Food Sci. Biotechnol. Vol. 18, No. 1, pp. 179 ~ 184 (2009)

Food Science and Biotechnology

© The Korean Society of Food Science and Technology

Onion Supplementation Inhibits Lipid Peroxidation and Leukocyte DNA Damage due to Oxidative Stress in High Fat-cholesterol Fed Male Rats

Jae-Hee Park, Bo-Young Seo, Kyung-Hea Lee[1], and Eunju Park*

Department of Food and Nutrition, Kyungnam University, Masan, Gyeongnam 631-701, Korea

[1]Department of Food and Nutrition, Changwon National University, Changwon, Gyeongnam 641-773, Korea

Abstract The aim of this study was to investigate effects of onion, red onion, or quercetin on plasma antioxidant vitamin, lipid peroxidation, and leukocyte DNA damage in rats fed a high fat-cholesterol diet. Forty SD male rats were assigned to normal control, high fat-cholesterol diet (HF), or HF+5% onion powder, HF+5% red onion powder, or HF+0.01% quercetin. The HF diet resulted in significantly higher plasma lipid peroxidation which decreased with onion, red onion, or quercetin supplementation. Leukocyte DNA damage induced by HF diet decreased significantly in rats fed onion and red onion, while quercetin supplementation had no effect on preventing leukocyte DNA damage. H_2O_2 induced leukocyte DNA damage exhibited a highly significant negative correlation with plasma retinol and tocopherols. These results suggest that onion or red onion powder exerts a protective effect with regard to DNA damage in rats fed HF diet. However, 0.01% quercetin in pure form might not be effective at preventing DNA damage.

Keywords: onion, red onion, quercetin, lipid soluble vitamin, lipid peroxidation, DNA damage, high fat-cholesterol diet

Introduction

Oxidative stress is known to cause oxidative damage to macromolecules such as DNA and lipids. Lipid peroxidation and oxidized DNA bases are considered important biological markers for carcinogenesis (1-3).

Vegetable and fruit intake is associated with a reduced risk of cancer and cardiovascular disease (4). Onion (*Allium cepa* L.) is one of the most widely used edible vegetables as a medicinal plant in the world (5). It is recognized as an important source of valuable phytonutrients such as flavonoids, fructooligosaccharides, thiosulfinates, and other sulphur compounds (6). In recent *in vitro* and animal experiments, onion exerts antioxidative, antidiabetic, and anticarcinogenic effects (5,7,8). The major flavonoid found in onion is quercetin, which is found in the plant structures below the surface (9). The protective effects of flavonoids in biological systems are ascribed to their capacity to transfer free radical electrons, chelate metal catalysts (10), activate antioxidant enzymes (11), and reduce α-tocopherol radicals (12). Quercetin scavenged oxygen free radicals (H_2O_2, O_2^-) and significantly inhibited lipid peroxidation of liver in a Fe^{2+} induced lipid peroxidation model (13). The biologically active fractions from onion are highly inhibitory toward angiotensin converting enzyme (14). Onions have clearly higher radical scavenging activities than garlic, with red onion having more activity than yellow onion (15). Quercetin and its dimeric form show high antioxidant activity which is comparable to that of α-tocopherol (16).

*Corresponding author: Tel: +82-55-249-2218; Fax: +82-55-244-6504
E-mail: pej@kyungnam.ac.kr
Received July 8, 2008; Revised September 2, 2008;
Accepted September 23, 2008

Recently, many people in Korea have begun to use onion as a nutraceutical food in powder or pill form because of its functional properties. However, little is known about the protective effects of onions with regard to lipid oxidation and genotoxic effects following its supplementation in various forms.

Therefore, the objective of this study was to evaluate the effects of onion powder, red onion powder, and quercetin on plasma antioxidant vitamin levels, lipid peroxidation, and leukocyte DNA damage in rats fed a high fat-cholesterol diet.

Materials and Methods

Reagents Casein, mineral mixture, and vitamin mixture were obtained from ICN Pharmaceuticals Inc. (Costa Mesa, CA, USA). Corn starch was obtained from Daesang Co. (Seoul, Korea) and corn oil and lard were obtained from Cheiljedang Co. (Seoul, Korea) and Shinhan Oil Co. (Gosung, Korea), respectively. Cyclohexane was purchased from Merck (Whitehouse Station, NJ, USA) and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Sample preparation and analysis Onion samples (green and red) were collected from the Changnyeong area and prepared by washing the skin after peeling, slicing, and chopping to the desired size, freezing, drying, and grinding them to a powder fine enough to pass through a #40 mesh. For analysis of quercetin, sample (1 g) was extracted with 50 mL of 80% methanol for 24 hr and filtered through a 0.45-μm filter. The high performance liquid chromatpgraphy (HPLC) condition for quercetin analysis was as follows, 4.6×250 mm i.d., Spherisorb ODS-2 (Altech Asso., Deerfoeld, IL, USA) 5 μm phase; solvent, 80% phosphate

Table 1. Experimental diet composition (g/kg)

	Control[1)]	HF	HF+O	HF+RO	HF+Q
Casein	150	150	150	150	150
Corn starch	576	548	498	498	548
Sucrose	100	100	100	100	100
Corn oil	60	60	60	60	60
Soybean oil	40	40	40	40	40
Lard	-	40	40	40	40
Cholesterol	-	10	10	10	10
Cellulose	25	-	-	-	-
Mineral mixture[2)]	35	35	35	35	35
Vitamin mixture[3)]	10	10	10	10	10
Choline chloride	2	2	2	2	2
DL-Methionine	1.8	1.8	1.8	1.8	1.8
Na-Taurocholate	-	3	3	3	3
Onion powder	-	-	50	-	-
Red onion powder	-		-	50	-
Quercetin	-				0.1
BHT	0.01	0.01	0.01	0.01	0.01
Total	1000	1000	1000	1000	1000

[1)]Control, AIN 93 based control diet group; HF, high fat-cholesterol diet group; HF+O, HF+5% onion powder group; HF+RO, HF+5% red onion powder group; HF+Q, HF+0.01% quercetin group.
[2)]AIN 93 mineral mixture contained (in g/kg of mixture), calcium phosphate, dibasic 500; sodium chloride 74; potassium citrate, monohydrate 220; potassium sulfate 52; magnesium oxide 24; manganous carbonate (43-48% Mn) 3.5; ferric citrate (16-17% Fe) 6; zinc carbonate (70% ZnO) 1.6; cupric carbonate (53-55% Cu) 0.3; potassium iodate 0.01; sodium selenite 0.01; chromium potassium sulfate 0.55; sucrose, finely powdered 118.03.
[3)]AIN 93 vitamin mixture contained (in g/kg of mixture): thiamine HCl 0.6; riboflavin 0.6; pyridoxine HCl 0.7; niacin 3; *d*-calcium pantothenate 1.6; folic acid 0.2; *d*-biotin 0.02; cyanocobalamin (vitamin B_{12}) 0.001; dry vitamin A palmitate (500,000 U/day) 0.8; dry vitamin E acetate (500 U/day) 10; vitamin D_3 trituration (400,000 U/g) 0.25; menadione sodium bisulfite complex 0.15; sucrose finely powdered 981.08.

buffer (pH 2.2) and 20% acetonitrile (0-15 min), 70% phosphate buffer (pH 2.2) and 30% acetonitrile (15-25 min), 60% phosphate buffer (pH 2.2), and 40% acetonitrile (25-30 min), 50% phosphate buffer (pH 2.2) and 50% acetonitrile (30-35 min); flow rate, 1 mL/min; and detection, 340 nm. The dehydrated quercetin was shown that 100 g of onion and red onion powders contain 236.0 and 87.8 mg of quercetin, respectively. The total flavonoid concentration was 277.3 and 189.0 mg/100 g of onion and red onion, respectively.

Animals and diets Eight-week-old male Sprague Dawley rats (SD, *n*=40) were purchased from Koatec Inc. (Pyeongtaek, Korea) and housed and cared for in accordance with the 'guide for care and use of laboratory animals' (Department of Health, Education, and Welfare, 1985). The rats were allowed free access to water and fed for the first week with a commercially prepared diet for adjustment. The rats were then randomly divided into 5 groups of 8 animals each and fed an AIN-93 based control diet, a high fat-cholesterol diet (HF), or HF supplemented with 5% onion (O), 5% red onion (RO), or 0.01% quercetin (Q) for 8 weeks (Table 1). Animals were monitored daily for general health, and body weights were recorded every week for the duration of the study. Feces were collected during the final 3 days and were used to determine fecal lipid profiles. At the end of the experimental period, the rats were anesthetized with ethyl ether, and blood was collected from the abdominal artery in a heparinated sterile tube. Plasma was obtained from the blood samples by centrifugation at 450×g for 30 min and stored at −80°C until required for further analysis.

Plasma lipid soluble vitamins Plasma concentrations of retinol and tocopherols were determined simultaneously by reversed phase-high pressure liquid chromatography (RP-HPLC) according to the method of Genser and Kang (17). Briefly, plasma proteins were precipitated with ethanol and lipids were extracted with *n*-hexane. After evaporation, the dry residue was dissolved in 150 μL of methanol-dichloromethane (85:15, v/v) and then 100 μL of this solution was injected into a guard-column (Merck LiChrospher 100 RP18, 10 μm, 250×4 mm). Samples were run at a flow rate of 1.0 mL/min. on a Dionex HPLC system (Summit™ HPLC; Sunnyvale, CA, USA). Absorption was monitored at 325 nm for retinol and at 295 nm for tocopherols. Concentrations were calculated from areas under the curve using an external calibration curve and then normalized to plasma total cholesterol (mg/dL). Plasma cholesterol was measured using enzymatic colorimetry with a commercial kit (Sigma-Aldrich) and a photometric autoanalyzer (CH-100 plus; SEAC, Firenze, Italy).

Baseline conjugated dienes in low density lipoprotein (LDL) The baseline LDL conjugated diene levels were determined according to the methods outlined by Ahotupa *et al.* (18), with slight modifications. Plasma (100 μL) was added to 700 μL of heparin citrate buffer (0.064 M trisodium citrate, 50,000 IU/L heparin, pH 5.05), and this suspension was incubated for 10 min at room temperature. The insoluble

lipoproteins were then sedimented by centrifugation at 1,000×g for 10 min. The pellet was resuspended in 100 μL of 0.1 M Na-phosphate buffer containing 0.9% NaCl (pH 7.4). Lipids were extracted from 100 μL of the LDL suspension with chloroform-methanol (2:1), dried under nitrogen, redissolved in cyclohexane, and analyzed at 234 nm using a spectrophotometer (Shimadzu, Tokyo, Japan). Ethylenediamine tetraacetic acid (EDTA) was added to the samples to prevent oxidation during sample preparation.

DNA damage determination by alkaline comet assay The alkaline comet assay was conducted according to Singh *et al.* (19) with little modification. The isolated lymphocytes were suspended in phosphate buffered saline (PBS) with 100 μM H_2O_2 for 5 min on ice to induce oxidative stress. Frosted slides (Fisher Scientific, Pittsburg, PA, USA) were prepared with a basal layer of 0.5% normal melting agarose, and lymphocytes mixed with 75 μL of 0.7% low melting agarose (LMA) were added to the slides. The slides were covered again with coverslips and kept in the refrigerator for 10 min. Then coverslips were removed and a top layer of 75 μL at 0.7% LMA was added and the slides were again kept cold for 10 min. After removal of the coverslips, the slides were immersed in a jar containing cold lysing solution [2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sodium laurylasarcosine (pH 10), 1% Triton X-100, and 10% dimethyl sulfoxide (DMSO) were added freshly] and stored in a refrigerator for 1 hr. After lysis, the slides were placed in a horizontal electrophoresis tank (Threeshine Co., Ltd., Daejeon, Korea). The slides were covered with fresh alkaline buffer (300 mM NaOH, 10 mM Na_2EDTA, pH 13.0) at 4°C for 40 min. To electrophorese the DNA, an electric current of 25 V/300±3 mA was applied for 20 min at 4°C. The slides were washed 3 times with neutralizing buffer (0.4 M Tris, pH 7.5) for 5 min at 4°C and then treated with ethanol for another 5 min. All of these steps were conducted under dark conditions to prevent additional DNA damage. Fifty μL of ethidium bromide (20 μL/mL) was added to each slide followed by analysis using a fluorescence microscope (Leica DMLB, Bensheim, Germany). Images of 100 randomly selected cells (50 cells from each of 2 replicate slides) were analyzed from each subject. Measurements were made by image analysis (Komet 5.0; Kinetic Imaging, Liverpool, UK) to determine the % DNA in the tail.

Statistical analysis Data were analyzed using the SPSS package for Windows (Version 12). Values were expressed as mean±standard error (SE). The data was evaluated by one-way analysis of variance (ANOVA) and the differences between the means were assessed using Duncan's multiple-range test. The differences were considered significant at $p<0.05$. Evaluation of the associations between parameters was carried out using Pearson's correlation.

Results and Discussion

Weight gain and food intake Body weight gain was not significantly different among the groups except for the HF+Q group (Table 2). Food intakes were significantly higher in HF+RO and HF+Q than the control, HF, or HF+O groups, however the food efficiency ratio (FER) was not significantly different among the groups. Therefore, body weight gain, food intake, and FER did not appear to be influenced by onion supplementation.

Retinol and tocopherols in plasma The antioxidant effects of some plant-derived compounds (e.g., α-tocopherol, carotenoids) provide plausible biological mechanisms by which high intake and plasma levels of these compounds can decrease the risk of coronary heart disease (20). The levels of plasma antioxidant vitamins are summarized in Table 3. Plasma retinol and γ- and α-tocopherol levels were significantly decreased in the HF groups compared to the

Table 2. Effect of onion, red onion, and quercetin supplementation on weight gain, food efficacy ratio of rats fed a high fat-cholesterol diet

	Control[1)]	HF	HF+O	HF+RO	HF+Q
Weight gain (g/8 week)	216.5±18.6[a2)]	231.3±23.8[ab]	208.1±23.0[a]	236.0±23.4[ab]	251.8±34.0[b]
Food intakes (g/day)	15.6±1.9[ab]	15.7±1.4[ab]	14.7±2.1[a]	16.3±1.9[b]	16.6±2.3[b]
FER[3)]	0.29±0.02[ns4)]	0.31±0.03	0.30±0.03	0.31±0.02	0.32±0.04

[1)]Control, AIN 93 based control diet group; HF, high fat-cholesterol diet group; HF+O, HF+5% onion powder group; HF+RO, HF+5% red onion powder group; HF+Q, HF+0.01% quercetin group.
[2)]Values are the mean±SEM for 8 animals in each group; values in the same row that do not share a common superscript are significantly different at $p<0.05$.
[3)]Food efficiency ratio (FER)=weight gain (g/day)/food intakes (g/day).
[4)]Not significant.

Table 3. Effect of onion, red onion and quercetin supplementation on lipid corrected plasma retinol and tocopherols in SD male rats fed a high fat-cholesterol diet

	Control[1)]	HF	HF+O	HF+RO	HF+Q
Retinol (μg/dL)	5.6±0.6[c2)]	2.6±0.4[ab]	2.7±0.2[ab]	3.2±0.5[b]	2.0±0.2[a]
γ-Tocopherol (μg/dL)	0.7±0.1[c]	0.3±0.0[ab]	0.3±0.0[ab]	0.2±0.0[a]	0.3±0.0[ab]
α-Tocopherol (μg/dL)	24.4±1.9[b]	9.5±0.9[a]	11.7±1.5[a]	9.2±1.0[a]	9.3±0.4[a]

[1)]Control, AIN 93 based control diet group; HF, high fat-cholesterol diet group; HF+O, HF+5% onion powder group; HF+RO, HF+5% red onion powder group; HF+Q, HF+0.01% quercetin group.
[2)]Values are the mean±SEM for 8 animals in each group; values in the same row that do not share a common superscript are significantly different at $p<0.05$.

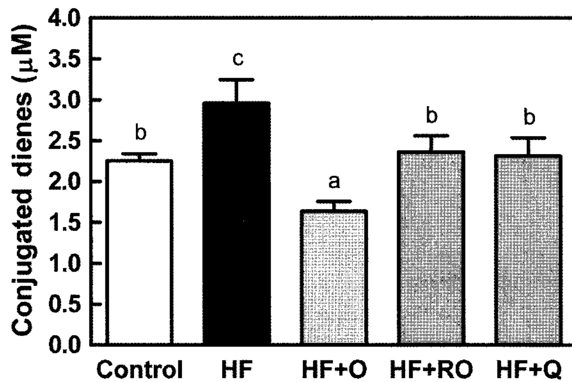

Fig. 1. Effect of onion, red onion, and quercetin supplementation on plasma lipid peroxidation (conjugated dienes) in SD male rats fed a high fat-cholesterol diet. [1]Control, AIN 93 based control diet group; HF, high fat-cholesterol diet group; HF+O, HF+5% onion powder group; HF+RO, HF +5% red onion powder group; HF+Q, HF+0.01% quercetin group. Each bar represents the mean±SE for 8 animals in each group. Bars with different superscript letters are significantly different at $p<0.05$ by Duncan's multiple-range test.

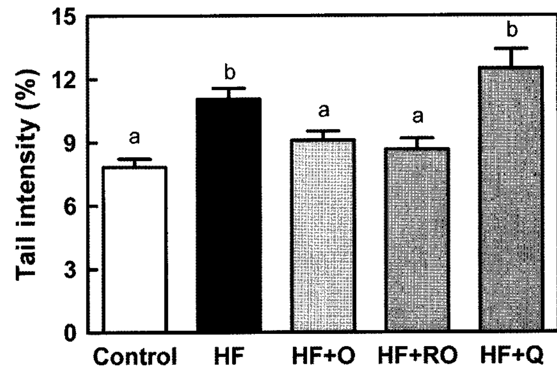

Fig. 2. Effect of onion, red onion, and quercetin supplementation on H_2O_2 induced DNA damage in leukocytes in SD male rats fed a high fat-cholesterol diet. [1]Control, AIN 93 based control diet group; HF, high fat-cholesterol diet group; HF+O, HF+5% onion powder group; HF+RO, HF+5% red onion powder group; HF+Q, HF+0.01% quercetin group. Each bar represents the mean±SE for 8 animals in each group. Bars with different superscript letters are significantly different at $p<0.05$ by Duncan's multiple-range test.

control group. This suggests that such antioxidant compounds are important for suppressing oxidative stress caused by a HF. However, plasma antioxidant vitamin levels were not improved by onion supplementation. It appears that the amount of onion supplementation in this study was insufficient to raise antioxidant vitamin levels in plasma. In agreement with our result Benito *et al.* (21) reported that status of plasma antioxidant vitamin did not increase after supplementation of 0.3% quercetin in SD rats, which is even higher than the quercetin concentration in our study (0.012% quercetin in HF+O group and 0.01% quercetin in HF+Q, respectively).

Lipid peroxidation (conjugated diene) Lipid peroxidation serves as a marker of cellular oxidative stress and has long been recognized as a major causative factor of oxidative DNA damage in such chronic diseases as atherosclerosis and cancer (22). A number of studies have reported increases in antioxidant capacity after the ingestion of flavonoid rich foods (14,23) and have suggested this may also have a protective effect against LDL oxidation, thereby slowing the progression of atherosclerosis (24). Conjugated dienes, a marker of lipid peroxidation, were significantly higher in the HF groups during the 8-week test period than in the control group (Fig. 1), while lipid peroxidation was significantly decreased in the onion, red onion, and quercetin supplemented groups. Onion exhibited a higher protective effect on lipid peroxidation than red onion, which might be ascribed to the higher contents of quercetin and total flavonoids in onion powder (daily consumed quercetin: 1.73 mg in HF+O, 0.72 mg in HF+RO, and 1.66 mg in HF+Q; daily consumed total flavonoid: 4.81 mg in HF+O and 1.35 mg in HF+RO). These results are consistent with the findings of Kim and Kim (25), who found that thiobarbituric acid reactive substance (TBARS) concentrations in LDL+VLDL fractions were significantly affected by onion peel powder containing high contents of total flavonoids. According to Yamamoto *et al.* (26), TBARS values in plasma were lower in the rats fed a high-fat high-sucrose diet containing welsh onion. However, the inhibition of LDL oxidation after HF+O supplementation was more effective than HF+Q, regardless of similar contents of quercetin. It could be ascribed to the various antioxidant compounds other than quercetin in onion.

Body cells and tissues are continuously threatened by damage caused by free radicals and reactive oxygen species, which are produced during normal oxygen metabolism or are induced by exogenous damage (27). Cells have evolved a complex network of defense barriers to counteract the generation of reactive oxygen species (ROS) by scavenging ROS and protecting against the oxidation of macromolecules. The dietary intake of antioxidants is thought to play a major role in this network (28). Compounds that have antioxidant properties, such as flavonoids, carotenoids, vitamin C, and vitamin E have been identified in large quantities in some natural foods (29). The major effect of flavonoids may be due to radical scavenging (30). By this action, flavonoids from onions could act as effective inhibitors of conjugated dienes due to the initial oxidation of LDL.

Leukocyte DNA damage The data in Fig. 2 show the effects of onion, red onion, and quercetin supplementation on DNA damage, expressed as tail intensity in rat peripheral blood cells based on the alkaline comet assay. The comet assay, also known as single cell gel electrophoresis assay has been used successfully for evaluating DNA damage and has been suggested as an excellent technique for use with biological markers in the detection, monitoring, and prognosis of chronic degenerative disease including certain types of cancer, diabetes, and atherosclerosis (31). When ROS are formed *in vitro* or by oxidative stress *in vivo*, several types of oxidative DNA lesions are formed (28). Increased oxidative stress and lipid peroxidation causing DNA damage and the disturbance of cell signaling pathways are being implicated in human cancers, neurodegenerative diseases, and atherosclerosis. It has been

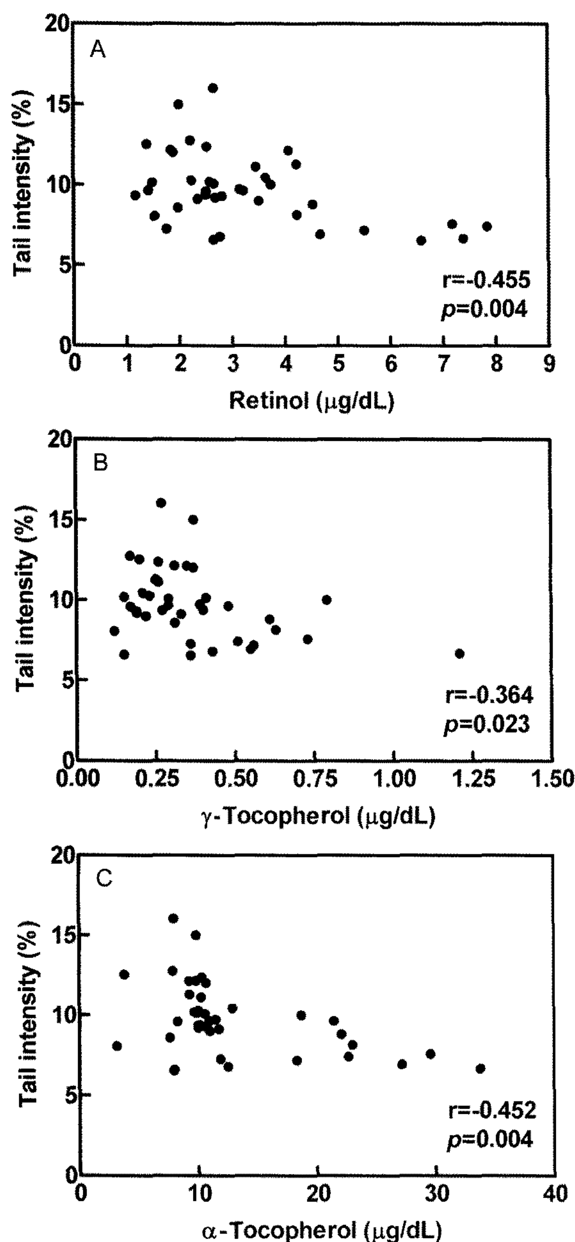

Fig. 3. Relationship between H_2O_2 induced DNA damage in leukocytes, lipid corrected plasma retinol (A), γ-tocopherol (B), and α-tocopherol (C). r=Pearson's correlation coefficient.

suggested that increased DNA damage in hypercholesterolemia is caused by the increased production of reactive oxidant species and decreased antioxidant capacity (32). Consistent with this hypothesis, the HF group showed greater leukocyte DNA damage than the control group in our study. On the contrary, leukocyte DNA damage was significantly reduced after onion or red onion supplementation. Onion and red onion appear to have exerted a greater antioxidant effect in the oxidant-challenged state. However, pure quercetin seems to have no protective effect on leukocyte DNA damage, although the quercetin content in the HF+Q group was similar to that in the HF+O group (daily consumed quercetin: 1.73 mg in HF+O vs. 1.66 mg in HF+Q).

According to Duthie *et al.* (33), incubation of cultured human cells (Caco-2, HepG2, and HeLa) and isolated lymphocytes with high concentrations of flavonols (quercetin at concentrations up to 100 μM) in the absence of an oxidative challenge increases DNA strand breakage. Johnson and Loo (34) also reported that high concentrations (100 μM) of quercetin by itself actually cause cellular DNA damage. Also, natural phytochemicals at the low levels present in fruits and vegetables offer health benefits, however these compounds might not be effective or safe when consumed in a pure form (35).

H_2O_2 induced leukocyte DNA damage showed a highly significant negative correlation with plasma retinol and tocopherol levels, although reduced plasma retinol and tocopherol levels with the HF were not affected by onion, red onion, or quercetin (Fig. 3 and Table 3). Costa and Nepomuceno (36) reported that increased plasma concentrations of antioxidant vitamins after multivitamin supplementation protects against genotoxic effects caused by a free radical generator. Serum levels of antioxidant vitamins and phytochemicals are also used as an indicator of protection against oxidative stress because they play a major role in the defense of cells and tissues (37).

In conclusion, supplementation with onion or red onion powder has significant health benefits, occurring via the inhibition of plasma lipid peroxidation and leukocyte DNA damage in rats fed a high fat-cholesterol diet.

Acknowledgments

This work is supported by the Kyungnam University Research Fund, 2009.

References

1. Frenkel K. Carcinogen-mediated oxidant formation and oxidative DNA damage. Pharmacol. Therapeut. 53: 127-166 (1992)
2. Shigenaga MK, Gimeno CJ, Ames B. Urinary 8-hydroxy-2-deoxyguanosine as a biological marker of *in vivo* oxidative DNA damage. P. Natl. Acad. Sci. USA 86: 9697-9701 (1989)
3. Floyd RA. The role of 8-hydroxyguanine in carcinogenesis. Carcinogenesis 11: 1447-1450 (1990)
4. Helim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism, and structure-activity relationships. J. Nutr. Biochem. 13: 572-584 (2002)
5. Kim SJ, Kim GH. Quantification of quercetin in different parts of onion and its DPPH radical scavenging and antibacterial activity. Food Sci. Biotechnol. 15: 39-43 (2006)
6. Slimestad R, Fossen T, Vagen IM. Onions: A source of unique dietary flavonoids. J. Agr. Food Chem. 55: 10067-10080 (2007)
7. Lee SK, Hwang JY, Kang MJ, Kim YM, Jung SH, Lee JH, Kim JI. Hypoglycemic effect of onion skin extract in animal models of diabetes mellitus. Food Sci. Biotechnol. 17: 130-134 (2008)
8. Han DS, Han JH, Oh SW, Rho SN. Effects of onion extracts on both normal and neoplastic cell proliferation and the inhibition of tumor growth in mouse. Food Sci. Biotechnol. 13: 269-273 (2004)
9. Patil BS, Pike LM. Distribution of quercetin content in different ring of various colored onion (*Allium cepa* L.) cultivars. J. Hortic. Sci. 70: 643-650 (1995)
10. Ferrali M, Signorini C, Caciotti B, Sugherini L, Ciccoli L, Giachetti D, Comporti M. Protection against oxidative damage of erythrocyte membranes by the flavonoid quercetin and its relation to iron chelating ability. FEBS Lett. 416: 123-129 (1997)
11. Elliott AJ, Scheiber SA, Thomas C, Tardini RS. Inhibition of glutathione reductase by flavonoids. Biochem. Pharmacol. 44: 1603-1608 (1992)
12. Hirano R, Sasamoto W, Matsumoto A, Itakura H, Igarashi O, Kondo K. Antioxidant ability of various flavonoids against DPPH

radicals and LDL oxidation. J. Nutr. Sci. Vitaminol. 47: 357-362 (2001)
13. Cai Q, Rahn RO, Zhang R. Dietary flavonoids, quercetin, luteolin, and geenistein, reduce oxidative DNA damage and lipid peroxidation and quench free radicals. Cancer Lett. 119: 99-107 (1997)
14. Serafini M, Ghiselli A, Ferro-Luzzi A. *In vivo* antioxidant effect of green and black tea in man. Eur. J. Clin. Nutr. 50: 28-32 (1996)
15. Nuutila AM, Puupponen-Pimiä R, Arani M, Oksman-Caldentey KM. Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. Food Chem. 81: 485-493 (2003)
16. Gülsen A, Markris DP, Kefalas P. Biomimetic oxidation of quercetin: Isolation of a naturally occurring quercetin heterodimer and evaluation of its *in vitro* antioxidant properties. Food Res. Int. 40: 7-14 (2007)
17. Genser D, Kang MH, Vogelsang H, Elmadfa I. Status of lipid-soluble antioxidants and TRAP in patients with Crohn's disease and healthy controls. Eur. J. Clin. Nutr. 53: 675-679 (1999)
18. Ahotupa M, Vasankari TJ. Baseline diene conjugation in LDL lipids: An indicator of circulating oxidized LDL. Free Radical Bio. Med. 27: 1141-1150 (1999)
19. Singh PN, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res. 175: 184-191 (1988)
20. Hennekens CH. Antioxidant vitamins and cardiovascular disease: Current knowledge and future directions. Nutrition 14: 50-51 (1998)
21. Benito S, Buxaderas S, Mitjavila MT. Flavonoid metabolites and susceptibility of rat lipoproteins to oxidation. Am. J. Physiol. Heart-C. 287: H2819-H2824 (2004)
22. Son IS, Kim JH, Sohn HY, Son KH, Kim JS, Kwon CS. Antioxidative and hypolipidemic effects of diosgenin, a steroid saponin of yam (*Dioscorea* spp.), on high-cholesterol fed rats. Biosci. Biotech. Bioch. 71: 3063-3071 (2007)
23. Whitehead TP, Thorpe GHG, Maxwell SRJ. Enhanced chemiluminescent assay for antioxidant capacity in biological fluids. Anal. Chem. 266: 265-277 (1992)
24. McAnlis GT, McEneny J, Pearce J, Young IS. Absorption and antioxidant effects of quercetin from onions, in man. Eur. J. Clin. Nutr. 53: 92-96 (1999)
25. Kim SK, Kim MK. Effect of dried powders or ethanol extracts of onion flesh and peel on lipid metabolism, antioxidative and antithrombogenic capacities in 16-month-old rats. Korean J. Nutr. 37: 623-632 (2004)
26. Yamamoto Y, Aoyama S, Hamaguchi N, Rhi GS. Antioxidative and antihypertensive effects of welsh onion on rats fed with a high-fat high-sucrose diet. Biosci. Biotech. Bioch. 69: 1311-1317 (2005)
27. de Groot H. Reactive oxygen species in tissue injury. Hepato-Gastroenterol. 41: 328-332 (1994)
28. Moller P, Loft S. Oxidative DNA damage in human white blood cells in dietary antioxidant intervention studies. Am. J. Clin. Nutr. 76: 303-310 (2002)
29. Halliwell B. Oxidative stress, nutrition, and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. Free Radical Res. 25: 57-74 (1996)
30. Nijveldt RJ, van Nood E, van Hoorn DEC, Boelens PG, van Noorren K, van Leeuwen PAM. Flavonoids: A review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr. 74: 418-425 (2001)
31. Collins AR, Gedik CM, Olmedilla B, Southon S, Bellizzi M. Oxidative DNA damage measured in human lymphocytes: Large differences between sexes and between countries, and correlation with heart disease mortality rates. FASEB J. 12: 1397-1400 (1998)
32. Harangi M, Remenyik E, Seres I, Varga Z, Katona E, Paragh G. Determination of DNA damage induced by oxidative stress in hyperlipidemic patients. Mutat. Res. 513: 17-25 (2002)
33. Duthie SJ, Johnson W, Dobson VL. The effect of dietary flavonoids on DNA damage (strand breaks and oxidaised pyrimidines) and growth human cells. Mutat. Res. 390: 141-151 (1997)
34. Johnson MK, Loo G. Effects of epigallocatechin gallate and quercetin on oxidative damage to cellular DNA. Mutat. Res. 459: 211-218 (2000)
35. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am. J. Clin. Nutr. 78: 517S-520S (2003)
36. Costa WF, Nepomuceno JC. Protective effects of a mixture of antioxidant vitamins and minerals on the genotoxicity of doxorubicin in somatic cells of *Drosophila melanogaster*. Environ. Mol. Mutagen. 47: 18-24 (2006)
37. Prior RL, Cao G. Analysis of botanicals and dietary supplements for antioxidative capacity: A review. J. AOAC. Int. 83: 950-956 (2000)