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)). 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.

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. Hence, there have been few studies both at home and abroad on the antioxidant capacity of a whole 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 PUFAprich 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 markets.

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

|   | Monday                        | Tuesday                  | Wednesday                 | Thursday                  | Friday                    | Saturday                  | Sunday                    |
|---|-------------------------------|--------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| KD | Sorghum rice                  | Rice with beans          | Mixed cereals rice        | Rice with beans           | Rice with red beans       | Rice with beans           | Rice with beans          |
|   | Spinach soup                  | Boiled chicken           | Sea mustard soup          | Soybean paste soup        | Mixed radish soup         | Baked bread               | Rice with beans          |
|   | Curtass fish (boiled down in soy sauce) | Boiled radish soup      | Steamed egg               | Grilled Spanish          | Steamed radish soup       | Grilled ham               | Radish soup              |
|   | Bellflower salad              | Laver                    | Cooked spinach            | Grilled Spanish           | Stir-fried oyster         | Vegetable stick           | Grilled mungbean         |
|   | Stir-fried Korean-leek        | Cooked crown daisy      | Cooked mungbean sprout    | Radish salat              | Radish salad              | 200 mL                    | sprout                    |

|   | Lunch                         |                         |                          |                           |                           |                           |                           |
|---|-------------------------------|--------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|   | Rice                          | Rice                     | Rice                     | Rice                      | Rice                     | Rice                      | Rice                      |
|   | Dried Pollack soup            | Rice with raw fish       | Soybean paste soup        | Soybean paste soup        | Soybean paste soup        | Soybean paste soup        | Mixed cereals rice        |
|   | Cooked soybean                | with winter              | with winter               | with winter               | with Chinese cabbage      | with Chinese cabbage      | Brown&Glutinous rice      |
|   | Grilled tofu                  | mushrooms                | mushrooms                | mushrooms                | Hotstone pot             | Pollock stew             | Brown&Glutinous rice      |
|   | Chinese cabbage kimchi        | Rakkyo                   | Rakkyo                    | Rakkyo                    | Bibimbap                 | Cooked spinach            | Brown&Glutinous rice      |
|   |                               |                          |                           |                           | Fried eggs               | Grilled saury             | Beef and mushroom         |
|   |                               |                          |                           |                           | Lettuce                  | Cooked bokchoy            | stew                      |
|   |                               |                          |                           |                           | Cucumber salat           |                           | Stir-fried anchovy         |
|   |                               |                          |                           |                           |                          |                           | Cooked eggplant           |

|   | Dinner                        |                          |                          |                           |                           |                           |                           |
|---|-------------------------------|--------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|   | Barley rice                   | Rice with beans          | Mixed cereals rice        | Rice with beans           | Brown&Glutinous rice      | Mixed cereals rice        |
|   | Soybean paste soup            | Kimchi stew              | Soft tofu stew            | Soft tofu stew            | Pollock stew             | Soybean paste stew        |
|   | with Chinese cabbage          | Grilled radish           | Grilled radish            | Grilled radish            | with tofu                | with tofu                 |
|   | Grilled yellow croaker        | saury                    | cooked bokchoy             | cooked bokchoy             | Pyeonyuk (steamed pork)  | Grilled coedoeck          |
|   | Squash pancake                | Grilled radish           |                            |                            | Fried Bellflower          | Dotorimuk (Acorn jelly)   |
|   | Chinese cabbage kimchi        | Grilled radish           |                            |                            | Water parsley salad      |                          |
|   |                               | Grilled radish           |                            |                            |                          |                          |                          |
|   | Leafy vegetables              | Mixed cereals rice       |                           | Mixed cereals             |                           |                          |                          |
|   | Green onions salad            | Dried radish             |                           | with Chinese cheese       |                           |                          |                          |

|   | Snack                         |                          |                          |                           |                           |                           |                           |
|---|-------------------------------|--------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|   | Milk                          | Milk                     | Liquid yogurt             | Yoghurt                   | Citrus fruit             | Milk                      |
|   | Apple                         | Kiwi                     | Strawberry                | Apple                     | Orange                   | Strawberry               |
|   | Citrus fruit                  | Persimmon                | Citrus fruit              | Pear                      |                          | Persimmon                |
|   | Injeolmi                      | Jeolpyeon                | Sweet potato              |                           |                          |                          |

References: 2010 Dietary Reference Intakes for Koreans first revision (The Korean Nutrition Society)

KD: Korean 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°C. 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% Na2CO3 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 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 IC50 (the half maximal inhibitory concentration) representing the concentration of the sample that removes the 50% DPPH radical.

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 sample was applied.

Table 2. Normal dietary pattern of American diet (a week, 2,000 kcal/day)\(^{1}\)

|           | Monday                  | Tuesday                | Wednesday               | Thursday                | Friday                   | Saturday                | Sunday                  |
|-----------|-------------------------|------------------------|-------------------------|-------------------------|--------------------------|-------------------------|-------------------------|
| Breakfast | Orange juice Milk Cereal | Scrambled egg Fruit cocktail Milk Pop tarts (cherry) | Cereal Butter Milk egg Wonder bread | English muffin Kolts’s berries Egg Milk | Cereal Peanut butter Milk Wonder bread Grapes | Mixed fruit (berries) Milk Chocolate muffin Grapes | Blueberries Waffles Milk Pancake Syrup |
| Lunch     | Tortilla chips Cheddar cheese Iceberg lettuce Avocado Salsa White kidney Grounded beef Crackers | Bun (hamburger) Sloppy Joe Ketchup Alexis 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 | Casserolettuna noodle Green peas Snickers | Fish fillet Sweet potatoes Salt | Pasta (rigatoni) Ground turkey Pasta Sauce Cheese Spinach Salad dressing Dinner roll Butter Oranges | Pepperoni pizza Pumpkin Pie Iceberg Salad dressing | Strogonoff Peppers Dinner roll Lemonade Juice | Chicken pot pie Pineapple Dinner roll |
| Snack     | Potato chips peaches Brownsies | Grapes Snickers Chocolate chip cookies Nutty Bars Grapes | Peppermint candy |

\(^{1}\)References: the National Institute of Agricultural Sciences of the Korea Rural Development Administration (KRDA)

\(^{2}\)AD: American diet
Troxol equivalent antioxidant capacity (TEAC) assay
To the test tube containing 990 μL of 7 mM ABTS solution with 2.45 mM K3Fe(CN)6, 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 IC50 (the half maximal inhibitory concentration) [33].

Oxygen radical absorbance capacity (ORAC)<sub>2,2’-azobis(2-amidinopropane) dihydrochloride (AAPH) as the protective effect. The pre-treated lymphocytes were then subjected to oxidative stress by suspending in phosphate-buffered saline with 100 μmol/L H2O2 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 Na2-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 α = 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 46.50 ± 0.67 mg/100 g respectively. The total phenolic content (AD6) was 62.89 ± 0.70 mg/100 g, and the lowest content (KDS) was 45.60 ± 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 IC50. As this value is low, it indicates that the level of antioxidant activity is high. In the KD menu, IC50 of KD4 was the lowest (2.65 ± 0.05 mg/mL), which indicates that its level of antioxidant activity was the highest. IC50 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, IC50 of AD3 was the lowest (3.04 ± 0.03 mg/mL), indicating that its level of antioxidant activity was the highest. IC50 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 IC50 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
Min Young Lee et al.

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

| Diet          | Total phenolics (mg/100g) | DPPH IC50 (mg/mL) | TEAC IC50 (mg/mL) | ORACROO (TE, μM) |
|---------------|---------------------------|-------------------|-------------------|------------------|
| Korean Diet   |                           |                   |                   |                  |
| KD1           | 61.03 ± 2.03a             | 2.81 ± 0.14bc     | 3.33 ± 0.07bc     | 2.29 ± 0.01c     |
| KD2           | 47.51 ± 0.62d             | 4.62 ± 0.12d      | 3.65 ± 0.24d      | 1.74 ± 0.07d     |
| KD3           | 60.01 ± 1.08d             | 2.97 ± 0.04d      | 3.03 ± 0.11d      | 2.18 ± 0.16d     |
| KD4           | 61.65 ± 2.30d             | 2.65 ± 0.05d      | 3.27 ± 0.25rc     | 3.21 ± 0.12d     |
| KD5           | 59.10 ± 0.06c             | 3.67 ± 0.04c      | 3.13 ± 0.09ms     | 1.44 ± 0.10c     |
| KD6           | 63.67 ± 1.77c             | 4.69 ± 0.16c      | 3.37 ± 0.12cs     | 1.87 ± 0.10c     |
| KD7           | 49.84 ± 0.62d             | 4.17 ± 0.03d      | 3.50 ± 0.16cd     | 2.66 ± 0.20d     |
| Total mean    | 57.19 ± 6.10              | 3.65 ± 0.82       | 3.33 ± 0.24       | 2.20 ± 0.57      |
| American Diet |                           |                   |                   |                  |
| AD1           | 56.13 ± 0.62c             | 4.74 ± 0.17d      | 4.14 ± 0.12d      | 1.61 ± 0.01c     |
| AD2           | 47.89 ± 0.13d             | 3.55 ± 0.06e      | 4.04 ± 0.18d      | 1.71 ± 0.06d     |
| AD3           | 60.87 ± 1.65b             | 3.04 ± 0.03a      | 3.31 ± 0.11a      | 2.02 ± 0.15b     |
| AD4           | 54.58 ± 0.49f             | 4.08 ± 0.17b      | 4.75 ± 0.02b      | 2.01 ± 0.12b     |
| AD5           | 46.50 ± 0.67d             | 4.55 ± 0.20f      | 3.94 ± 0.18e      | 1.72 ± 0.10f     |
| AD6           | 62.89 ± 0.70c             | 3.24 ± 0.06e      | 3.19 ± 0.08e      | 2.53 ± 0.11c     |
| AD7           | 47.66 ± 1.28d             | 4.57 ± 0.12d      | 3.34 ± 0.04c      | 1.91 ± 0.11h     |
| Total mean    | 53.79 ± 6.35cc            | 3.97 ± 0.66e      | 3.81 ± 0.55*      | 1.93 ± 0.31ns    |

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; ORACROO, Oxygen radical absorbance capacity.

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 IC50 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 IC50 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 IC50 of KD meals was 3.39 ± 0.11 mg/mL while that of AD meals was 3.94 ± 0.08 mg/mL, which indicates

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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 (KD2) 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).

**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 H2O2. 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,
As the Korean society is industrialized, however, the dietary patterns and the risk of chronic diseases have been changing with the increased consumption of high-calorie foods such as preserved foods, etc. The menu of the control group included 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 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 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 H2O2 [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 H2O2 after the pre-treatment with flavonoid elements (kaempferol or quercetin) of horseradish and Armoracia rusticana extract (AE). However, 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 H2O2, 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 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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