rate of body weight change and food intake in SD rats fed diets containing different parts of grapes with or without Cd.¹

Group ²	Rate of body weight change				Food intake				Epididymal fat pad weight			
	Age	16 mo (g/mo) ⁶	18 mo (g/mo) ⁷	20 mo (g/mo) ⁸	16 mo (g/d) ¹⁰	18 mo (g/d) ¹¹	20 mo (g/d) ¹²	16 mo (mg/g BW)	18 mo (mg/g BW)	20 mo (mg/g BW)		
C(-)	19.3±7.30a ³	4.6±2.95ab	4.6±2.95ab	12.9±3.24ab	22.8±0.91a	19.3±0.65bc	17.6±0.58 ^{NS9}	19.46±2.70a	22.60±1.54ab	15.61±0.95b		
W(-)	2.5±4.12bc	24.4±11.32a	24.4±11.32a	20.9±16.75a	20.1±0.49ab	23.1±1.17a	18.3±0.95	13.66±0.83b	22.82±1.31a	16.15±2.62b		
P(-)	3.0±5.42bc	1.6±8.93ab	1.6±8.93ab	11.2±7.74ab	19.3±1.88ab	20.0±0.48abc	18.1±0.64	16.52±2.45ab	19.99±1.85abc	19.91±2.84ab		
J(-)	14.6±3.18ab	-4.5±8.35bc	-4.5±8.35bc	9.2±8.27ab	21.2±1.36ab	17.3±1.04c	19.4±0.89	14.94±1.97ab	19.97±0.76abc	26.22±2.44a		
C(+)	-4.1±3.33c	-10.0±5.14bc	-10.0±5.14bc	-10.7±8.99b	19.7±0.56ab	18.4±1.17c	17.7±0.51	12.58±0.94b	15.83±1.59c	15.96±2.48b		
W(+)	-0.6±3.74c	-10.6±1.97bc	-10.6±1.97bc	-13.5±11.33b	18.4±1.06b	19.1±0.81bc	17.9±1.49	14.62±0.68ab	16.27±1.75c	14.11±3.20b		
P(+)	2.1±4.84bc	-5.0±12.30bc	-5.0±12.30bc	-12.1±4.02b	20.9±1.54ab	22.1±1.03ab	17.8±1.37	12.80±1.50b	15.78±1.73c	17.21±1.58b		
J(+)	-7.2±2.59c	-28.8±6.67c	-28.8±6.67c	-14.7±5.94b	23.0±1.52a	20.1±1.73abc	18.8±1.92	10.68±1.48b	16.94±2.83bc	14.16±1.30b		
Significant factor (2-way) ⁴		B, A*B	B	B	—	A, A*B	—	B	B	B		
Significant factor (3-way) ⁵			B			C, A*B, A*C			B, C, A*B*C			

¹ Mean ± standard error (n=5). ² See Table 1. ³ Values with different leathers within the column are significantly different at $\alpha=0.05$ by Duncan's multiple range test.

⁴ Statistical significance of dietary factors was calculated based on 2-way ANOVA. A: Effect of grapes was significant at $\alpha=0.05$, B: Effect of Cd was significant at $\alpha=0.05$, A*B: Interaction of grapes and Cd was significant at $\alpha=0.05$.

⁵ Statistical significance of dietary factors was calculated based on 3-way ANOVA. Significant factor notations used for 3-way ANOVA are as follows: A: Effect of grapes was significant at $\alpha=0.05$, B: Effect of Cd was significant at $\alpha=0.05$, A*B: Interaction of grapes and Cd was significant at $\alpha=0.05$, A*C: Interaction of grapes and age was significant at $\alpha=0.05$, B*C: Interaction of Cd and age was significant at $\alpha=0.05$, A*B*C: Interaction of grapes and Cd and age was significant at $\alpha=0.05$.

⁶ Data are calculated by dividing weight changes for 3 mo (14–16 mo-aged) by 3.

⁷ Data are calculated by dividing weight changes for 2 mo (17–18 mo-aged) by 2.

⁸ Data are calculated by dividing weight changes for 2 mo (19–20 mo-aged) by 2.

⁹ Not significant at $\alpha=0.05$ by Duncan's multiple range test.

¹⁰ Daily food intakes are calculated by dividing total intakes for 3 mo (14–16 mo-aged) by 3.

¹¹ Daily food intakes are calculated by dividing total intakes for 2 mo (17–18 mo-aged) by 2.

¹² Daily food intakes are calculated by dividing total intakes for 2 mo (19–20 mo-aged) by 2.

Table 4. Organ weight in SD rats fed diets containing different parts of grapes with or without Cd.¹ (mg /g BW)

Group ²	Liver				Kidney				Spleen			
	Age	16 mo	18 mo	20 mo	16 mo	18 mo	20 mo	20 mo	16 mo	18 mo	20 mo	
C(-)	22.67±0.65b ³	13.06±0.76bc	22.79±0.68bc	5.52±0.37b	5.57±0.32c	6.35±0.46abc	1.66±0.14 ^{NS,6}	1.86±0.14ab	1.57±0.13b			
W(-)	23.91±1.37b	22.52±1.02c	23.71±0.77bc	5.98±0.25ab	5.59±0.27c	5.67±0.32bc	1.48±0.06	1.53±0.13b	1.92±0.26ab			
P(-)	23.30±0.32b	22.76±1.22bc	23.61±0.80bc	5.78±0.39ab	5.50±0.39c	6.32±0.38abc	1.64±0.11	1.69±0.15ab	2.02±0.25ab			
J(-)	24.92±0.61b	22.19±0.61c	21.55±1.36c	6.10±0.20ab	5.99±0.31bc	5.09±0.45c	1.72±0.14	1.71±0.11ab	1.42±0.13b			
C(+)	22.85±1.35b	23.45±0.80abc	24.20±0.71bc	6.17±0.18ab	6.09±0.09bc	6.55±0.18ab	1.54±0.11	1.80±0.12ab	1.76±0.12b			
W(+)	24.58±0.46b	23.43±1.05abc	28.69±1.90a	6.08±0.06ab	6.24±0.38bc	7.50±0.89a	1.62±0.06	1.92±0.06a	2.52±0.44a			
P(+)	23.28±0.88b	25.81±0.84ab	25.54±0.65b	6.10±0.21ab	7.21±0.26a	6.43±0.31ab	1.60±0.07	1.91±0.07a	1.84±0.10ab			
J(+)	27.72±0.98a	26.16±1.11a	25.45±0.96b	6.65±0.45a	6.59±0.26ab	6.65±0.34ab	1.72±0.25	1.80±0.07ab	1.94±0.27ab			
Significant factor (2-way) ⁴	A	B	A, B	B	B	B	—	—	—			
Significant factor (3-way) ⁵	A, B, A*C			B, A*B*C			B, C, A*C					

¹ Mean±standard error (n=5). ² See Table 1. ³⁻⁵ See Table 3. ⁶ Not Significant at $\alpha=0.05$ by Duncan's multiple range test.

Table 5. Plasma and Liver TBARS level in SD rats fed diets containing different parts of grapes with or without Cd.¹

Group ²	Plasma TBARS (nmol/dL plasma)				Liver TBARS (nmol/g wet liver)			
	Age	16 mo	18 mo	20 mo	16 mo	18 mo	20 mo	
C(-)	555.79±27.48ab ³	652.81±30.49cd	757.45±87.26ab	8.83±0.74ab	11.84±1.06 ^{NS,6}	10.61±0.52abc		
W(-)	499.14±41.49b	610.83±27.80cde	612.22±82.21bc	8.89±0.62ab	10.63±1.13	9.49±0.75c		
P(-)	459.82±17.65b	472.17±15.18e	540.29±48.38c	7.06±0.92b	9.53±0.59	8.60±0.56c		
J(-)	498.83±38.11b	543.74±18.57de	670.06±88.64abc	8.61±0.40ab	10.88±0.47	8.61±0.62c		
C(+)	660.48±59.61a	915.26±61.65a	867.30±38.57a	10.88±1.53a	12.23±0.64	12.26±0.77a		
W(+)	569.76±37.44ab	822.39±75.46ab	745.02±39.62ab	9.23±0.67ab	10.63±0.71	10.06±0.96bc		
P(+)	512.44±31.19b	655.42±31.32cd	617.22±35.34bc	9.15±0.36ab	9.83±0.66	9.82±0.37c		
J(+)	644.03±12.09a	737.48±81.69bc	775.22±56.58ab	9.25±0.78ab	12.16±1.11	12.04±0.81ab		
Significant factor (2-way) ⁴	A, B	A, B	A, B	B	A	A, B		
Significant factor (3-way) ⁵	A, B, C, B*C			A, B, C				

¹ Mean±standard error (n=5). ² See Table 1. ³⁻⁵ See Table 3. ⁶ Not significant at $\alpha=0.05$ by Duncan's multiple range test.

ther grape intake or Cd administration except in 18-mo-old animals.

EFP weights per BW of experimental animals were affected significantly by both age and Cd administration, therefore, it was the highest in 18-mo-old groups and non-Cd groups had higher levels than Cd groups.

Organ weight of experimental animals

Liver, kidney, and spleen weights per BW of experimental animals are shown in Table 4. In 16-mo-old animals, the Cd-grape juice [J(-)] group had a significantly higher level than other groups. In both 18- and 20-mo-old animals, the Cd groups showed higher liver weights per BW than non-Cd groups. For all but the 18-mo-old Cd(-) groups, grape groups showed higher weights than their control groups in all 16-, 18- and 20-mo-old experimental animals.

In all 16-, 18- and 20-mo-old animals, kidney weights per BW were significantly affected by Cd administration but neither by grape intake nor by age factor; therefore, Cd groups showed higher weights than non-Cd groups.

Generally, spleen weight per BW was slightly increased with age and Cd groups had higher levels than non-Cd groups. In addition, there were no significant differences among 16-mo-old groups and in 18-mo-old animals, and Cd(+) grape groups showed higher levels than Cd(-) grape groups. In 20-mo-old animals, Cd groups had higher levels than non-Cd groups except the grape pomace group.

Lipid peroxide content in plasma and liver

The results of plasma and liver lipid peroxide (Thiobarbituric Acid Reactive Substances: TBARS) content is shown in Table 5. In 16-mo-old animals, grape groups had lower contents of plasma lipid peroxide than their control groups and Cd groups had lower plasma TBARS levels than non-Cd groups. These tendencies continued in 18-mo-old animals and grape intake decreased peroxide contents in particular; grape pomace was the most effective in decreasing peroxide contents. In addition, there was a large difference between Cd groups and non-Cd groups as Cd administration increased this content significantly. In 20-mo-old animals, grape-containing groups showed lower TBARS levels than their control group and Cd administration significantly increased lipid peroxide contents.

In conclusion, in all the 16-, 18-, and 20-mo-old animals, the control group showed a higher level of plasma TBARS than the grape-containing groups and Cd groups showed a higher concentration of plasma lipid peroxide than non-Cd groups. However, Cd+grape groups were lower than the Cd-control group and the Cd+grape pomace group showed similar levels to the non-Cd control group. The plasma content of lipid peroxides increased with age, but 20-mo-old Cd-groups showed lower levels than 18-mo-old groups except the grape juice group.

The content of lipid peroxide in the liver in 16-mo-old animals did not show a significant difference by grape intake. However, Cd groups showed slightly higher liver peroxide contents than Cd(-) groups. Even though 18-

mo-old experimental groups did not show any significant difference in the content of lipid peroxides, grape intake groups showed a little lower liver peroxide content than the control group. The grape intake groups of 20-mo-old animals also showed lower contents than control groups. Cd groups showed higher liver peroxide contents than Cd(-) groups.

Generally, similar to plasma, the contents of liver lipid peroxides of all aged grape groups were lower than control groups; in particular grape pomace groups showed the lowest contents. Cd intake groups showed higher content of liver lipid peroxides than non-Cd groups in all age groups.

The contents of lipid peroxide in both plasma and liver were increased through Cd administration. Grape intake groups showed lower contents of lipid peroxide than control groups without regard to Cd administration. Grape pomace intake is the most effective for inhibiting the formation of lipid peroxide in the body. In addition, the content of plasma lipid peroxide showed an increase with age but the content of liver lipid peroxide was the highest for 18-mo-old animals.

Antioxidative enzyme activity in RBC

The results of the RBC SOD, which converts a superoxide anion to H₂O₂ at the initial stage of free radical formation, are shown in Table 6. In 16-mo-old animals, the RBC SOD activities in Cd groups were lower than those in non Cd groups. Grape groups showed an increasing trend in the activity when compared to each control group; in particular, grape pomace intake groups showed the highest activity. Even though Cd administration in 18-mo-old animals did not result in any significant difference, the activity was increased by grape intake; in particular, the activity in the Cd(-) grape pomace intake group was significantly higher than that in the Cd control group. In 20-mo-old animals, Cd administration did not create any significant difference but the grape pomace intake group showed significantly higher activity than the Cd(+) control group. Generally, the SOD activities in all aged grape intake groups were higher than in the control; in particular, grape pomace intake groups showed the highest activity, but Cd-control groups showed the lowest activity. Since the significant difference in the activity was shown according to age, the activity in 16-mo-old animals was the lowest and increased markedly in 18-mo-old animals, but there was no change in activity between 18-mo-old animals and 20-mo-old animals.

The results of RBC catalase activity of experiment animals are shown also in Table 6. The activities in 16-mo-old grape intake groups were a little higher than those in each control group; in particular, the activity for grape pomace intake groups was significantly higher. Cd administration made a difference in the activity and non-Cd groups showed higher activity than Cd-administration groups. However, in 16-mo-old animals, Cd administration did not effect any significant difference in the activity except in relation to grape intake. Grape intake groups showed an increasing trend of the activity when compared with control groups.

Table 6. RBC Antioxidative Enzyme activity in SD rats fed diets containing different parts of grapes with or without Cd.¹

Group ²	Superoxide dismutase (unit/min/mg protein) ⁶				Catalase (μ mol/mg protein) ⁷				Glutathione peroxidase (unit/min/mg protein) ⁸				
	Age	16 mo	18 mo	20 mo	16 mo	18 mo	20 mo	16 mo	18 mo	20 mo	16 mo	18 mo	20 mo
C(-)		7.54 \pm 0.70c ³	21.73 \pm 2.10ab	20.09 \pm 3.94ab	6.18 \pm 0.30bcd	5.14 \pm 0.39b	5.39 \pm 0.48abc	4.09 \pm 0.27b	3.54 \pm 0.05bc	3.11 \pm 0.14b			
W(-)		18.85 \pm 0.57ab	27.32 \pm 3.93ab	23.99 \pm 2.81ab	6.90 \pm 0.33bc	6.25 \pm 0.56ab	6.01 \pm 0.14ab	4.56 \pm 0.19ab	3.90 \pm 0.23ab	3.90 \pm 0.28a			
P(-)		21.33 \pm 1.97a	30.01 \pm 4.05a	29.22 \pm 3.32a	8.44 \pm 0.44a	6.58 \pm 0.28a	6.34 \pm 0.66a	5.23 \pm 0.47a	4.16 \pm 0.20a	3.58 \pm 0.20ab			
J(-)		10.90 \pm 2.62bc	27.04 \pm 2.00ab	24.29 \pm 1.81ab	7.15 \pm 0.23b	5.97 \pm 0.49ab	5.54 \pm 0.80abc	4.78 \pm 0.39ab	3.79 \pm 0.16ab	3.49 \pm 0.20ab			
C(+)		5.99 \pm 1.53c	20.47 \pm 2.94b	18.41 \pm 1.23b	5.61 \pm 0.52d	4.93 \pm 0.35b	4.87 \pm 0.56abc	4.12 \pm 0.14b	3.02 \pm 0.26c	2.88 \pm 0.18b			
W(+)		11.81 \pm 2.30bc	22.05 \pm 2.28ab	23.53 \pm 2.69ab	5.93 \pm 0.18cd	5.84 \pm 0.30ab	4.71 \pm 0.51abc	4.53 \pm 0.30ab	3.15 \pm 0.21c	3.39 \pm 0.30ab			
P(+)		16.96 \pm 4.58ab	28.80 \pm 2.01ab	27.87 \pm 3.41a	7.10 \pm 0.37b	6.04 \pm 0.14ab	4.39 \pm 0.25bc	4.69 \pm 0.19ab	3.48 \pm 0.13bc	3.48 \pm 0.25ab			
J(+)		7.19 \pm 2.26c	23.54 \pm 1.02ab	25.68 \pm 2.00ab	5.67 \pm 0.33d	5.51 \pm 0.64ab	3.85 \pm 0.72c	4.52 \pm 0.21ab	3.17 \pm 0.17c	3.18 \pm 0.24ab			
Significant factor(2-way) ⁴		A, B	A	A	A, B	A	B	A	A, B	A			
Significant factor(3-way) ⁵			A, B, C			A, B, C			A, B, C				

¹ Mean \pm standard error ($n=5$). ² See Table 1. ³⁻⁵ See Table 3.⁶ SOD activities are expressed as units per minute per mg protein (One unit inhibits cytochrome *c* reduction rate by 50% in a coupled system with xanthine oxidase at pH 7.8 and 25°C in a 3.0 mL reaction volume).⁷ Catalase activities are expressed as μ mol formaldehyde utilized as standard per mg protein.⁸ GSH-Px activities are expressed as unit per mg protein (One unit catalyzes the oxidation by H₂O₂ of 1.0 μ mol of reduced glutathione to oxidized glutathione per min at pH 7.0 and 25°C).

Table 7. Liver antioxidative enzyme superoxide dismutase activity in Sprague-Dawley rats fed diets containing different parts of grapes with or without Cd.¹

Group ²	Superoxide dismutase (unit/min/mg protein) ⁶				Catalase (μ mol/mg protein) ⁷				Glutathione peroxidase (unit/min/mg protein) ⁸			
	Age	16 mo	18 mo	20 mo	16 mo	18 mo	20 mo	16 mo	18 mo	20 mo	16 mo	18 mo
C(-)		34.19 \pm 3.46 ^{NS 3}	27.72 \pm 1.94 ^{NS}	24.94 \pm 1.92 ^{NS}	25.95 \pm 1.26b ⁹	32.95 \pm 2.85ab	25.48 \pm 0.85a	27.86 \pm 0.83 ^{NS}	28.90 \pm 2.43abc	23.43 \pm 0.72ab		
W(-)		34.34 \pm 1.49	28.67 \pm 1.22	25.39 \pm 1.42	28.77 \pm 1.44ab	31.82 \pm 3.07abc	25.64 \pm 2.88a	28.36 \pm 2.05	26.69 \pm 2.75abc	24.65 \pm 1.69ab		
P(-)		38.56 \pm 6.13	31.16 \pm 1.42	27.56 \pm 0.98	31.04 \pm 1.27a	37.48 \pm 5.24a	26.01 \pm 1.47a	29.90 \pm 2.94	34.32 \pm 1.55a	28.26 \pm 2.39a		
J(-)		35.72 \pm 3.49	28.54 \pm 0.90	25.62 \pm 1.85	26.80 \pm 1.73ab	30.91 \pm 2.59abc	23.65 \pm 1.17ab	28.00 \pm 2.87	33.70 \pm 3.19ab	24.79 \pm 3.14ab		
C(+)		31.81 \pm 1.38	27.73 \pm 1.03	24.53 \pm 1.09	18.67 \pm 2.19c	22.68 \pm 2.95c	21.27 \pm 2.18ab	26.91 \pm 1.48	21.64 \pm 2.84c	14.39 \pm 2.03c		
W(+)		34.50 \pm 1.29	29.34 \pm 1.56	25.98 \pm 0.75	25.30 \pm 1.21b	24.19 \pm 1.66bc	21.58 \pm 0.51ab	29.61 \pm 1.78	24.94 \pm 2.98bc	15.98 \pm 1.20c		
P(+)		37.43 \pm 1.69	31.37 \pm 0.42	26.04 \pm 1.26	27.51 \pm 0.95ab	25.84 \pm 2.24bc	22.56 \pm 0.78ab	31.81 \pm 2.27	31.13 \pm 3.70ab	19.90 \pm 2.62bc		
J(+)		32.23 \pm 1.72	29.70 \pm 1.16	24.91 \pm 1.26	24.55 \pm 1.36b	23.08 \pm 1.68bc	19.28 \pm 2.05b	27.26 \pm 2.09	28.84 \pm 2.35abc	15.67 \pm 2.44c		
Significant factor (2-way) ⁴		—	—	—	A, B	B	B	—	A, B	B		
Significant factor (3-way) ⁵			A, C			A, B, C, B*C			A, B, C, B*C			

¹ Mean \pm standard error ($n=5$) ² See Table 1. ³ Not significant at $\alpha=0.05$ by Duncan's multiple range test. ⁴⁻⁵ See Table 3.

⁶ SOD activities are expressed as units per minute per mg protein (One unit inhibits cytochrome *c* reduction rate by 50% in a coupled system with xanthine oxidase at pH 7.8 and 25 °C in a 3.0 mL reaction volume).

⁷ Catalase activities are expressed as μ mol formaldehyde utilized as standard per mg protein.

⁸ GSH-Px activities are expressed as unit per mg protein (One unit catalyzes the oxidation by H₂O₂ of 1.0 μ mol of reduced glutathione to oxidized glutathione per min at pH 7.0 and 25 °C).

⁹ Values with different letters within the column are significantly different at $\alpha=0.05$ by Duncan's multiple range test.

Even though there was no significant difference due to grape intake, 20-mo-old Cd(+) groups showed a decrease in the activity when compared to non-Cd groups; in particular, Cd-grape intake groups showed a little lower activity than their control groups. Generally, RBC catalase activity showed a slight decreasing trend with age and the 20-mo-old Cd(+) groups showed the lowest activity. In 20-mo-old Cd(+) groups, grape intake groups showed slightly lower activity than their control groups but the increasing effect of catalase activity in grape groups was not shown significantly. As there was significant difference in the activity due to Cd administration, the activity in all age groups was decreased through Cd administration. Grape intake groups, except the 20-mo-old Cd-grape group, showed a little higher activities than their control groups.

The results of RBC GSH-Px activity of experiment animals are shown in Table 6. In 16-mo-old animals, grape intake groups showed slightly higher activities than control groups; in particular, non-Cd-pomace [P(-)] groups showed significantly higher activities than control groups but there was no difference in the activity due to Cd administration. In 18-mo-old animals, Cd administration groups showed significantly lower activity than non-Cd groups. The activities in grape intake groups were a little higher than those in control groups; in particular, the activity of grape pomace intake group was the highest. However, there was no difference due to Cd administration in 20-mo-old animals. Grape intake groups showed slightly higher activity when compared to control groups. RBC GSH-Px activity generally decreased in all age groups when Cd was administered and this activity decrease recovered a little in grape intake groups; in particular, the activity in grape pomace intake groups recovered the most. GSH-Px activity according to age showed the same changes as catalase activity. GSH-Px activity decreased slightly with age.

Antioxidative enzyme activity in liver

The results of liver SOD, catalase, and GSH-Px activity in experiment animals are shown in Table 7. Liver SOD activity did not show any significant difference between experimental groups in any of the 16-, 18-, or 20-mo-old animals but grape intake groups showed higher activity when compared to control groups regardless of Cd administration. In particular, the activity in grape pomace intake groups was the highest. In addition, liver SOD activity decreased with age.

Liver catalase activities in all age groups showed significant difference due to Cd administration and were decreased by Cd administration. Grape intake groups in 16-mo-old animals showed higher activity when compared to each control group and the grape pomace intake group showed the highest activity amongst them. The grape pomace intake group of 18-mo-old animals did not show any significant difference but had a little higher activity than control group. However, there was no effect of grape intake on 20-mo-old animals. In general, the increased liver catalase activity due to the effect of grape intake was shown only in 16-

mo-old animals. However, the decreased activity due to Cd administration was shown in all age groups and the activity in 18-mo-old animals was shown to be the highest because of activity differences due to age.

Liver GSH-Px activity in experimental animals did not show any significant difference in 16-mo-old animal groups. However, the activity in 18-mo-old animals was decreased through Cd administration, and grape intake groups showed higher activity than control groups; in particular, the effects in grape pomace intake groups was shown to be the most conspicuous. The GSH-Px activity in 20-mo-old animals decreased more than in 16- and 18-mo-old animals; in particular, the decreased activity of Cd administration groups was more conspicuous because of a significant difference in the activity due to Cd administration. The effect of grape intake was shown only in grape pomace groups and was larger than in any other group.

Concentration of 8-Hydroxy-2'-deoxyguanosine in kidney tissues

The results of the number of 8OHdG per 100,000 normal deoxyguanosines used as an indication for DNA damage in kidney tissues by HPLC are shown in Table 8. Grape intake in 16-mo-old animals showed lower 8OHdG levels than in control groups. Because the level in Cd groups was increased due to Cd administration, Cd groups, except Cd(+) grape pomace groups, showed higher levels than Cd(-) groups. Due to the grape intake of 18-mo-old animals, the 8OHdG levels were inclined to decrease, in particular, grape pomace intake

Table 8. Formation of 8-hydroxy-2'-deoxyguanosine in cellular DNA of kidney tissue of SD rats fed diets containing different parts of grapes with or without Cd.¹

Group ²	(80 HdG/10 ⁵ dG)		
	Age		
	16 mo	18 mo	20 mo
C(-)	11.46±1.70ab ³	20.08±4.83ab	19.16±2.42 ^{NS4}
W(-)	8.10±1.02b	19.38±1.27ab	16.77±3.10
P(-)	8.04±1.0b	11.49±2.54b	10.13±1.81
J(-)	9.36±1.67ab	14.98±1.86ab	14.46±3.77
C(+)	12.62±1.70a	23.28±5.49a	19.21±2.77
W(+)	10.33±0.75ab	20.78±1.51ab	17.77±2.98
P(+)	7.30±0.86b	10.97±1.78b	17.08±3.29
J(+)	10.27±0.96ab	15.85±1.11ab	18.75±3.35
Significant factor (2-way) ⁵	A	A	—
Significant factor (3-way) ⁶		A, C	

¹ Mean±standard error (n=5). ² See Table 1.

³ Values with different letters within the column are significantly different at $\alpha=0.05$ by Duncan's multiple range test.

⁴ Not significant at $\alpha=0.05$ by Duncan's multiple range test.

⁵⁻⁶ See Table 3.

groups showed the most conspicuous activity decrease. The 8OHdG levels except for Cd-grape pomace intake group were a little higher due to Cd administration. There was no significant difference among 20-mo-old experimental groups.

Generally, the 8OHdG concentration levels in kidney tissues were higher in 18- and 20-mo-old animals when compared to 16-mo-old animals due to age, but there was little difference between 18-mo-old animals and 20-mo-old animals. The concentration in grape intake groups was lower than that of control groups; therefore, grape intakes showed the effect of decreasing the accumulation of 8OHdG, and in particular, the effect in the grape pomace intake group was the highest.

DISCUSSION

To see how Cd administration-induced oxidative stress and grape intake affect age-related changes in antioxidative capacity, lipid peroxidation, and DNA damage, we raised 13-mo-old rats with experimental diets for a total of 7 mo and measured body and organ weights, food intake, plasma and lipid peroxide contents, RBC and liver antioxidative enzyme activity and DNA damage in the kidney.

Body weight was affected significantly by Cd administration as in another study (28) and weight loss appeared in groups administered with Cd, but weight increases mostly appeared in non-Cd groups. Such weight loss and growth decline due to Cd administration are general symptoms shown in Cd poisoning and the reason for the loss and decline is thought to be that food intake was decreased due to appetite decline or food efficiency was decreased due to the effect of Cd on the nutrient absorption and metabolism. However, when Cd was fed at 0.02% levels in this study, food intake was not significantly decreased. Even though food intake was decreased with age, rates of body weight change were not affected by age. Additionally, as the food intake of grape intake groups in 18-mo-old animals was higher than that of control groups regardless of Cd administration but rates of BW change in 18-mo-old animals were not affected by grape intake, it was shown that a decrease or increase of food intake was not related to rate of BW change. Therefore, this study showed that the weight loss in Cd groups would be caused by Cd's interference in nutrient absorption or metabolism rather than by a decrease in food intake.

Liver, kidney, spleen, and EFP weights per BW showed significant differences depending on Cd administration. Liver, kidney, and spleen weights per BW with Cd administration were a little higher than those without Cd administration but EFP weights per BW showed the opposite trend. Such changes in organ and tissue weights per BW with Cd administration may be caused by organ sclerosis due to Cd poisoning (28, 29). As the effect of grape intake was shown only in the liver, liver weight per BW was increased largely when both Cd and grapes were administered to the SD rats. The weights of spleen and EFP per BW showed a significant change

according to age. Spleen weight per BW increased with age and EFP weight per BW was the heaviest in 18-mo-old animals and decreased in 20-mo-old animals.

In the result of this study, grape intake groups maintained lower lipid peroxide levels by promoting the activities of antioxidative enzymes such as SOD, catalase, and GSH-Px, as well as by the non-enzymatic antioxidative mechanisms of the many antioxidative components in grape powders. It was thought that this enzymatic promotion and the non-enzymatic antioxidative mechanisms contribute to the prevention of the formation of lipid peroxide in both plasma and liver. The antioxidative effect of grapes had not been shown in *in vivo* nutrient intervention studies, where grape was directly administered to experimental groups, but, *in vitro* study results using grapes, wine extracts, and flavonoid components of grapes have been as follows. According to Noroozi's report (30), DNA damage in human lymphocytes decreased in proportion to the concentration of injected flavonoids and especially quercetin, myricetin, and kaempferol, which are contained mainly in grapes, showed excellent antioxidative capacities. Karakaya et al. (31) measured the total phenolic compounds of some foods and determined the total antioxidant activity of the phenolic extracts from food samples. Their results showed that total phenolic compounds were higher especially in grape molasses and red wine and total antioxidant activities were strongly correlated with total amounts of phenolic compounds.

Because the high correlation between phenolic compounds containing flavonoids and antioxidative function measured *in vitro* has been well-demonstrated and the lipid peroxide accumulation in groups fed on grape pomace powder, of which total flavonoids content and antioxidative component content are the highest among the three experimental grape samples, was more effectively restricted than in the control group and whole grape, or grape juice intake group and the activities of antioxidative enzymes including SOD in pomace groups were promoted significantly, it is suggested that flavonoids and antioxidative components are effective in proportion to their contents not only *in vitro* but also *in vivo*.

Tedesco et al. (2) reported in a study comparing antioxidative effects of wines that RBC oxidation induced by H_2O_2 was more effectively restricted by treatment with red wine extracts rather than by treatment with white wine extracts. RBC functions as an oxygen carrier that contains a relatively large amount of polyunsaturated fatty acids in cell membranes and must be more susceptible to oxidative damage by ROS than other cells. If hemoglobin is converted into an oxidative type such as methemoglobin, it may work as a strong oxidation promoter in the body. Furthermore, it is known that free hemoglobin exposed to H_2O_2 undergoes a heme decomposition step to emit an iron ion and the emitted iron ion initiates free radical reaction and lipid peroxidation.

We concluded that the increase of RBC SOD activity in grape intake groups caused to convert superoxide

anions to H_2O_2 at the initial stage of free radical formation and then the increase of RBC catalase and GSH-Px activities caused to reduce H_2O_2 to H_2O , therefore, the increases of RBC antioxidative enzymes resulted in protection of cells from oxidative stress and restriction of iron discharge during heme decomposition. Additionally both fat-soluble vitamins and flavonoids which are especially rich in grape pomace along with the increase of RBC GSH-Px activity led to decrease the amount of lipid peroxides of cell membranes by scavenging free radicals.

It is thought that the decreased activity of antioxidative enzymes due to Cd administration is caused by the fact that Cd acts as a strong antagonist in both absorption and body utilization of Cu and Zn, which are components of SOD in cytosol, and of Se located in the active site of GSH-Px. When Cd is accumulated in the body, it acts as an oxidation catalyst to promote the formation of free radicals in the body and as an electrophile to promote both cell and organ damage and aging and furthermore as a carcinogenic chemical (5). When the RBC suspension was prepared for the determination of RBC enzyme activity in this experimental process, almost the same quantity of blood was collected from all experimental animals; however, the quantity of RBC in Cd groups was conspicuously less than that of non-Cd groups. Therefore, it was shown that hematocrit was decreased by Cd administration and it is thought that the decrease of RBC volume ratio to whole blood in Cd-administered groups is caused by inducing Fe deficiency in which Cd curtails both the absorption and utilization of Fe.

In this study, Cd induced oxidative stress in the body to increase lipid peroxide contents and to decrease antioxidative enzyme activities. That seems to be caused by flavonoids and dietary fiber of grape powders in the Cd-grape-administered groups forming stable metal ion complexes with free Fe and Cu ions which can promote free radical damage, or flavonoids and dietary fiber of grape powders scavenging free radicals such as superoxide anion, hydroxyl radical, and peroxy radical directly and showing antioxidative action to effectively inhibit the accumulation of lipid peroxide contents in both plasma and the liver increased by Cd administration.

When antioxidative enzyme activities in the liver were compared to those in RBC, liver SOD activity was not significantly increased by the grape diet. RBC SOD activity increased with age; however, liver SOD activity showed lower levels at 18 and 20 mo than at 16 mo and showed an opposite trend to RBC SOD activity, while no changes in liver SOD activity by Cd administration were shown at any age. When catalase and GSH-Px activities in the liver were compared to the control group, they were increased by grape intake and Cd-administered groups showed lower activity than non-Cd groups. Since GSH-Px activity was changed according to age, the activity at 20 mo was largely decreased rather than at 16 or 18 mo. Such a change of liver antioxidative enzyme activity affects lipid peroxide content

in the liver, so grape intake causes a decrease of liver lipid peroxide content as well as an increase in three antioxidative enzyme activities in the liver, and Cd administration decreases liver catalase and GSH-Px activities and increases liver lipid peroxide contents. When compared to the Cd-control group, the intake of both Cd and grape restricted the accumulation of liver lipid peroxide because grape intake promotes non-enzymatic and enzymatic antioxidative mechanisms.

Bobek's study (32), in which male rats of the Wistar species had been raised by feeding a diet with added grape pomace at the 5% dietary level for 8 wk, reported that the liver GSH-Px activity was increased by 38% when compared to the control group which was administered the same quantity of cellulose instead of grape pomace. Bobek's study showed the same trend as this study, that liver antioxidative enzyme activity was increased by grape intake. Therefore, it is thought from our study and Bobek's study that the increase of liver antioxidative enzyme activity is caused by a complicated interrelationship among several antioxidative components contained within a grape diet, not by dietary fiber like cellulose.

When Palomero et al. (33) administered cyclosporin A (CyA) used as an immunosuppressive agent to both 24- and 4-mo-old Wistar rats and compared the result of 24-mo-old rats with that of 4-mo-old rats, the liver lipid peroxide accumulation in the 24-mo-old rats was more greatly increased than in the 4-mo-old when compared to each month's control groups that were not injected with CyA. Additionally, the liver SOD and catalase activities in the 24-mo-old rats were more greatly decreased than in the 4-mo-old when compared to each month's control groups and furthermore there was no significant change of antioxidative enzyme activity in the 4-mo-old. That is, CyA poisoning was shown to be different according to age and was more lethal in 24-mo-old rats than in 4-mo-old rats. Therefore, because in this study, Cd was administered to over-12-mo-old rats and the adaptability to oxidative stress in old rats can be lower than that in young rats, these results that the antioxidative enzyme activity was decreased and lipid peroxide accumulation was increased may be regarded as more significant.

The 8OHdG content as an index for DNA damage of kidney tissues, was lower in grape intake groups at all ages than in control groups, and the grape pomace group showed the lowest content. The content was increased a little due to the Cd administration but the increase was not significant. The content was increasing with age and was higher in 18- and 20-mo-old groups than in the 16-mo-old groups. The reason why this study investigated DNA damage especially in kidney tissues is that increased DNA damage was more conspicuous in kidney tissues than in other organs and Cd poisoning was the most destructive in kidney according to the results of other previous studies which selected 8OHdG as an indicator for DNA damage.

Chronic infections, smoking, and a high-fat diet, which are known as factors to cause cancer, actually

increased the level of oxidative DNA damage in the body and especially smoking and the high-fat diet promoted the formation of 8OHdG in animal experiments (34, 35). It is expected that oxidative DNA damage is in inverse proportion to antioxidative components including flavonoids and antioxidative vitamins. In this study, the decrease of 8OHdG accumulation in kidney tissues due to grape administration seems to be similar to a decrease of oxidative DNA damage in human or animal tests in the case of the intake of brussels sprouts (36, 37), tomatoes (38) or vegetable juices (39). Thus, it is thought that the decrease of DNA damage in the case of taking vegetable or fruits like grapes and tomatoes is caused by a kind of association of various antioxidative components including phytochemicals, not a specific antioxidative component included in fruits or vegetables, or by a complex effect of several factors of glycoside or free form in flavonoids in the mechanism to decrease DNA damage.

The aging process causes a decrease in the function of cells, tissue, and organs, and induces metamorphoses and due to these malfunctions and transformations, various degenerative diseases may occur and the quality of life may fall. Therefore, it is thought that, through both sufficient and balanced diet and the consumption of various physiologically active materials, antioxidative enzyme activity can be promoted to prevent cellular and DNA damage. How to maintain both the function and health of body may be future topics awaiting solution in the study of aging.

This study investigated the changes in antioxidative ability and DNA damage of SD rats according to aging and the effect of both grape-containing antioxidative components and Cd administration on the change of antioxidative capacity, lipid peroxidation, and DNA damage. Conclusively, among three different grape formulations, namely whole grape, grape pomace, and grape juice, the administration of grape pomace containing the highest content of flavonoids, fat-soluble vitamins, and dietary fiber conspicuously decreased lipid peroxide contents in both plasma and the liver as well as DNA damage to kidney tissues, and increased greatly antioxidative enzyme activity in both RBC and the liver. These antioxidative effects of grape pomace administration were also shown under oxidative stress by Cd and aging. When the old SD rats ingested both grape pomace and Cd, lipid peroxide and DNA damage were decreased to the level of the control group of the same age. As a result, it is expected that grape formulations, especially grape pomace, significantly can delay the onset of various degenerative diseases such as cancer and the aging process, which are caused by the accumulation of free radical damage.

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