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Comparison of lymphocyte DNA damage levels and total antioxidant capacity in Korean and American diet

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BACKGROUND/OBJECTIVE: This study aims to measure the *in vitro* antioxidant capacity of Korean diet (KD) with American diet (AD) as a control group and to examine the *ex vivo* DNA damage reduction effect on human lymphocytes.
MATERIALS/METHODS: The KD applied in this study is the standard one-week meals for Koreans (2,000 kcal/day) suggested by 2010 Dietary Reference Intakes for Koreans. The AD, which is the control group, is a one-week menu (2,000 kcal/day) that consists of foods that Americans would commonly take in according to the National Health and Nutrition Examination Survey. The antioxidant capacity of each menu was measured by means of the total phenolic assay and 3 *in vitro* antioxidant activity assays (2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, trolox equivalent antioxidant capacity (TEAC), Oxygen radical absorbance capacity ($ORAC_{ROO\cdot}$)), while the extent of *ex vivo* lymphocyte DNA damage was measured by means of the comet assay.
RESULTS: When measured by means of TEAC assay, the *in vitro* antioxidant capacity of the KD of the day was higher than that of the AD ($P < 0.05$) while there was no significant difference in total phenolic contents and DPPH and ORAC assays. The *ex vivo* lymphocyte DNA damage protective effect of the KD was significantly higher than that of the AD ($P < 0.01$). As for the one-week menu combining the menus for 7 days, the total phenolic assay ($P < 0.05$) and *in vitro* antioxidant capacity ($P < 0.001$, DPPH; $P < 0.01$, TEAC) of the KD menu were significantly higher than those of the AD menu. Likewise, the *ex vivo* DNA damage reduction rate of the Korean seven-day menu was significantly higher than that of the American menu ($P < 0.01$).
CONCLUSION: This study demonstrates that the high antioxidant capacity and DNA damage protective effect of KD, which consists generally of various plant foods, are higher than those of typical AD.

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INTRODUCTION

Dietary antioxidants function to protect the body from chronic diseases [1,2] such as cardiovascular diseases, diabetes, and cancer by removing free radicals (ROS) and controlling LDL oxidation [3]. Plant foods contain a wealth of dietary antioxidants such as vitamin C, vitamin E, carotenoids, and phenolic compound. It is widely known that taking in a large quantity of fruits and vegetables increases the activity of a human body's antioxidant defense system, contributing to preventing chronic diseases related to oxidative stress [4-7].

Since Korean diet (KD) consists traditionally of side dishes including soup and plant foods in addition to boiled rice, it features a high content of carbohydrate, low content of fat, and rich plant ingredients [8-10]. Nutritional epidemiologists have reported that the plant food-centered dietary patterns of Koreans are advantageous in reducing the risk of chronic diseases [11-15]. This is probably because Korean food menus consist mainly of plant foods such as whole grains, fruits, vegetables, and seaweeds, which contain a wealth of elements that involve antioxidant activity. The Mediterranean diet [16], which is well-known for its effect to reduce the risk of chronic diseases, has been reported to show outstanding antioxidant activity, and thus it is expected that the antioxidant activity of KD would also be quite significant. Existing researches on the excellence of KD include studies on its nutritional epidemiology that clarifies the relation between Korean dietary patterns and chronic diseases [11], case-control studies [17] that examine the Korean dietary patterns of normal individuals and patients and their relations with the risk of diseases, and nutritional intervention studies [18] that observe changes in the risk of chronic diseases after the KD. In contrast, there have been few studies focusing on the antioxidant activity of the KD especially in relation with its DNA damage reduction effect.

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Existing researches at home and abroad on antioxidant activity focus mainly on individual foods or specific components [19]. Antioxidants in food may lead to synergy or inhibitive activity depending on whether they are mixed harmoniously when taken in. In order to compare Korean and American diets in terms of antioxidant activity, therefore, it is of significance to examine a food group or a whole menu rather than individual foods. However, there have been few studies both at home and abroad on the antioxidant capacity of a whole diet or a dietary pattern rather than of individual foods. Yang *et al*. [20] utilized the data of the National Health and Nutrition Examination Survey (NHANES 2001-2002) in order to analyze the level of antioxidant capacity that is common in American diet. They estimated the total dietary antioxidant capacity of a whole diet in reference to the food data of this diet recall survey and existing measurements of each food's antioxidant capacity. Russnes *et al*. [21] examined foods that the Spanish population would take in by means of the food frequency questionnaire (FFQ), and then they estimated the total dietary antioxidant capacity of a whole diet in reference to the existing FRAP value data of each food to analyze correlation with prostate cancer. Recently, the result [16] of the *in vitro* measurement of the total dietary antioxidant capacity in the Spanish Mediterranean diet has come out. The author *et al*. have measured and reported the antioxidant activity and phenolic content in KD, not those of individual foods that are included in KD [22].

In general, foods that feature a high level of antioxidant activity or a high phenolic content have outstanding DNA damage reduction effects, and such foods contribute to controlling cancer cell reproduction or preventing chronic diseases [23-25]. There are a number of nutritional intervention studies that specify the relation between dietary patterns and DNA damage reduction effects [26-28].

In an experiment, a healthy diet with vegetables and PUFA-rich plant oil was applied to patients with type 2 diabetes for 8 weeks and, as a result, DNA damage was reduced [26,28]. It was also reported that a menu with a wealth of whole grains, fruits, and vegetables in daily life can reduce DNA damage due to oxidative stress [27]. Such researches demonstrate that foods of high antioxidant capacity or diets with such foods can protect DNA from damage and control oxidative stress. However, there has been no research on the effect of KD on human DNA damage reduction.

Thus, this study aims to examine the *in vitro* antioxidant capacity and *ex vivo* lymphocyte DNA damage reduction effect of KD in comparison with those of American diet (AD) in order to clarify the excellence of KD in terms of antioxidant activity.

MATERIALS AND METHODS

Diet

As for KD, this study applies the one-week standard diet menu for Koreans as suggested in the 2010 Dietary Reference Intakes for Korean (2010 KDRIs) [29] issued by the Korean Nutrition Society. This menu was prepared in consideration of the recommended number and amount of each food group per day with 2,000 kcal as the standard for a day. In addition, standards for nutrient intakes such as vitamin, minerals, and fiber were taken into account in addition to the appropriate amount of calories. A basic adult dietary pattern with a standard amount of milk and dairy goods per meal was reflected (Table 1). Fresh ingredients for the KD menu were purchased at local

Table 1. Normal dietary pattern of Korean diet (a week, 2,000 kcal/day)[1)]

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
	KD[2)]1	KD 2	KD 3	KD 4	KD 5	KD 6	KD 7
Breakfast	Sorghum rice Spinach soup Cutlassfish (boiled down in soy sauce) Bellflower salad Stir-fried Korean-leek	Rice with beans Beef radish soup Boiled chicken Laver Cooked crown daisy	Mixed cereals rice Sea mustard soup Steamed egg Cooked spinach Cooked mungbean sprout	Rice with beans Soybean soup Grilled Spanish mackerel Stir-fried oyster mushrooms Radish salsd	Toasted bread Grilled ham Vegetable stick Milk (200 mL)	Rice with red beans Soybean paste soup with cabbage Grilled mackerel Cooked squash Cooked mungbean sprout	Rice with beans Radish soup Grilled sole (flatfish) Cooked leaf beet Japchae
Lunch	Rice Dried Pollack soup Cooked soybean Grilled tofu Chinese cabbage kimchi	Rice Yukgaejang Soft tofu Cooked sweet potato stem Green laver salad	Rice with raw fish Soybean paste soup with winter mushrooms Rakkyo	Rice Soybean paste soup with dried radish leaves Bulgogi Lettuce Cucumber salad	Soybean paste soup with chinese cabbage Hotstone pot Bibimbap Fried eggs Cucumber kimchi	Rice Seolleongtang Cooked dried radish leaves Cubed radish kimchi	Noodle soup with clams Fried fish paste Small radish kimchi
Dinner	Barley rice Soybean paste soup with chinese cabbage Fried spicy pork Leafy vegetables Green onions salad	Brown&Glutinous rice Curled mallow soup Grilled yellow croaker Squash pancake Chinese cabbage kimchi	Rice with beans Kimchi stew Cooked cabbage Grilled saury Cooked bokchoy	Mixed cereals rice Soft tofu stew Cooked chwinamul Na bak kimchi	Brown&Glutinous rice Pollack stew Cooked spinach Grilled deodeok Dotorimuk (Acorn jelly salad)	Mixed cereals rice Soybean paste stew with tofu Pyeonyuk (steamed pork) Fied Bellflower Water parsley salad	Brown&Glutinous rice Beef and mushroom stew Stir-fried anchovy Cooked eggplant
Snack	Milk Apple Citrus fruit Injeolmi	Milk Kiwi Persimmon Jeolpyeon	Liquid yogurt Strawberry Citrus fruit Sweet potato	Yoghurt Apple Pear Potato	Citrus fruit Banana	Milk Orange	Milk Strawberry Persimmon

[1)] Reference: 2010 Dietary Reference Intakes for Koreans first revision (The Korean Nutrition Society)
[2)] KD: Korean diet

Table 2. Normal dietary pattern of American diet (a week, 2,000 kcal/day)[1)]

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
	AD[2)]1	AD 2	AD 3	AD 4	AD 5	AD 6	AD 7
Breakfast	Orange juice Milk Cereal	Scrambled egg Fruit cocktail Milk Pop tarts (cherry)	Cereal Butter Milk egg Wonder bread	English muffin Kontt's berries Egg Milk	Cereal Peanut butter Milk Wonder bread Grapes	Mixed fruit (berries) Milk Chocolate muffin	Blueberries Waffles Milk Pancake Syrup
Lunch	Tortilla chips Cheddar cheese Iceberg lettuce Avocado Salsa White kidney Grounded beef Crackers	Bun (hamburger) Sloppy Joe Ketchup Alexia oven reds Peppermint candy	Bun (hamburger) Chicken tender Lettuce Tomatoes Ketchup Potato chips Peaches	Bun (hot dog) Ball park hot dogs Slice cheese Baked beans Peppermint candy	Wonder bread Turkey breast Slice cheese Tomatoes Lettuce Butter Mayonnaise dressing Potato chips	Macaroni & Cheese Orange Ball park hot dogs Bun (hot dog) Ketchup	Dinner roll Chili con carne Peaches Chocolate chip cookies
Dinner	Roseli Ravioli Pasta Sauce butter Dinner roll Brownies	Casserole(tuna noodle) Green peas Snickers	Fish fillet Sweet potatoes Salt Vanilla Ice Cream Raspberry chocolate syrup	Pasta (rigatoni) Ground turkey Pasta Sauce Cheese Spinach Salad dressing Dinner roll Butter Oranges	Pepperoni pizza Pumpkin Pie Iceberg Salad dressing	Stroganoff Peppers Dinner roll Lemonade Juice	Chicken pot pie Pineapple Dinner roll
Snack	Potato chips	peaches	Brownies	Grapes Snickers	Chocolate chip cookies	Nutty Bars Grapes	Peppermint candy

[1)] Reference: the National Institute of Agricultural Sciences of the Korea Rural Development Administration (KRDA)
[2)] AD: American diet

stores in Daejeon. Kinds and quantities of various ingredients used in each menu met the standard of CAN-pro 4.0, and the standard amounts of the main ingredients specified in the sample menu were applied.

The applied AD menu is a typical American dietary menu that was used in the assignment entitled "The Effect of KD on the Health and Nutritional Condition and a Development of a Simplified Safety Test Technology," which was conducted to compare the nutritional excellence of the recommended KD and AD by the National Institute of Agricultural Sciences, the Rural Development Administration [13]. The applied AD menu is 2,000 kcal per day. This is a menu that consists of foods that are commonly prepared for Americans according to the data of NHANES-2001-2004 (Table 2). To purchase foods that Americans would actually eat, products of the specific brands specified in the menu were purchased, and vegetables and fruits were purchased at local stores in Daejeon. Since the AD menu consisted mostly of processed foodstuffs, fruits, salads, and bread that could be taken in without additional cooking, no further ingredients except those indicated in the menu were required. The 'daily meals' sample menus were made by combining various foods that were prepared for each meal in a day. The 'one-week meals' sample menus were made by combining various foods that were prepared for each meal in one-week.

Freeze-drying of Korean and American dietary foods and production of extract samples

Freeze-drying is a way of drying foods as they are with no physical or chemical change. Since there is no protein denaturation in this process, it is possible to preserve the unique savor of foods and to store them for a long period of time [30]. Prepared Korean and American dietary foods went through the freeze-drying process, were pulverized, analyzed, and stored in a freezer at -20℃. Extracts of the Korean and American dietary foods were used to produce methanol-acetone extract samples by means of 50% methanol and 70% acetone.

Antioxidant activity assay (in vitro)

Total soluble phenolic content assay

Total soluble phenolic content was determined according to the method of Randhir *et al*. [31]. The test sample dissolved in distilled water (1 mL) was mixed with 1 mL of ethanol (95%, v/v). To each sample, 0.5 mL of Folin-Ciocalteu reagent (50%, v/v) and 1 mL of 5% Na_2CO_3 were added. The reaction mixture was left in the dark for 1 h. Its absorbance was then read at 725 nm with 99% ethanol as the blank using a spectrophotometer (Shimadzu Inc. Kyoto, Japan). A standard curve was established using various concentrations of gallic acid in 99% ethanol. Finally, the absorbance values were converted to the mg of total soluble phenolics per g of test sample.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity

In accordance with the method of Chen *et al*. [32], the test sample (10 μL) was mixed with 190 μL of 120 μM DPPH in ethanol and transferred to a microplate well. After incubation at 37°C for 30 min, the absorbance of the resulting solution was measured at 517 nm using an ELISA reader (Tecan Austria, Salzburg, Austria). The DPPH scavenging capacity of the extract was expressed as IC_{50} (the half maximal inhibitory concentration) representing the concentration of the sample that removes the 50% DPPH radical.

Trolox equivalent antioxidant capacity (TEAC) assay

To the test tube containing 990 μL of 7 mM ABTS solution with 2.45 mM $K_2S_2O_8$, 10 μL of test sample was added, and the solution was mixed. The ABTS solution was kept in the dark until use. Its absorbance was read at 734 nm using a spectrophotometer (Shimadzu Inc., Kyoto, Japan) for 6 min at 25°C. Antioxidant activity of the TEAC was represented in IC_{50} (the half maximal inhibitory concentration) [33].

Oxygen radical absorbance capacity ($ORAC_{ROO\cdot}$) assay

The ORAC assay was carried out on a Tecan GENios multi-functional plate reader (Tecan Austria, Salzburg, Austria) with fluorescent filters (excitation wavelength: 485 nm; emission filter: 535 nm). In the final assay mixture, 40 μM fluorescein was used as the target of free radical attack with either 20 mM 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) as the peroxyl radical generator in a peroxyl radical scavenging capacity ($ORAC_{ROO\cdot}$) assay [34]. Trolox (1 μM) was used as the standard control and prepared fresh daily. The analyzer was programmed to record fluorescence every 2 min after AAPH was added. All fluorescence measurements were expressed relative to the initial reading. The final results were calculated using the difference of the area under the fluorescence decay curve between the blank and the sample. $ORAC_{ROO\cdot}$ was expressed as μM of Trolox equivalents (TE). One ORAC unit is equivalent to the net protection area provided by 1 μM Trolox.

Ex vivo DNA damage determination by the alkaline comet assay

Selection of blood-gathering subjects and blood-collecting

Blood-gathering subjects included 5 healthy female adults in their 20 s, each of whom voluntarily agreed with the blood collecting process. Subjects were selected among those who had no history of chronic diseases that might cause DNA damage such as hypertension, diabetes, and cardiovascular disease, had not smoked, and had taken no vitamin or health functional food at all. The collected blood was carried in a 10 mL heparinated sterile tube (Vacutainer Becton Dickinson Co.) to the laboratory, and then the lymphocytes were separated within 3 hours. Since this was an experiment on a human body which involved the process of collecting blood, separating lymphocytes from it, processing KD and AD extracts, and comparing DNA damage reduction effects, an approval from the Institutional Review Board at Hannam University (South Korea) was gained (2013-07k) before the experiment was conducted.

Comet assay

The alkaline Comet assay was conducted as described by Singh *et al*. [35] with little modifications. Lymphocytes were separated by using 200 μL of Histopaque 1077 after mixing 100 μL fresh whole blood in 1 mL RPMI (10% FBS). The isolated lymphocytes were incubated for 30 min at 4°C with 250 μg/mL of sample extracts that showed the maximum DNA damage protective effect. The pre-treated lymphocytes were then subjected to oxidative stress by suspending in phosphate-buffered saline with 100 μmol/l H_2O_2 for 5 min at 4°C. The lymphocytes were mixed with 75 μL of 0.85% low-melting agarose and added to the slides pre-coated with 0.75% normal melting agarose. The slides were then immersed in lysis solution and were then placed into an electrophoresis tank containing 300 mmol/L NaOH and 10 mmol/L Na_2-EDTA (pH 13.0) for 40 min. The slides were electrophoresed for 20 min, washed three times with a neutralizing buffer and then treated with ethanol for another 5 min before staining with ethidium bromide to view under fluorescent microscope (Leica, Wetzlar, Germany). The image of each nucleus received through the CCD camera (Nikon, Tokyo, Japan) was analyzed by the computer-equipped comet image analyzing system (Kinetic Imaging, Liverpool, UK) [36]. The DNA damage of lymphocyte was observed by tail moment (TM), which is the multiplied value of TL (or "tail length," the distance of the DNA fragment moved from the nucleus) and % DNA in tail. The DNA damage degree was measured by a total of 150 lymphocytes or 50 cells from each of the three replicate slides.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS-PC+ v22.0, Inc., an IBM Company, Chicago, Illinois, USA) software for Windows. The significance of the mean comparison between the Korean and AD was tested by independent paired t-test. Also, a comparison of the means in 7 days was performed by one-way analysis of variance with a post-hoc comparison using the least significant difference test. Values were expressed as mean values ± SD or SE, and all the statistical significance was evaluated at the level of $\alpha = 0.05$.

RESULT

Antioxidant capacity of daily meals in Korean and American diet

The total phenolic content and *in vitro* antioxidant activity of daily meals in the one-week KD and AD menus were measured, and the results are presented in Table 3. In the one-week KD menu, the highest total phenolic content (KD6) was 63.67 ± 1.77 mg/100 g, and the lowest content (KD2) was 47.51 ± 0.62 mg/100 g respectively. In the one-week AD menu, the highest total phenolic content (AD6) was 62.89 ± 0.70 mg/100 g, and the lowest content (KD5) was 46.50 ± 0.67 mg/100 g respectively. The average phenolic content of the one-week meals was a bit higher than that of the AD (53.79 ± 6.35 mg/100 g) although the difference was insignificant. The DPPH radical scavenging capacity of a daily KD/AD menu was indicated with IC_{50}: As this value is low, it indicates that the level of antioxidant activity is high. In the KD menu, IC_{50} of KD4 was the lowest (2.65 ± 0.05 mg/mL), which indicates that its level of antioxidant activity was the highest. IC_{50} of KD6 was the highest (4.69 ± 0.16 mg/mL), indicating that its level of antioxidant activity was the lowest. In the AD menu, IC_{50} of AD3 was the lowest (3.04 ± 0.03 mg/mL), indicating that its level of antioxidant activity was the highest. IC_{50} of AD1 was the highest (4.74 ± 0.17 mg/mL), indicating that its level of antioxidant activity was the lowest. As for the average DPPH radical scavenging capacity of one-week meals of KD/AD, the IC_{50} value of the Korean meals was lower than the other, which indicates that the DPPH radical scavenging capacity of the KD was generally higher than that of the AD although the difference was statistically insignificant. As ABTS values as well were

Table 3. Total phenol content and *in vitro* antioxidant capacity of 1 day meals in Korean diet and American diet

Diet	Total phenolics (mg/100 g)	DPPH IC_{50} (mg/mL)	TEAC IC_{50} (mg/mL)	$ORAC_{ROO\cdot}$ (TE, μM)
Korean Diet (KD)				
KD1	61.03 ± 2.03[1)b2)]	2.81 ± 0.14[ab]	3.33 ± 0.07[abc]	2.29 ± 0.01[c]
KD2	47.51 ± 0.62[d]	4.62 ± 0.12[e]	3.65 ± 0.24[d]	1.74 ± 0.07[d]
KD3	60.71 ± 1.08[b]	2.97 ± 0.04[b]	3.03 ± 0.11[a]	2.18 ± 0.16[c]
KD4	61.65 ± 2.30[ab]	2.65 ± 0.05[a]	3.27 ± 0.25[abc]	3.21 ± 0.12[a]
KD5	55.90 ± 0.00[c]	3.67 ± 0.04[c]	3.13 ± 0.09[ab]	1.44 ± 0.10[e]
KD6	63.67 ± 1.77[a]	4.69 ± 0.16[e]	3.37 ± 0.12[bcd]	1.87 ± 0.10[d]
KD7	49.84 ± 0.62[d]	4.17 ± 0.03[d]	3.50 ± 0.16[cd]	2.66 ± 0.20[b]
Total mean	57.19 ± 6.10	3.65 ± 0.82	3.33 ± 0.24	2.20 ± 0.57
American Diet (AD)				
AD1	56.13 ± 0.62[c]	4.74 ± 0.17[d]	4.14 ± 0.12[b]	1.61 ± 0.01[c]
AD2	47.89 ± 0.13[d]	3.55 ± 0.06[b]	4.04 ± 0.18[b]	1.71 ± 0.06[c]
AD3	60.87 ± 1.65[b]	3.04 ± 0.03[a]	3.31 ± 0.11[a]	2.02 ± 0.15[b]
AD4	54.58 ± 0.49[c]	4.08 ± 0.17[c]	4.75 ± 0.02[c]	2.01 ± 0.12[b]
AD5	46.50 ± 0.67[d]	4.55 ± 0.20[d]	3.94 ± 0.18[b]	1.72 ± 0.10[c]
AD6	62.89 ± 0.70[a]	3.24 ± 0.06[a]	3.19 ± 0.08[a]	2.53 ± 0.11[a]
AD7	47.66 ± 1.28[d]	4.57 ± 0.12[d]	3.34 ± 0.04[a]	1.91 ± 0.11[b]
Total mean	53.79 ± 6.35[NS3)]	3.97 ± 0.66[NS]	3.81 ± 0.55*	1.93 ± 0.31[NS]

[1)] All values are expressed as Mean ± SD.
[2)] Means with different superscripts in the same column are significantly different by the least significant difference test.
[3)] Comparison of the means between Korean and American diet was performed by t-test, NS: Not Significant, * $P<0.05$
DPPH, 2,2-diphenyl-1-picrylhydrazyl; TEAC, trolox equivalent antioxidant capacity; $ORAC_{ROO\cdot}$, Oxygen radical absorbance capacity.

indicated with IC_{50}, a low value indicates a high level of antioxidant activity. As in the case of KD, there was no difference in different days of the week for the AD too. As for the average ABTS IC_{50} of the one-week meals of the KD and AD, however, that of the Korean menu was 3.33 ± 0.24 mg/mL while that of the American menu was 3.81 ± 0.55 mg/mL, which indicates that the level of antioxidant activity of the Korean menu was significantly high ($P = 0.01$). The $ORAC_{ROO\cdot}$ assay result shows that the average values of the one-week KD and AD menus showed no significant difference (Table 3).

Comparison of antioxidant capacity in one-week meals of Korean and American diet

For the total phenolic content and *in vitro* antioxidant capacity of the one-week meals of KD and AD, each meal containing the amount of food for one individual was measured and compared. The result is presented in Fig. 1. The total phenolic content in the KD is 55.04 ± 0.48 mg/100 g, which is significantly higher than that of the AD (52.94 ± 0.55 mg/100 g) (Fig. 1A). According to the result of the DPPH radical scavenging capacity assay that examined the antioxidant capacity of the one-week meals of KD and AD, the value of IC_{50} of the KD was 3.7 ± 0.05 mg/mL while that of the AD was 4.47 ± 0.08 mg/mL. Thus, the antioxidant capacity of KD meals, whose value of IC_{50} was relatively low, was significantly higher than that of AD meals ($P < 0.001$) (Fig. 1B). In the ABTS assay as well, the value of IC_{50} of KD meals was 3.39 ± 0.11 mg/mL while that of AD meals was 3.94 ± 0.08 mg/mL, which indicates

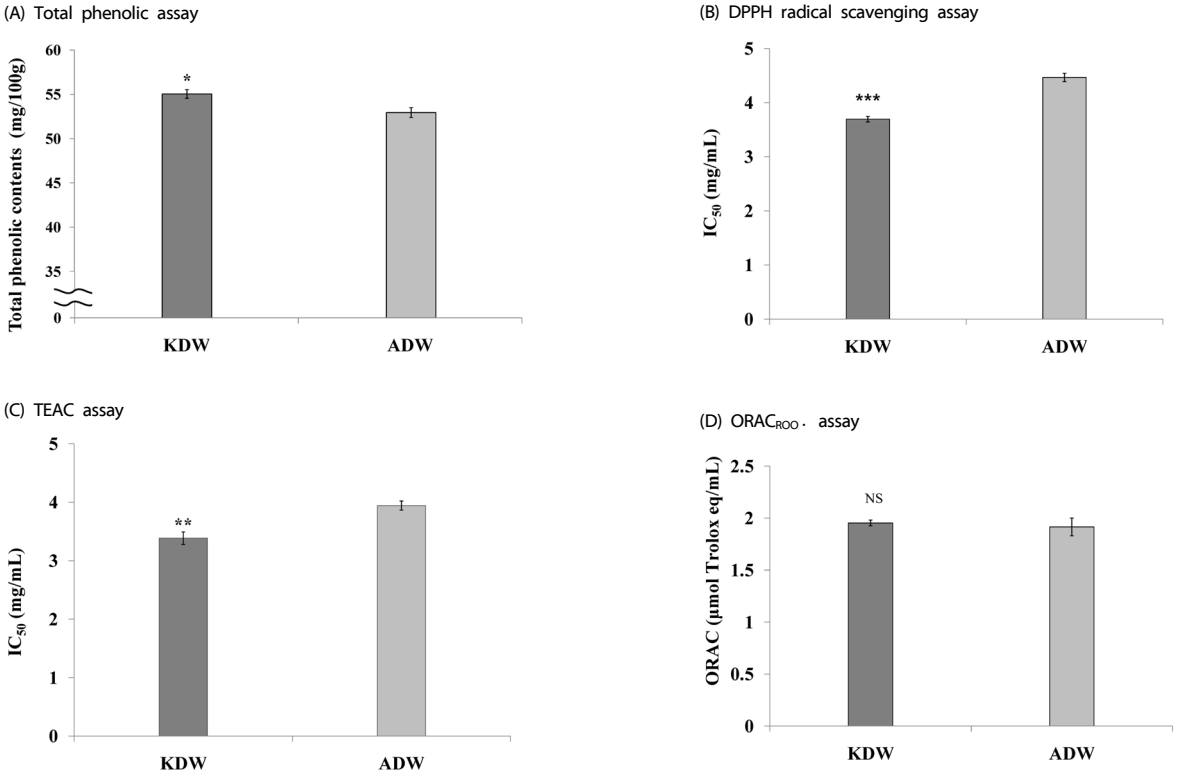

Fig. 1. Comparison of antioxidant capacity in one-week meal of Korean diet and American diet from (A) total phenolic content assay, (B) DPPH, (C) TEAC, (D) $ORAC_{ROO\cdot}$ assay. KDW, one-week Korean diet; ADW, one-week American diet. Mean ± SD, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, NS: Not Significant (t-test)

Table 4. Levels of lymphocyte DNA damage as TM, TL, TD of 1 day meals in Korean and American diet

Diet	Tail moment	Tail length	% DNA in tail
Korean Diet (KD)			
KD1	23.7 ± 1.34[1)bc2)]	31.63 ± 2.05[bc]	36.08 ± 2.04[cd]
KD2	30.78 ± 1.98[d]	41.10 ± 2.51[e]	38.90 ± 2.11[d]
KD3	19.98 ± 0.94[b]	30.26 ± 0.98[b]	28.79 ± 1.18[b]
KD4	15.02 ± 1.01[a]	23.42 ± 0.85[a]	23.53 ± 1.06[a]
KD5	25.70 ± 1.09[c]	37.47 ± 1.81[de]	33.42 ± 2.15[bc]
KD6	25.99 ± 1.50[c]	38.84 ± 1.70[de]	33.55 ± 1.57[bc]
KD7	23.34 ± 1.38[bc]	35.68 ± 1.42[cd]	29.91 ± 1.40[b]
Total mean	23.50 ± 0.67	34.07 ± 0.83	32.03 ± 0.78
American Diet (AD)			
AD[2)]1	27.85 ± 0.81[ab]	38.69 ± 1.61[bc]	34.64 ± 1.90[a]
AD2	27.15 ± 1.54[ab]	31.88 ± 1.14[a]	45.07 ± 2.59[c]
AD3	26.68 ± 1.40[ab]	35.14 ± 1.55[ab]	36.71 ± 2.16[ab]
AD4	29.36 ± 1.79[ab]	39.09 ± 2.09[bc]	39.45 ± 1.74[abc]
AD5	38.40 ± 1.19[c]	42.62 ± 2.02[c]	52.49 ± 1.77[d]
AD6	25.51 ± 1.60[a]	31.50 ± 1.59[a]	36.98 ± 0.83[ab]
AD7	31.43 ± 2.83[b]	35.71 ± 1.11[ab]	42.96 ± 4.41[bc]
Total mean	29.48 ± 0.74***[3)]	36.38 ± 0.70*	41.18 ± 1.05***

[1)] Mean ± SE; all values are relative score (%) for the positive control (H_2O_2), the maximum amount of DNA damage.
[2)] Means with different superscripts in the same column are significantly different by the least significant difference test.
[3)] Comparison of the means between Korean and American diet was performed by t-test, * P< 0.05, *** P< 0.001

that the level of antioxidant activity of the KD menu was significantly higher than that of the AD menu (P < 0.01) (Fig. 1C). As the antioxidant capacity of one-week meals of KD and AD was compared by means of the $ORAC_{ROO}$ assay, it turned out that there was no significant difference between the KD (1.95 ± 0.03 TE, μM) and the AD (1.91 ± 0.09 TE, μM) (Fig. 1D).

Lymphocyte DNA damage in daily meals in Korean and American diet

Table 4 shows the result of comparing the daily *ex vivo* DNA damage reduction effect of the one-week KD and AD menus. All the three indicators of DNA damage measured by means of the Comet assay showed significant difference among days of the week. As for the Korean menu, the value of TM was 15.02 ± 1.01 (KD4) to 30.78 ± 1.98 (KD2), the value of TL was 23.42 ± 0.85 (KD4) to 41.10 ± 2.51 (KD2), and the value of TD was 23.53 ± 1.06 (KD4) to 38.90 ± 2.11 (KD2). In contrast, the value of TM in the American menu was 25.51 ± 1.60 (AD6) to 38.40 ± 1.19 (AD5), the value of TL was 31.50 ± 1.59 (AD6) to 42.62 ± 2.02 (AD5), and the value of TD was 34.64 ± 1.90 (AD1) to 52.49 ± 1.77 (AD5), indicating that both KD and AD menus showed difference in the extent of DNA damage among days of the week. Thus, it turned out that the extent of DNA damage could be varied significantly depending on the foods and their combinations in a menu. The average extent of DNA damage per day in the one-week meals of KD (TM, 23.50 ± 0.67; TL, 34.07 ± 0.83; TD, 32.03 ± 0.78) was significantly lower than that of the one-week meals of AD (TM, P < 0.001; TD, P < 0.001; TL, P < 0.05).

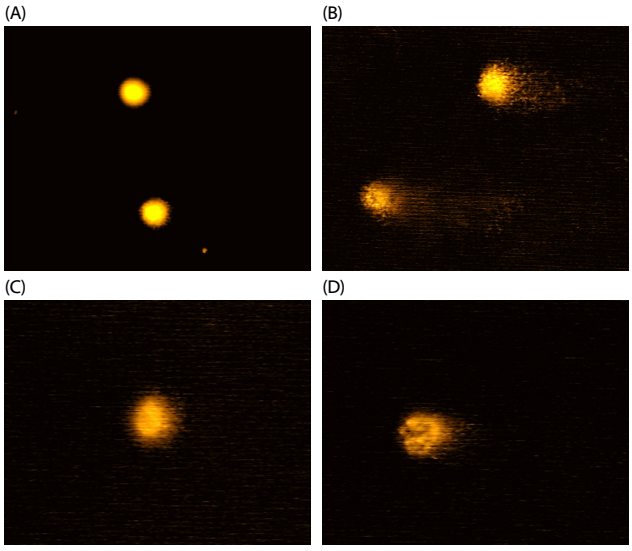

Fig. 2. Images of comets obtained by alkaline comet assay representing different degrees of DNA damage in Korean diet and American diet. (A) No damage, (B) Positive control (by H_2O_2), (C) one-week Korean diet, (D) one-week American diet

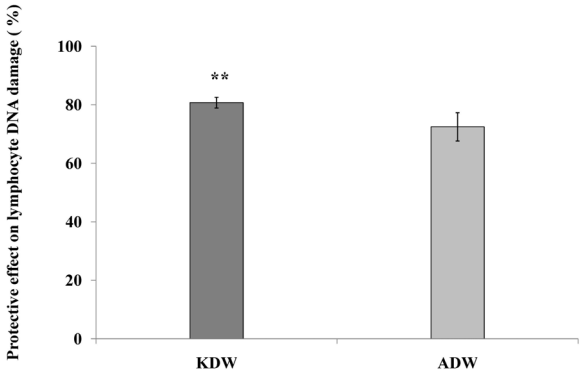

Fig. 3. Comparison of protective effect on lymphocyte DNA damage in one-week meal of Korean and American diet by tail moment. KDW, one-week Korean diet; ADW, one-week American diet. Mean ± SE, ** P< 0.01, t-test.

DNA damage reduction effect in one-week meals of Korean and American diet

For the DNA damage reduction effect of one-week meals of KD and AD, each meal containing the amount of food for one individual was measured and compared. The result is presented in Fig. 2 and 3. The level of DNA damage was reduced in the one-week meals of KD (Fig. 2C) and AD (Fig. 2D) compared to that of DNA damage (Fig. 2B) by H_2O_2. In particular, the extent of reduction in the KD was significantly higher than that in the AD. The reduction rate of DNA damage in one-week meals of KD was 80.7 ± 1.84% while that in one-week meals of AD was 72.5 ± 4.84, which indicates that the DNA damage reduction effect of the KD was significantly higher than that of the AD (P < 0.01) (Fig. 3).

DISCUSSION

A traditional Korean menu consists mainly of boiled rice, soup,

and side dishes. Rice is boiled with mixed grains such as barley and millet, while soup is made of various ingredients such as meat, fish, vegetables, etc. Side dishes include kimchi, which is a major fermented food, vegetables, meat, shellfish, etc. [37]. As the Korean society is industralized, however, the dietary composition of Koreans changes gradually: The traditional dietary pattern of carbohydrate and plant foods is changing with healthy Korean dietary patterns of grains, vegetables, fruits, eggs, fish, and dairy goods in the proper ratio. In fact, there is no specific concept of what is a typical Korean dietary pattern.

Recently, many researches on the relation between Korean dietary patterns and the risk of chronic diseases have been released [11,14,15]. In addition to such observation studies that classify dietary patterns and observe risk factors of chronic disease, intervention studies that observe changes in risk factors of chronic diseases after letting subjects have KD meals for a certain period of time were also conducted recently [12,13]. Jung *et al*. [12] observed changes in risk factors of cardiovascular diseases after letting a group of patients with hypertension and diabetes maintain KD for a certain period of time. This study applied the "Korean traditional diet" (total content of fat: 19%) that was common in the 1970s. The meal includes boiled rice and at least three side dishes of soup, kimchi, and fermented foods (made from fermented beans). It may include raw or cooked vegetables, steamed or roasted fish or meat, dried and preserved foods, etc. The menu of the control group included American dietary items such as bread, milk, and dairy goods and was prepared according to the basic principles of diet suggested by the Korean Diabetes Association. These two different menus were served to patients with hypertension and diabetes for 12 weeks. As a result, the group to which the KD menu was applied showed positive changes in risk factors of cardiovascular diseases such as blood pressure, BMI, waist measurement, heart rate, LDL cholesterol, etc. Schroeder *et al*. [13] provided adults who were overweight and obese (risk factors of cardiovascular diseases) with the KD menu, Dietary Guidelines for Americans (DGA), and the typical American diet (TA) for 4 weeks in order to examine the effect of Korean dietary patterns. The KD menu included five food groups: grains (whole grains and refined grains)/meat, fish, eggs, beans/vegetables/fruits/milk, and dairy goods in consideration of the dietary composition recommendation of 2010 KDRIs. The menu of the 2010 DGA is recommended by the U.S. Department of Agriculture. It presents various dietary patterns with vegetables, fruits, whole grains, fiber, seaweeds, lean meat, beans, nuts, low-fat dairy goods, and so forth. The TA consists of foods that Americans commonly take in. This menu was prepared based on the result of the NHANES. The percentage of vegetables, fruits, whole grains, fiber, and dairy goods is relatively low while the percentage of sodium, saturated fat, and refined grains is high. One nutritional intervention study [13] included a 4-week experiment, where the total cholesterol level and LDL decreased in the KD and DGA groups.

According to one case-control study on the relation between Koreans' dietary patterns and colorectal cancer, the Traditional pattern incorporating vegetables, mushrooms, seaweeds, beans, and fish and the Prudent pattern using fruits, milk, dairy goods, whole grains, nuts, and kimchi decreased the risk of colorectal cancer because of the intake of many fruits and dairy goods [17]. It may be difficult to summarize such existing research findings in a few words, but in general, traditional KD menus that present carbohydrate, protein, and fat in the proper composition ratio or the Acceptable Macronutrient Distribution Range (AMDR) and include a large amount of grains, vegetables, fruits, kimchi, seaweeds, fish, beans, and fermented foods have positive effects in preventing risk factors of chronic diseases, compared to Western diet menus that center on fat and protein.

The above-mentioned studies all show the relation between the risk of chronic diseases and Korean dietary patterns while there are few researches on the antioxidant activity of KD. It is well-known that when the antioxidant status is improved, oxidative stress decreases and then the risk of chronic diseases decreases accordingly. Thus, if the antioxidant activity of subjects had been analyzed in the studies mentioned above, there would have been positive results. In addition, although the Korean dietary patterns in nutritional intervention studies above were different, most of them were a type of Korean healthy diet. Accordingly, this study, which compares the antioxidant activity and DNA damage reduction effect of KD with those of AD, applies the KD menu suggested by the 2010 KDRIs as a Korean healthy dietary pattern. This menu was prepared with six food groups: grains, meat/fish/eggs/beans, vegetables, fruits, milk/dairy goods, and oil/sugars. Each meal contains an amount of food recommended for one individual each day.

In this study, the average value of *in vitro* antioxidant activity of the KD daily meals measured by means of the ABTS assay was higher than that of AD. As for the one-week meals that combined all the daily meals as well, those of KD showed a significantly higher level of antioxidant activity than those of AD when measured by means of the phenolic content analysis method ($P < 0.05$), DPPH radical scavenging capacity (assay) ($P < 0.001$), and the TEAC assay ($P < 0.01$). This result indicates that the antioxidant capacity of KD is superior to that of AD. However, one-week meals of both KD and AD consisted of different foods each day, and their levels of antioxidant activity were also different accordingly. Further, it may be difficult to affirm that the one-week meals used in this study are typical menus of KD and AD. Thus, it is necessary to compare the antioxidant activity of KD and AD in a broader range for a longer period of time. Still, this study is of significance in that it attempts to measure the level of antioxidant activity of combination diets in comparison of KD and AD while existing domestic and foreign researches on food antioxidant activity focused on individual foods or specific ingredients of individual foods while there have been few attempts to measure the antioxidant activity of one meal with various kinds of foods, one-day diets with three meals and one snack, or one-week diets that combine menus for each day. Studies on the antioxidant capacity of one meal and one-day, one-week, or one-month diets need to be conducted continually in a broader range.

The ratio of plant foods in the KD is higher than that of AD. In particular, plant foods in KD such as fruits, vegetables, mushrooms, seaweeds, nuts, potato, grains, beans, kimchi, and oils contribute a lot to its relatively high level of antioxidant capacity [22]. As for the quantity of vegetables and fruits in

the KD and AD used in this study, the average amount of vegetables and fruits (including fruit juice) per day in the case of KD was 586 g while that of AD was 339 g, which indicates that KD includes more vegetables and fruits in each meal compared to the AD. It is thought that such differences in dietary patterns result in a different level of antioxidant capacity.

Other dietary patterns that present a sufficient amount of fruits, vegetables, and whole grains and thus feature a high level of antioxidant capacity are those of the Mediterranean diet. To examine the effect of Mediterranean dietary patterns on the state of antioxidant capacity, Zamora *et al*. [38] conducted a nutritional intervention study among subjects in their 50 to 80 s with risk factors of cardiovascular diseases (smoking, hypertension, use of hyperlipidemia drug, obesity, etc.) for one year. As they measured the antioxidant capacity of plasma thereafter, the level of plasma Non-enzymatic Antioxidant Capacity increased. This result indicates that the Mediterranean diet consists mainly of plant foods of great antioxidant capacity such as fruits, vegetables, nuts, and whole grains unlike other diets [16]. KD is similar to the Mediterranean diet in that both are in a high level of antioxidant activity [22], but a deeper study needs to be conducted on other aspects that show the excellence of the KD.

There have been a number of studies on measuring the effect of DNA damage reduction of food extracts by means of the comet assay where lymphocytes were separated, food extracts went through the pre-treatment, and DNAs were intentionally damaged by means of H_2O_2 [23]. Many view that this kind of *ex vivo* experimental method is advantageous, compared to other common *in vitro* experimental methods, in applying it to human bodies [39]. Hence, this study adopts the *ex vivo* method using lymphocytes to examine the DNA damage reduction effect of one-day meals and one-week meals of KD and AD in the way of comet assay. The comet assay experiment produced two interesting results: First, there was difference in DNA damage reduction effects among the days of the week in both KD and AD, and this difference was more significant than that among *in vitro* experimental methods. This indicates that *ex vivo* DNA damage protective effect may be far more sensitive than the *in vitro* antioxidant activity depending on the daily diet in this study. In general, it is also viewed that *ex vivo* DNA damage reduction effect is a more sensitive parameter than *in vitro* antioxidant activity. Thus, the comet assay that utilizes lymphocytes is better than common *in vitro* assay methods in measuring food's antioxidant capacity.

Second, in both daily meals ($P < 0.001$) and one-week meals of diet ($P < 0.01$), the DNA damage protective effect of KD was significantly higher than that of AD. This result can be a basis for future study to demonstrate the excellence of KD in terms of antioxidant activity. It has been reported that foods of outstanding antioxidant activity show a relatively high level of DNA damage protective effect [23,24]. According to Park *et al*. [23], flavonoid compounds and antioxidant vitamins show a high level of DNA damage protective effect, and foods of a high level of DNA damage protective effect play an important role in preventing cancer or chronic diseases. Gafrikova *et al*. [24] measured the protective effect on DNAs damaged by H_2O_2 after the pre-treatment with flavonoid elements (kaempferol or quercetin) of horseradish and Armoracia rusticana extract (AE). As a result, it turned out that as for AE, DNA damage was reduced from 78% to 35.75% while as for kaempferal and quercetin, DNA damage was reduced from 83.3% to 19.4% and 16.2% respectively. A number of existing researches demonstrated that there is a high correlation between phenolic content and antioxidant capacity [16,22]. In addition, Jayakumar and Kanthimathi [25] reported that as the phenolic content of various spices was high, the DNA damage protective effect, as well as cancer cell reproduction control effect, were high accordingly. Such studies correspond to the finding of this study that the phenolic content, antioxidant activity, and DNA damage protective effect of KD are all better than those of AD.

Switzeny *et al*. [28] conducted an experiment among patients with type II diabetes where they maintained the diet with 300 g of vegetables and 25 mL of vegetable oil at each meal for 8 weeks, and the extent of DNA repair was analyzed. As a result, it turned out that the rich antioxidant substances in the meals removed free-radicals and increased the level of DNA restoration compared to the baseline. In addition, this study demonstrated a high level of correlation between DNA strand breaks and the methylation level as well. These findings verify that foods and dietary patterns of high antioxidant capacity can protect DNAs from damage and control oxidative stress. These studies correspond to the finding of this study that KD can enhance antioxidant activity compared to AD, and that the former can protect DNAs from damage by H_2O_2, having many health benefits.

However, this study has limitations in that it uses the one-week menus as samples of KD and AD to show the antioxidant activity and DNA damage reduction effect, but one week is insufficient to represent the typical dietary patterns of KD and AD. Since we eat different foods every day, either in KD or AD, it is difficult to compare KD and AD only based on one-week menus. Thus, a future study needs to prepare menus of KD and AD for a longer period to measure the antioxidant activity and DNA damage reduction effect.

This study aims to measure the *in vitro* antioxidant capacity of KD and AD, compare the DNA damage reduction effect, and clarify the excellence of KD. The findings of this study are of significance in that the excellence of KD is proven not only in terms of antioxidant activity but also DNA damage reduction effect on lymphocytes, which is methodologically more sensitive. However, more study needs to be conducted to verify if *in vitro* and *ex vivo* experiments would produce the same results in *in vivo* or nutritional intervention studies [23]. Thus, *in vivo* study needs to be conducted to demonstrate if KD substantially increases the level of plasma antioxidant capacity or decreases DNA damage in lymphocytes. Nonetheless, the findings of this study can be used as a basis to further prove the chronic disease preventive and curative effects of KD demonstrated in a number of former researches. In addition, this study measures the dietary antioxidant capacity and DNA damage reduction effect of KD not for individual foods but for one-day or one-week meals of diet. Thus, this attempt will be an important turning point for future studies on the antioxidant functions of the KD.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interests.

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