Onion Flesh and Onion Peel Enhance Antioxidant Status in Aged Rats

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(Received July 3, 2006)

Summary This study was designed to investigate the effects of dietary onion flesh or onion peel on lipid peroxides and DNA damage in aged rats. Sprague Dawley male rats (n=40, 16 mo old) were blocked into five groups and raised for 3 mo with either an onion-free control diet or onion diets (Allium cepa L., intermediate-day variety) containing either 5% (w/w) powdered dried onion flesh, 5% (w/w) powdered dried onion peel or ethanol extracts of the two powdered forms of onion. Total antioxidant status (TAS) and levels of total polyphenols and quercetin were greatest in onion peel ethanol extract, followed by onion peel powder, onion flesh ethanol extract, and onion flesh powder. Plasma quercetin and isorhamnetin levels were markedly increased by onion peel powder and onion peel ethanol extract. Rats fed onion flesh powder or onion peel powder had a higher plasma TAS than rats fed the control diet. Onion peel powder reduced liver thiobarbituric reactive substances relative to those of the control diet in aged rats (p<0.05). Brain 8-isoprostane levels were markedly decreased by all four onion diets and the decrease was significant for the onion flesh powder and onion peel powder diets (p<0.05). There was no significant decrease in cellular DNA damage in the kidney or brain tissue among rats fed the four onion diets. Onion flesh or onion peel enhanced antioxidant status in aged rats and may be beneficial for the elderly as a means of lowering lipid peroxide levels.

Key Words onion flesh, onion peel, lipid peroxide, DNA damage, aged rats

Flavonoids are polyphenolic compounds that are widely distributed throughout the plant kingdom (1). They occur naturally in fruits and vegetables and are therefore an integral part of the human diet (2, 3). The dietary intake of flavonols and flavone aglycones in Western Europe is about 26 mg per day (4). Approximately 70% of total flavonoid intake consists of quercetin (4). Recently, a survey of food intake in Korea revealed that the average daily intakes of quercetin, kaempferol, lutein, myricetin, and apigenin were 19.0, 10.4, 1.1, 2.4, and 0.8 mg, respectively (5). The major dietary sources of quercetin vary considerably between locations and cultures: they are red wine in Italy, tea in China, and onions in the United States, Japan, and Korea (5, 6).

Onions (Allium cepa L.) are rich in two chemical groups that have perceived health benefits for humans: flavonoids and alk(en)yl cysteine sulfoxides (ACSOs). Onions contain flavonols such as quercetin and its derivatives, which are responsible for the yellow flesh and brown skins of many yellow types of onions (7). ACSOs are flavor precursors that generate the characteristic odor and taste of onions when cleaved by the enzyme alliinase (8). Sixteen different flavonols consisting of aglycones and glycosylated derivatives of quercetin, isorhamnetin and kaempferol have been identified in onions (9). Onion peel contains over 20 times more quercetin than onion flesh (1). Although onion peels have high levels of flavonoids, they are usually discarded before onions are processed for human consumption. Takahama and Hirota (10) suggested that quercetin is formed by the deglucosidation of its glucosides, followed by autoxidation to produce protocatechuic acid. Recently, some anti-platelet and membrane-rigidifying flavonoids were isolated from the outer scales of onions and identified as quercetin, quercetin dimers, and quercetin 4′-glucoside (11, 12). Although it has been shown that extracts from the outer scales of onions have potent radical scavenging activities in vitro (13, 14), the in vivo antioxidative capacity of onion peel is still unclear.

The structure of flavonoids varies widely within the major classifications and substitutions include hydroge- nation, hydroxylation, methylation, manoloylation, sulphation, and glycosylation. Low- and intermediate-molecular weight flavonoids can be extracted with solvents such as water, alcohol, and aqueous acetone. High-molecular weight flavonoids over 5,000 Dalton usually remain insoluble in these solvents (15). Several in vitro studies have been conducted to elucidate the antioxidative effect of various types of onion extracts (11, 13, 14). However, the in vivo antioxidative capacity of various onion preparations is not elucidated yet. Therefore, this study was designed to ascertain whether feeding powder or ethanol extract of onion flesh or onion

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Table 1. Classification of experimental groups. (g/kg diet)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>C</th>
<th>FP</th>
<th>FE</th>
<th>PP</th>
<th>PE</th>
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<tbody>
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<tr>
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<tr>
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<td>1.8</td>
<td>1.8</td>
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<tr>
<td>Onion powder/ethanol extract with starch</td>
<td>—</td>
<td>50</td>
<td>50</td>
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<td>50</td>
</tr>
</tbody>
</table>

1 C: onion free control group, FP: onion flesh powder group, FE: onion flesh ethanol extract group, PP: onion peel powder group, PE: onion peel ethanol extract group.
2 Mineral mix (AIN-93M) (g/kg mixture): calcium phosphate, dibasic (CaHPO₄·2H₂O) 500, sodium chloride (NaCl) 74, potassium citrate monohydrate (K₃C₆H₅O₇·H₂O) 220, Potassium sulfate (K₂SO₄·24), Manganese carbonate (43–48%, Mn 3.5, ferric citrate (16–17% Fe) 6.06, zinc carbonate (70% ZnO) 1.6, cupric carbonate (53–55%, Cu 0.3, potassium iodate (KI) 0.01, sodium selenite (Na₂SeO₃·H₂O) 0.01, chromium potassium sulfate (Cr₂(KSO₄)₂·2H₂O) 0.55, sucrose finely powdered, to make 1,000 g.
3 Vitamin mix (AIN-93M) (mg/kg mixture): nicotinic acid 3,000, calcium pantothenate 1,600, pyridoxine-HCl 700, Thiamin-HCl 600, riboflavin 600, folic acid 200, β-Biotin 20, vitamin B₁₂ (cyanocobalamin) (0.1% in mannitol) 2.5, vitamin E (all-rac-α-tocopheryl acetate) (500 IU/g) 1,500, vitamin A (all-trans-retinyl palmitate) (500,000 IU/g) 800, vitamin D₃ (cholecalciferol) (400 IU/g) 250, vitamin K (phyloquinone) 75.0, sucrose finely powdered, to make 1,000 g.
4 Each ethanol extract of the corresponding powder was adjusted with cornstarch to make up the weight difference between the freeze-dried and ethanol extract type. (1) FP: flesh powder 50 g/kg diet, (2) FE: flesh ethanol extract 10.42 g/kg diet + cornstarch 39.58 g/kg diet, to make 50 g/kg diet, (3) PP: peel powder 50 g/kg diet, (4) PE: peel ethanol extract 5.56 g/kg diet + cornstarch 44.44 g/kg diet, to make 50 g/kg diet.

peel enhances the antioxidative capacity of aged rats.

METHODS AND MATERIALS

Animals. Male 16-month-old Sprague Dawley rats (Sam:TaC[N(SD)BR, inbred, originating from Samtaco Bio-Korea, Korea) weighing 618 ± 6 g were placed in individual stainless steel wire-mesh cages in an automatically controlled room. The room had a 12:12 h light-dark cycle, a temperature of 22–24°C and 45 ± 5% humidity. This study was conducted in the nutrition laboratory of Ewha Women’s University in compliance with the guidelines for the Guide for the Care and Use of Laboratory Animals (16) recommended by Ewha Women’s University institutional animal care and use committee.

Diet. The rats were fed a commercial pelleted diet (Zeigler Rodent, USA) and deionized water for the first 10 d for acclimation. After acclimation, they were fed the (AIN)-93M diet (17) of the American Institute of Nutrition for 10 d, then randomly blocked into five groups according to body weight and raised for 3 mo on the experimental diets (powdered dried onion flesh and onion peel or an ethanol extract of powdered dried onion flesh and onion peel). The experimental design and the composition of diets are shown in Table 1. The diets were formulated according to the nutrient content of the AIN-93M diet with slight modifications. Cornstarch (Daesang Co., Korea) was the only source of carbohydrate in the diets. Corn oil (CJ Co., Korea) and soybean oil (Ottogi Foods Co., Korea) were used as sources of lipid.

Processing of onions. Onions were purchased from a local market in Seoul, Korea. The onions were of the intermediate-day variety (Allium cepa L., Muan County, Jeolla NamDo, Korea), which is widely consumed in South Korea. Onion flesh and onion peel were separated, washed, cut into 4 × 4 mm pieces, frozen, lyophilized, and ground to pass through a 40-mesh sieve. Onion powders contained less than 5% water and were stored at −70°C until they were used in the experimental diets. Freeze-dried powder of onion flesh or onion peel was extracted three times with five volumes of 95% (v/v) ethanol at 80°C for 1 h and filtered using a vacuum. The filtrate was centrifuged at 10,000 × g for 30 min (RT 6000B, Sorvall, USA), the resulting supernatant was collected and ethanol was evaporated from the supernatant by a rotary vacuum evaporator (N-N series, EYELA, Japan). After concentration, extracts were frozen, lyophilized using a vacuum-tray freeze dryer (Ishin Lab. Co. Ltd., Korea), and ground to pass through a 40-mesh sieve. Yields from freeze-drying and/or ethanol extraction of fresh onion flesh or onion peel were calculated, ethanol extracts were adjusted with cornstarch to make up the weight difference between the freeze-dried and ethanol extracts (Table 1).

Specimen collection. At the end of the experimental period, the animals were deprived of food for 12 h and sacrificed after anesthetization with ethyl ether. After
collecting blood samples directly from the heart with a heparinized or EDTA-treated syringe, the brain was removed by decapitation, weighed, and cut into small pieces in a dry-ice bath as described by Glowinski and Iversen (18). Subsequently, tissue samples were collected and cut into small pieces, frozen over dry ice and stored at −70°C. Plasma was separated by centrifugation at 4°C at 1,000 × g for 30 min (Union 55R, Hanil, Korea).

**Determination of total polyphenol, quercetin, and total antioxidant status in onion samples.** Total polyphenol content was determined using the method of Folin-Denis (19). Quercetin content was determined using the method of Merken and Beecher (20) and Hertog et al. (21). Briefly, 1 g of dried sample was extracted with 25 mL methanol (stabilized with 0.5 g/L butylhydroxytoluene, BHT) and hydrolyzed with 6 N HCl at 90°C for 2 h. After cooling, the extract was made up to 100 mL with methanol, sonicated, filtered using a 0.20 μm filter (Nylon 66 syringe filter, Whatman, USA), and analyzed immediately using high performance liquid chromatography (HPLC). The chromatograph (Waters 2690 Separation Module, Waters, USA) was equipped with a UV detector set to 370 nm. A standard curve was compiled from serial dilutions of quercetin (Q0125, Sigma, USA) in methanol. The average recovery of a quercetin standard was 92% (n = 3). HPLC analysis of quercetin was performed under isocratic conditions using a Lichrospher 100-RP 18 column (5 μm, 4.6×125 mm; SRD, Austria). The mobile phase consisted of 75% 0.033 M H₃PO₄ (concentration of H₃PO₄ in final solution=0.025 M) and 25% acetonitrile containing 0.05% trifluoroacetic acid to adjust the pH to 2.4. The run time was 25 min and the flow rate was 1.0 mL/min.

Sample preparation for measuring the total antioxidant status (TAS) of the onion samples was performed as described by McCusker and Fitzgerald (22) and Marklund and Marklund (23) with slight modifications. In brief, 1 g onion samples were extracted with 50 mL 95% ethanol at 90°C for 2 h, cooled, and filtered under reduced pressure. The TAS of the filtrate was then analyzed using a TAS kit (Randox Laboratories Ltd., UK) according to the manufacturer’s instructions.

**Measurement of plasma TAS and determination of quercetin metabolites in plasma.** Plasma TAS was analyzed using a TAS kit (Randox Laboratories Ltd.), based on the trolox equivalent antioxidant capacity (TEAC) assay in the present study. The assay was conducted according to the manufacturer’s instructions. Quercetin metabolites were measured using the method of Manach et al. (24, 25). Heparinized plasma was acidified to pH 4.9 with 0.1 volumes of 0.58 M acetic acid and incubated at 37°C for 30 min in the presence of 8×10⁶ IU β-glucosidase plus 2.5×10³ IU sulfatase (G0876, Sigma). Aglycones were then extracted with 2.7 volumes of acetone. After centrifugation, 20 μL of the supernatant was injected into the column for HPLC analysis. The average recovery rates of quercetin and isorhamnetin were 87% and 85% (n = 3), respectively. HPLC analysis of quercetin was performed under isocratic conditions with a Lichrospher 100-RP 18 column (5 μm, 4.6×125 mm; SRD). The mobile phase consisted of 73% water H₂PO₄ (99.5 : 0.5) and 27% acetonitrile. The flow rate was 1.5 mL/min and the UV detector was set at 370 nm.

**Lipid peroxide levels in liver and brain tissue.** Liver malondialdehyde content was determined by measuring the concentration of thiobarbituric acid reactive substances (TBARS) as described by Buckingham (26). Brain 8-isoprostane levels were measured using a kit (516351, Cayman Chemical, USA). The brain tissue preparations were done in accordance with the method of Hoffman et al. (27).

**DNA damage in the kidney and brain tissue.** The degree of DNA damage in the kidney was estimated from the concentration of 8-hydroxy-2'-deoxyguanosine (8-OHdG). DNA was extracted from tissues using a Wako DNA extractor kit (Wako, Japan). After extraction, DNA was hydrolyzed and the 8-OHdG concentration of the resulting solution was determined using an enzyme linked immunosolvent assay (New 8-OHdG Check, Japanese Institute for the Control of Aging, Japan). Absorbance was measured using a Spectra Max 340 (Molecular Devices, USA).

**Statistical analysis.** All results were expressed as means±standard errors (SEs). The data were analyzed

| Table 2. Yields and the composition of antioxidant substances in onion samples. |
|-----------------|-----------------|-----------------|-----------------|
| Constituent      | Type of powder  |                |                |
| Yields from fresh onion flesh or peel (% of each fresh form (w/w)). | Onion flesh powder | Onion flesh ethanol extract | Onion peel powder | Onion peel ethanol extract |
| Yield (%)¹      | 9.14            | 1.90 (20.84²)  | 32.27           | 3.59 (11.11)     |
| Total polyphenol (mg/g powder) | 13.98          | 27.96          | 133.62          | 288.99           |
| Quercetin (mg/g powder) | 1.12           | 2.35           | 20.50           | 99.68            |
| Total antioxidant status (mmol/g powder) | 0.04          | 0.05           | 0.12            | 0.12             |

¹ Yields from fresh onion flesh or peel, % of each fresh form (w/w).
² The yield of ethanol extraction from the flesh or peel powdered type of onion, % of the corresponding powder (w/w).
by one-way analysis of variance (ANOVA) and mean differences among the experimental groups were evaluated using Duncan’s multiple range tests at the p<0.05 level.

RESULTs

Yields and the contents of antioxidative substances in onion samples are shown in Table 2. The lyophilizing yields of onion flesh and onion peel powders were 9.14% (w/w) and 32.27% for fresh onion flesh and onion peel, respectively. The lyophilizing yield of onion peel powder was about 3.5 times greater than that of onion flesh powder. The yields of ethanol extraction of onion flesh powder and onion peel powder were 20.84% and 11.11%, respectively. Expressed as percentages of the mass of fresh onion, yields were as follows: onion flesh powder, 9.14% (w/w); onion flesh ethan-ol extract, 1.90%; onion peel powder, 32.27%; onion peel ethanol extract, 3.59%.

Total polyphenol content was 13.98 mg/g for onion flesh powder, 27.96 mg/g for onion flesh ethanol extract, 133.62 mg/g for onion peel powder and 288.99 mg/g for onion peel ethanol extract. The total polyphenol content of onion peel powder was tenfold higher than that of onion flesh powder and those of the ethanol extracts were twice those of their corresponding freeze-dried powders. The quercetin content of onion samples was 1.12 mg/g for onion flesh powder, 2.35 mg/g for onion flesh ethanol extract, 20.50 mg/g for onion peel powder and 99.68 mg/g for onion peel ethanol extract. The quercetin content of onion peel powder was about 20 times greater than that of onion

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**Fig. 1.** Plasma total antioxidant status level in aged rats fed various onion diets. Each bar represents the mean±SE: C: onion free control group; FP: onion flesh powder group; FE: onion flesh ethanol extract group; PP: onion peel powder group; PE: onion peel ethanol extract group. a) Bars with different letters are significantly different at a=0.05 by Duncan’s multiple range test.

**Fig. 2.** Plasma quercetin metabolites in aged rats fed various onion diets. Each bar represents the mean±SE: C: onion free control group; FP: onion flesh powder group; FE: onion flesh ethanol extract group; PP: onion peel powder group; PE: onion peel ethanol extract group. a) Bars with different letters are significantly different at a=0.05 by Duncan’s multiple range test.
flesh powder. About 8% of total polyphenols in onion flesh samples was quercetin, but in onion peel samples, about 15% (onion peel powder) and 35% (onion peel ethanol extract) of total polyphenols were quercetin. The TAS of onion peel samples (onion peel powder 0.12 mmol/g; onion peel ethanol extract 0.12 mmol/g) was more than double those of onion flesh samples (onion flesh powder 0.04 mmol/g; onion flesh ethanol extract 0.05 mmol/g). From the antioxidant composition of the onion samples, it is evident that the TAS and the content of total polyphenols and quercetin were greatest in the onion peel powder diet followed by the onion peel ethanol extract diet, the onion flesh powder diet, and the onion flesh ethanol extract diet.

Plasma TAS and levels of plasma quercetin metabolites are shown in Figs. 1 and 2. Onion flesh powder and onion peel powder elevated plasma TAS (p<0.05). Rats fed onion flesh or onion peel ethanol extract diets tended to have a higher plasma TAS than rats fed the control diet. Rats fed onion peel diets had more than 20 times the plasma quercetin concentrations of rats fed onion flesh diets. The plasma isorhamnetin level of the onion peel powder group was the highest of the groups fed onions. The isorhamnetin level was about 5–10 times greater than the quercetin level.

Levels of liver TBARS and brain 8-isoprostane are given in Figs. 3 and 4. Levels of liver TBARS were significantly lowered by onion peel powder. The brain 8-isoprostane level was markedly decreased by the onion flesh powder diet and the onion peel powder diet. 8-OHdG levels in DNA from kidney and brain tissue are shown in Fig. 5. Kidney and brain 8-OHdG levels did not differ among the experimental groups. There was no significant difference between the control group and
DISCUSSION

The total polyphenol content of onion samples was 13–289 mg/g dried powder. The total polyphenol content of onion peel ethanol extract was more than 20 fold greater than that of onion flesh powder and double that of onion peel powder. On the other hand, the quercetin content of the onion samples was 1.12–99.68 mg/g dried powder; onion peel ethanol extract had the greatest content followed by onion peel powder, onion flesh ethanol extract, and onion flesh powder. Quercetin accounts for approximately 8% to 35% of total polyphenols in onion samples.

Bilyk et al. (9) reported that the quercetin content of the edible portion of onions is 60–1,000 mg/kg fresh weight. Furthermore, they reported that the quercetin content accounts for 2.5–6.5% of the dry matter of onion peel. Hertog et al. (2) reported that the quercetin content of onion flesh was 347 mg/kg fresh weight. The total quercetin content of 55 yellow onion samples in the United States was 54–286 mg/kg fresh weight (28). In our study, onion flesh contained 102 mg of quercetin per kg fresh weight and onion peel contained approximately 2.1% quercetin of dried powder, which is consistent with the results of Bilyk et al. (9). In this study, the quercetin content of onion samples was estimated from the aglycone content after acid hydrolysis. Further research is needed to elucidate the in vivo effects of onion diets according to their quercetin and glucoside contents because the composition of these diets affects absorption, metabolism, and antioxidant status in vivo.

The TAS of onion peel powder and onion peel ethanol extract, which contained high levels of quercetin, was more than double that of onion flesh powder and onion flesh ethanol extract. This result indicates that high quercetin content may be associated with a high TAS. Noroozi et al. (29) recently estimated the intake of flavonols and quercetin in middle-aged men from dietary records. The range of quercetin intakes in their study was 18–82 mg/d (0.26–1.17 mg per kg body weight per day; body weight 70 kg basis). In addition, Hertog (6) reported that the consumption of total flavonoids varied from 6–64 mg/d in seven countries. Assuming that the food intake of the rats in our study was 20 g/d, quercetin consumption was 1.12 mg/d (1.81 mg per kg body weight per day) in rats fed onion flesh powder, 0.49 mg/d (0.79 mg per kg body weight per day) in rats fed onion flesh ethanol extract, 20.5 mg/d (33.06 mg per kg body weight per day) in rats fed onion peel powder, and 11.08 mg/d (17.87 mg per kg body weight per day) in rats fed onion peel ethanol extract diet. Therefore, rats fed onion flesh powder consumed an amount of quercetin similar to that reported by Noroozi et al. for humans (29). Rats fed onion peel powder consumed double the quercetin consumed by rats fed onion peel ethanol extract, 18-fold higher than that consumed by rats fed onion flesh powder and 30-fold higher than that consumed by rats fed onion flesh ethanol extract.

In onions, quercetin exists mostly in the O-glucoside form, which contains a sugar bound to carbons 3 or 4 (30). The major glycosides are quercetin 4′-O-β-glucoside and quercetin 3,4′-O-β-diglucoside (31). The quercetin content of the experimental diets was 0.006% for the onion flesh powder diet, 0.003% for the onion flesh ethanol extract diet, 0.1% for the onion peel powder diet and 0.05% for the onion peel ethanol extract diet. Each ethanol extract contained half of the quercetin content of the corresponding powder. The ethanol extraction method used in this study only extracted half the quercetin present in the onion powders.

Rats fed the onion peel powder diet had the highest plasma quercetin level of all groups and a significantly higher plasma TAS than rats fed the control diet (p<0.05). Several studies have shown that there is a strong positive correlation between plasma TAS and...
plasma quercetin concentration in humans and rats (32, 33). According to Morand et al. (33), a diet containing 0.2% quercetin elevated TAS from 0.61 mmol/L to 0.96 mmol/L in rats. In our study, plasma concentrations of quercetin metabolites were markedly increased by onion peel diets, especially the onion peel powder diet, which contained the highest concentration of quercetin. Even though the plasma quercetin levels of the onion flesh groups were significantly lower than those of the onion peel groups, plasma TAS did not differ significantly between the onion flesh and onion peel groups. Indeed, plasma TAS was elevated by onion flesh powder as well as by onion peel powder.

Several factors might have contributed to the lack of differences between elevated plasma TAS in rats fed onion samples. The composition of the four onion samples differed. Even though levels of plasma quercetin metabolites in rats fed onion peel or its extract were significantly higher than in rats fed onion flesh or its extract, the biological activity of the metabolites in rats fed onion peel or its extract may not have been sufficient to induce differences between the groups. Yamamoto et al. (34) reported that the introduction of a conjugate group at the dihydroxy group in the B ring decreases free radical scavenging activity. Quercetin-3′-glucuronide, isorhamnetin-3-O-glucuronide, and quercetin-3′-O-sulfate are the main conjugates present in human subjects who have consumed onions (35). Plasma of rats in the onion peel groups may have contained greater amounts of the less active forms of metabolites than that of the onion flesh groups. Other antioxidative compounds in onion flesh and peel such as sulfur- and selenium-containing compounds and quercetin derivatives may have contributed to the enhanced plasma TAS.

In plasma, quercetin is not present as an aglycone but occurs only in conjugated forms. Generally, about 20–40% of quercetin is methylated in the 3′-position, yielding isorhamnetin (35–37). Mullen et al. (38, 39) identified quercetin-3′-sulphate, quercetin-3′-glucuronide, isorhamnetin-3′-glucuronide, a quercetin diglucuronide, and a quercetin glucuronide sulphate in quantifiable amounts and six additional quercetin metabolites in trace quantities in humans after ingestion of onion diets. Methylation of quercetin seems to reduce its antioxidant capacity because isorhamnetin is known to be less potent than quercetin (24, 34). The methylated form of quercetin is mainly synthesized by the liver. The concentrations of conjugated quercetin and conjugated isorhamnetin as quercetin metabolites in the plasma increased markedly (quercetin 8 µmol/L and isorhamnetin 45 µmol/L, respectively) in Wistar rats fed a 0.25% quercetin diet for 14 d (25). Azuma et al. (40) also reported that almost all of the quercetin metabolites in the plasma of Wistar rats fed onion-based diets (onion intake 1 g/d; quercetin aglycone equivalent 3.9 mg/d) for 1 or 2 wk were sulfate/glucuronide conjugates of quercetin and isorhamnetin. The conjugated derivative forms of quercetin are believed to be more powerful antioxidants using a lipid oxidation in vitro model than the water-soluble form of vitamin E, even though they are less powerful than quercetin aglycone (33, 41). On the other hand, isorhamnetin has been reported to have lower antioxidative activity compared to quercetin (24, 34).

In this study, plasma quercetin levels increased markedly with the consumption of onion peel, especially with the consumption of the onion peel powder diet. This result was caused by the high quercetin content of onion peel diets compared to onion flesh diets. In addition, the ratio of isorhamnetin to quercetin in the plasma of rats fed onion peel was about 10 : 1, which was twofold higher than that of rats fed onion flesh. Manach et al. (25) suggested that a diet high in quercetin increases the ratio of isorhamnetin to quercetin in the plasma. Thus, this study also supports the notion that the active form of quercetin is effectively modulated by the liver according to quercetin availability. However, further studies are necessary to measure fecal and urinary contents of quercetin metabolites as well as plasma levels of quercetin metabolites to understand the bioavailability of quercetin in vivo.

Malondialdehyde (MDA), a major secondary product of lipid peroxidation, is toxic (42). Since Slater (43) developed the TBARS method for measuring MDA, the TBARS assay has been the most popular assay for measuring lipid peroxidation. Recently, an enzyme immunoassay was developed for measuring the concentration of 8-isoprostanes, nonenzymatically produced prostaglandins. The 8-isoprostane enzyme immunoassay is regarded as a very sensitive lipid peroxidation assay. It is useful for measuring lipid peroxide levels in brain tissue, which is highly vulnerable to free radical attack (44, 45). In the present study, the onion peel powder diet lowered the liver TBARS level significantly (p<0.05). Other onion diets also tended to lower liver TBARS levels. This antioxidant effect was more apparent for brain 8-isoprostane levels than for liver TBARS levels. The brain 8-isoprostane level was decreased markedly by the onion flesh powder or the onion peel powder diet (p<0.05). Ethanol extraction diets of onion flesh or onion peel contained less effective substances than their powder counterparts. Therefore, the powder forms of onion flesh or onion peel would be more beneficial for reducing lipid peroxides in the elderly than the ethanol extracts.

8-OHdG is generated by nucleic acid modification, predominantly from hydroxide attacks on guanidine (46). The central nervous system (CNS) appears to be particularly vulnerable to damage by reactive oxygen species (ROS). A number of factors contribute to the relatively high vulnerability of the CNS to oxidative damage, including low levels of the natural antioxidant, glutathione, in neurons (47), a high proportion of polyunsaturated fatty acids in membranes (48), and a relatively high requirement for oxygen because of the high metabolic activity of the brain (49). Furthermore, this vulnerability may increase further with aging (49). On the other hand, Schmerold and Niedermuller (50) reported that the increase in DNA damage with aging is
more conspicuous in kidney tissue than in other types of tissue. Therefore, this study investigated DNA damage in kidney and brain tissue.

Noroozi et al. (51) used the comet assay to study the effect of flavonoids and vitamin C on oxidative DNA damage in human lymphocytes. They revealed that flavonoids have a more protective effect than vitamin C on oxidative DNA damage and free flavonoids are more protective than conjugated flavonoids. Even though in vitro studies revealed the antioxidative capacity of onions, convincing in vivo evidence for a consistent effect of ingestion of onion or quercetin metabolites is lacking (52). In our study, onion diets did not decrease DNA damage significantly. The discrepancy between in vitro and in vivo studies may be partly attributable to the absorption and metabolism of onion ingredients, including quercetin derivatives.

The observed changes in antioxidative capacity resulting from onion diets differed according to the part of the onion used and the preparation method. The onion flesh powder diet enhanced antioxidative capacity by reducing lipid peroxide levels in brain tissue. Moreover, the onion flesh powder diet increased plasma TAS. The onion flesh ethanol extract diet tended to increase plasma TAS and quercetin levels and decrease brain 8-isoprostane levels. The onion peel powder diet enhanced antioxidative capacity by increasing plasma TAS and quercetin levels and by decreasing lipid peroxide levels in liver and brain tissue. The onion peel ethanol extract diet tended to increase TAS and quercetin levels and decrease brain 8-isoprostane levels.

Onion peel contains a large amount of total phenols and quercetin. As these compounds could suppress lipid oxidation in vivo, onion peel could also be a useful resource for the functional food industry. The powdered form of onion flesh or onion peel may be more effective in lowering lipid peroxide levels than their ethanol extracts. We concluded that onion flesh or onion peel enhances antioxidant status in aged rats and may be beneficial for the elderly as a means of lowering lipid peroxide levels.

Acknowledgments

This paper was supported by SangWoo Corporation as a part of the project, ‘A Study on elucidating new candidates of dietary supplements’ and supported by the Ministry of Education and Human Resources Development as a part of the second stage of Brain Korea 21 project in 2006.

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