INTRODUCTION

Metabolic syndrome (MetS) is a cluster of risk factors for cardiovascular disease (CVD), type 2 diabetes mellitus, and cancer.\(^1\,\,^2\) The components of MetS include abdominal obesity, hyperglycemia, dyslipidemia, and hypertension, and there are complexly interconnected with each other.\(^3\,\,^5\) There are several different diagnostic criteria for MetS,\(^6\) but the worldwide prevalence is known to be about 20%–25%.\(^7\) The prevalence of MetS in Korea has reached about 32% among adults aged 30 and older\(^8\) and about 3.19% among children and adolescents.\(^9\) Therefore, an effective strategy for the prevention and treatment of MetS is needed.

Recently, oxidative stress has emerged as one of the most important features of MetS.\(^10\) A number of studies demonstrated

Antioxidant-Rich Dietary Intervention Improves Cardiometabolic Profiles and Arterial Stiffness in Elderly Koreans with Metabolic Syndrome

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Purpose: Oxidative stress plays an important role in the pathogenesis of chronic metabolic diseases. This study investigated the effect of the antioxidant-rich dietary intervention on oxidative stress, metabolic parameters, and arterial stiffness in elderly Koreans with metabolic syndrome (MetS).

Materials and Methods: Thirty-one subjects with MetS were enrolled and randomly divided into dietary intervention group and control group. Subjects in the intervention group received three meal boxes prepared with antioxidant-rich ingredients every day for 4 weeks, and subjects in the control group maintained their usual diets. Anthropometric and various biochemical parameters related to oxidative stress, inflammation, and MetS were assessed. Brachial-ankle pulse wave velocity (baPWV) and fat measurement using computed tomography were also conducted before and after 4 weeks.

Results: There were significant differences in waist circumference, visceral to subcutaneous fat ratio, lipid peroxidation, oxidized low density lipoprotein (oxLDL), systolic and diastolic blood pressure, lipid parameters, advanced glycation end products, and baPWV between before and after the study in the experimental group (all \(p<0.05\)). Significant inter-group differences were observed between the experimental and control group in terms of the differences in body mass index, waist circumference, oxygen radical absorbance capacity, protein carboxylation, lipid peroxidation, oxLDL, blood pressure, lipid parameters, and baPWV between before and after the study (all \(p<0.05\)).

Conclusion: Antioxidant-rich dietary intervention for a 4-week period ameliorated the state of oxidative stress and improved the components of MetS including central obesity, dyslipidemia, hypertension, and arterial stiffness in elderly Koreans with MetS.

Key Words: Diet therapy, oxidative stress, metabolic syndrome, vascular stiffness
that oxidative stress, along with the inflammatory state, leads to the development of MetS. One case-control study proved that oxidative stress measured by serum malondialdehyde is strongly associated with increased risk of MetS. Another cross-sectional study with polycystic ovary syndrome patients reported that MetS aggravates the oxidative stress process and reduces the anti-oxidative capacity, leading to increased levels of triglyceride (TG) and low density lipoprotein (LDL)-cholesterol. Moreover, it has been proposed that measuring oxidative stress markers may be used to identify those who are at a higher risk for CVD, and thus require more intensive care, among subjects with MetS. Considering the association between oxidative stress and MetS, a high intake of dietary antioxidants, including vitamin A, C, and E, may have positive effects on MetS. Several studies have proven beneficial effects of dietary antioxidants on alleviating oxidative stress. An experimental study showed that antioxidant from nutrients and phytochemicals may reduce the oxidative damage on DNA and LDL-cholesterol in healthy subjects. Another study on diabetes patients demonstrated that a triple antioxidant therapy consisting of vitamin E, lipoic acid, and vitamin C attenuated the oxidative stress of methemoglobin formation in vitro and reduced the hemoglobin glycation in vivo. In animal studies with diabetes mouse models, vitamin E could retard the coronary atherosclerosis by attenuating macrophage oxidative stress, and curcumin had protective effects on diabetic nephropathy by reducing renal oxidative stress.

Taken together, antioxidant-rich diet intervention may have beneficial effects on relieving oxidative stress and metabolic diseases. However, most of the previous studies were conducted with a single nutrient supplementation, such as antioxidant-rich fruit, vegetable, or a specific extract, while the patients maintained their usual main meals. In this study, we investigated the effects of well-controlled antioxidant-rich meal diet on oxidative stress and arterial stiffness in elderly Koreans with MetS.

MATERIALS AND METHODS

Study population
MetS was defined according to the updated National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP) III with the application of Asia-Pacific Region standard for abdominal obesity. The subjects who met three or more of the following criteria were enrolled: waist circumference (male ≥90 cm, female ≥85 cm); serum fasting plasma glucose ≥100 mg/dL; serum TG ≥150 mg/dL or taking triglyceride-lowering medication; serum high density lipoprotein (HDL)-cholesterol (male <40 mg/dL, female <50 mg/dL) or on medication for low HDL-cholesterol; systolic blood pressure (SBP) ≥130 mm Hg; diastolic blood pressure (DBP) ≥80 mm Hg or taking anti-hypertensive drugs. Subjects with any of the following conditions were excluded: under the age of 20 years; diagnosed with diabetes or taking glucose lowering drugs; current smoker; drinker of more than one glass alcoholic beverage per day (355-mL beer, 5-oz wine, 1.3 glass of hard liquor); have lost more than 5% of total weight in the past month; allergic or hypersensitive to certain foods; chronic or acute infectious state or history of malignancy. A total of 31 subjects were randomly assigned to experimental group (n=16) and control group (n=15) using computerized method of random list generation. The subjects were recruited from March 8, 2018 until June 20, and the final visit was August 3, 2018. All patients gave their written informed consent, and the study was approved by the Institutional Review Board (IRB) of Gangnam Severance Hospital (3-2018-0010). This clinical trial was registered at the International Clinical Trials Registry Platform (KCT0004549).

Dietary intervention
For experimental group, three meal boxes were delivered to the patient’s home every day. The food in the meal boxes were made from ingredients with high antioxidant capacity. Oxygen radical absorbance capacity (ORAC) value was used as a reference parameter to measure antioxidant capacities of the meal box. The daily values of ORAC, which were measured to determine the antioxidant capacities of the meal box, are shown in Table 1. The ingredients and cooking methods were selected by food nutritionist and cook from the central laboratory of corporation Our-Home (Seoul, Korea). The Korean Diabetes Association (KDA) recommends a daily intake of 1800 kcal for men and 1600 kcal for women with diabetes or MetS who are 60 kg in weight. Nutrients and calories in the lunch boxes were provided in accordance with these guidelines. To control any effects of other foods, the consumption of any other food was restricted in the experimental group over the duration of the study, and the control group maintained their usual diet. We checked the compliance of diet by telephone and instructed subjects in the experimental group to only eat the delivered lunch boxes and nothing else. One subject in the experi-

Table 1. Antioxidant Capacities of Daily Meals Provided to Experimental Group Measured by ORAC

| Day     | Time | Meal Box | ORAC (μg TE/100 g) |
|---------|------|----------|---------------------|
| Day 1   | 11, 21 lunch box | 3955.07 |
| Day 2   | 12, 22 lunch box | 3425.27 |
| Day 3   | 13, 23 lunch box | 3820.37 |
| Day 4   | 14, 24 lunch box | 2534.37 |
| Day 5   | 15, 25 lunch box | 3397.34 |
| Day 6   | 16, 26 lunch box | 3112.99 |
| Day 7   | 17, 27 lunch box | 2818.60 |
| Day 8   | 18, 28 lunch box | 2548.56 |
| Day 9   | 19 lunch box    | 2348.78 |
| Day 10  | 20 lunch box    | 4229.02 |

ORAC, oxygen radical absorbance capacity.
mental group was dropped out due to low compliance to the diet intervention. All subjects were trained to maintain their usual lifestyle, such as exercise and sleeping patterns, and were instructed to take medication as usual. The dietary intervention was carried out for 4 weeks.

Anthropometric and metabolic parameters
The body weight and height of subjects were measured to the nearest 0.1 kg and 0.1 cm, respectively. Body mass index (BMI) was calculated as weight (kg)/height squared (m²). The measurement of the waist circumference was made using a tape measure while the subject was standing after a full exhalation. The measuring position was the midpoint between the lowest of the ribs and the highest of the pelvis. Blood pressure (BP) was measured using electronic BP meter (HEM-7121, Omron, Kyoto, Japan) after the subjects were given rest for at least 5 minutes, and the upper arm was placed stably at the heart level.

Blood samples were taken from the antecubital vein after at least 8 hours of fasting. The samples were immediately centrifuged, and the plasma and serum samples were stored at -70°C until analysis. The fasting serum glucose concentrations were measured by Hexokinase-G6P-DH method (AU5800, Beckman Coulter, Atlanta, GA, USA), and glycated hemoglobin (HbA1c) was measured by Ion exchange-high performance liquid chromatography method (HLC-723G8, Tosoh, Tokyo, Japan). The total cholesterol, HDL-cholesterol, LDL-cholesterol, and TGs were measured enzymatically using a chemical analyzer and enzymatic immuno-inhibition method (AU5800, Beckman Coulter). Apolipoprotein-B was measured by immune-turbidimetric method (AU5800, Beckman Coulter).

Oxidized LDL (oxLDL) (10-1143-01, Mercodia, Uppsala, Sweden), advanced glycation end products (AGEs) (CSB-E09412h, Cusabio Biotech, Wuhan, China), small dense LDL (sdLDL) (MBST00740, MyBioSource, San Diego, CA, USA), tumor necrosis factor-alpha (DTA00D, R&D systems, Minneapolis, MN, USA), ORAC assay (STA-345, Cell Biolabs, San Diego, CA, USA), lipid peroxidation assay (MBS400415, MyBioSource), and protein carbonylation (STA-310, Cell Biolabs) were performed respectively. All measurements were performed according to the respective enzyme-linked immunosorbent assay manuals, and absorbance was measured using VersaMax microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Brachial-ankle pulse wave velocity
Brachial-ankle pulse wave velocity (baPWV) measurements were obtained using VP-1000 PLUS (BP-203RPE III, Omron). Study subjects were stabilized for at least 5 minutes on supine position, and the arterial pulse waves in both forearm and ankle arteries were measured simultaneously using the oscillometric method. The baPWV was calculated automatically by time phase analysis, and the distance between the upper arm and ankle was estimated based on the height. The baPWV was obtained from the right and left measurements.

Fat measurement computerized tomography
Fat measurement computerized tomography (fat CT) scan were performed on all subjects in the supine position using INGENIUTY CORE 128 (Philips, Amsterdam, the Netherlands). The single-slice CT imaging method was used to obtain the area of visceral and subcutaneous fat. The area of visceral and subcutaneous fat in the level of lumbar spine level 4 to 5 was obtained. The fat area was obtained in the attaining range of -30 to -190 Hounsfield units. The ratio of visceral fat area to subcutaneous area (V/S ratio) was used as an indicator of the amount of fat accumulation in the abdominal cavity.

Statistical analysis
The SPSS software package (Version 23.0; IBM Corp., Armonk, NY, USA) was used to perform statistical analysis. Data were presented as the mean±standard deviation for continuous variables. Baseline characteristic parameters of the experimental and control groups were compared using the Kolmogorov-Smirnov test and Shapiro-Wilk test method, which are known as the independent sample t-test method. In addition, various parameters measured before and after the study were compared using the paired test method for each group. The significance of the difference between the parameters of experimental group versus control group was analyzed by the analysis of variance test. p-values less than 0.005 were considered statistically significant.

RESULTS

Baseline characteristics
There were no differences in baseline characteristics, including age, sex, BMI, waist circumference, and visceral fat area, between the experimental and control groups (Table 2). Among 16 subjects in the experimental group, all 16 subjects had hypertension according to the NCEP-ATP III criteria, including four subjects who were on BP lowering medication. Among 15 subjects in the control group, all 15 subjects had hypertension, including five on BP-lowering medication. In terms of dyslipidemia, eight subjects in the experimental group and eight in the control group had hypertriglyceridemia or were taking statin or omega-3 fatty acids. Three subjects in both the experimental and control groups had low HDL-cholesterol level. While eight subjects in the experimental group and 11 subjects in the control group had impaired fasting glucose, none of the subjects was diagnosed with diabetes or taking glucose-lowering medication. There were no significant differences in the prevalence of hypertension and dyslipidemia nor medication history among the groups.

Changes in obesity parameters after dietary intervention
After 4 weeks of intervention, waist circumference (p=0.001)
and L4/L5 V/S ratio ($p=0.037$) showed significant differences in the experimental group (Table 2). In contrast, no statistically significant differences were observed in the control group. There were significant inter-group differences in the changes in BMI ($p=0.011$) and waist circumference ($p<0.001$) between the experimental and control groups (Table 2).

**Changes in cardiometabolic parameters and PWV**

SBP ($p=0.004$), DBP ($p=0.001$), total cholesterol ($p<0.001$), LDL-cholesterol ($p=0.001$), HDL-cholesterol ($p=0.001$), apolipoprotein-B ($p=0.001$), sdLDL ($p<0.001$), and baPWV ($p=0.031$) showed significant differences in the experimental group (Table 2). No such differences were found in the control group. Inter-group differences were observed in the changes in SBP ($p=0.011$), DBP ($p=0.001$), total cholesterol ($p=0.002$), LDL-cholesterol ($p=0.010$), HDL-cholesterol ($p=0.041$), apolipoprotein-B ($p=0.034$), sdLDL ($p=0.015$), right baPWV ($p=0.009$), and left baPWV ($p=0.032$) between the experimental and control groups. Glucose metabolism parameters showed no significant difference after 4 weeks in both groups (Table 3).

**Changes in parameters related to oxidative stress and inflammation**

There were significant differences in several oxidative stress markers and inflammatory parameters in the experimental group after dietary intervention (Table 4). Lipid peroxidation ($p<0.001$), AGEs ($p=0.002$), and oxLDL ($p<0.001$) were significantly decreased after intervention. Significant inter-group differences were observed in the changes in protein carboxylation ($p=0.030$), lipid peroxidation ($p=0.001$), and oxLDL ($p=0.001$) between the experimental and control groups (Table 4).

**DISCUSSION**

In this study, we noted that a strict, well-controlled 4-week antioxidant-rich dietary intervention led to improvements in various metabolic parameters, including abdominal obesity, BP, and dyslipidemia, as well as markers of oxidative stress and inflammation in elderly Koreans with MetS. To our knowledge, this is the first study to assess the effects of comprehensive antioxidant-rich meal diet on metabolic parameters and arterial stiffness.

Among the components of MetS, antioxidant-rich diet led to significant improvements in abdominal obesity and BP. While there were no differences in body weight or BMI, there were significant decreases in the waist circumference and the L4/L5 V/S ratio, which is a standard method for assessing visceral fat mass in the experimental group.25 This suggested that the antioxidant-rich meal is effective in improving not only abdominal obesity but also visceral obesity. As for TG and fasting glucose, although without a statistical significance, there was a tendency toward improvements. Lastly, in terms of HDL-cholesterol, the dietary intervention lowered both LDL- and HDL-cholesterol levels in this study. The fact that low-fat diet lowers both LDL-cholesterol and HDL-cholesterol may explain the reduced HDL-cholesterol in the current study.25 These results were in agreement with those of previous studies conducted with anti-oxidant rich fruits or vegetables. There are many large population studies that demonstrated the inverse associations between fruit and vegetable consumption with adiposity, BMI, or waist circumference.26-28 Also, there are various data to support the relationship between antioxidant diet and hyperlipidemia29,30 or hypertension.31,32 Recent meta-analysis on food groups and risk of hypertension showed an inverse association for the risk of hypertension for 100 g of fruits/d (relative risk: 0.97).34

When we checked the frequency of MetS after 4 weeks, we...
### Table 3. Changes in Cardiometabolic Parameters and PWV after 4 Weeks of Dietary Intervention

| Parameters | Baseline | After 4 weeks | Change | p value | p value 1 | p value 2 |
|------------|----------|---------------|--------|---------|-----------|-----------|
| SBP (mm Hg) | 140.31±12.76 | 130.73±12.77 | -9.69 | 0.032 | 0.011 | 0.006 |
| DBP (mm Hg) | 85.89±15.61 | 81.00±15.82 | -4.89 | 0.001 | 0.002 | 0.003 |
| FPG (mmol/L) | 5.57±0.47 | 5.62±0.47 | 0.15 | 0.799 | 0.799 | 0.799 |
| HbA1c (%) | 5.88±0.36 | 5.79±0.36 | -0.12 | 0.041 | 0.041 | 0.041 |
| TG (mmol/L) | 1.30±0.62 | 1.06±0.58 | -0.25 | 0.084 | 0.084 | 0.084 |
| HDL-cholesterol (mmol/L) | 0.26±0.13 | 0.26±0.13 | 0.00 | 1.000 | 1.000 | 1.000 |
| LDL-cholesterol (mmol/L) | 0.76±0.26 | 0.73±0.26 | -0.32 | 0.069 | 0.069 | 0.069 |
| ORAC (μM) | 7202.67 | 6920.17 | -282.50 | 0.081 | 0.081 | 0.081 |
| Protein carboxylation (nmol/mg) | 0.52±0.72 | 0.70±0.91 | -0.18 | 0.193 | 0.193 | 0.193 |
| Lipid peroxidation (μM/mL) | 0.13±0.46 | 0.14±0.46 | 0.01 | 0.611 | 0.611 | 0.611 |
| AGEs (ng/mL) | 32.08±15.39 | 22.20±10.88 | -9.88 | 0.064 | 0.064 | 0.064 |
| Oxidized LDL (μg/mL) | 4.47±1.53 | 3.07±1.28 | -1.40 | 0.017 | 0.017 | 0.017 |

### Table 4. Changes in Parameters Related to Oxidative Stress and Inflammation after 4 Weeks of Dietary Intervention

| Parameters | Baseline | After 4 weeks | Change | p value | p value 1 | p value 2 |
|------------|----------|---------------|--------|---------|-----------|-----------|
| CRP (mg/L) | 0.88±0.15 | 0.61±0.13 | -0.27 | 0.002 | 0.002 | 0.002 |
| TNF-α (pg/mL) | 4.47±1.53 | 3.07±1.28 | -1.40 | 0.017 | 0.017 | 0.017 |

Data are presented as means ± SD. *p* paired t-test between before and after 4-weeks; †p independent t-test for after 4-weeks measurement between experimental and control groups; ‡p repeated-measures ANOVA for intergroup difference for changes between before and after the study.
found that 10 subjects in the experimental group and nine subjects in the control group no longer had MetS after the intervention. In contrast to individual components of MetS, a comparable percent of subjects in the experimental and control groups had remission, and there are several possible explanations for this discrepancy. Most of the subjects satisfied three components and few with four components of MetS; and in many cases, the values for each component were slightly higher than the diagnostic criteria at the time of baseline study. Therefore, a slight improvement in metabolic parameters in the experimental group led to the remission of MetS.

In addition to conventional parameters, antioxidant-rich diet improved other markers of CVD, including apolipoprotein-B and sdLDL. sdLDL is known to have greater atherogenic potential compared to other LDL subfractions and LDL-cholesterol itself, and is considered to predict CVD better than total LDL-cholesterol. Moreover, antioxidant-rich diet lowered baPWV, which is a non-invasive, widely used method for assessing arterial stiffness and predicting CVD. Previous studies have demonstrated that circulating sdLDL readily undergoes multiple atherogenic modifications in blood plasma, such as desialylation, glycation, and oxidation, which further increase its atherogenicity.

There were significant decrements of oxiLDL, lipid peroxidation, and AGEs in the experimental group with significant intergroup differences in these parameters between the experimental and control groups. These results imply that antioxidant-rich diet for 4 weeks could reduce oxidative stress and increase antioxidant capacity. Previous studies reported similar results to our study. Subjects with higher intake of fruits and vegetables showed lower levels of plasma oxLDL, and inverse relationships were noted between the amount of fruit and vegetable intake and oxidative stress in adolescents, middle-aged men, and premenopausal women. In addition, carotenoid consumption, which is a representative dietary antioxidant, was related to lowering of oxidative stress markers, lipid oxidation, and DNA damage.

The underlying mechanism for the improved metabolic parameters, arterial stiffness, and oxidative stress is not elucidated in this study. However, considering the altered antioxidant and inflammatory status of subjects with MetS, the abundant intake of various functional nutrients, such as vitamins, minerals, phytochemicals, and dietary fibers, in our prepared meal box may have contributed to reducing the damage caused by oxidative stress. This is supported by the World Health Organization recommendation which encourages eating >400 g of fruits and vegetables per day to prevent chronic diseases.

Several limitations should be mentioned in this study. First, since the sample size of this study was relatively small, large-scale study with a cross-over study design is needed to confirm the effect of our results. Second, we did not check the long-term effect of diet after the study. Also, we could not confirm whether the dietary effects return to normal after the end of intervention. In addition, since our study subjects were Korean adults with MetS, we cannot generalize our results to other population. Finally, the meal box was not provide to the control group. Although the control subjects were told to resume their usual diet, their diet was not controlled like the experimental group. Despite these limitations, our study has several strengths distinguished from previous studies. While previous studies investigated the effects anti-oxidant rich diet by providing nutritional education or supplementing of one or two fruits or vegetables, our study provided three comprehensive antioxidant-rich meal box every day for 4 weeks. Since this was a well-controlled, prospective study, it enabled us to demonstrate the cause and effect of antioxidant-rich diet on metabolic health. Also, we analyzed various metabolic parameters along with antioxidant capacity and oxidative stress, as well as arterial stiffness, in this study.

In conclusion, a 4-week comprehensive antioxidant-rich dietary intervention improved the components of MetS, including central obesity, hypertension, and dyslipidemia, as well as arterial stiffness and oxidative stress in elderly Korean adults with MetS.

ACKNOWLEDGEMENTS

Se Jin Kim and Jung Yun Choi from OUTHOME provided the support for antioxidant meal preparation.

This work was supported by the Food Research Institute, OURHOME Co., Ltd.

AUTHOR CONTRIBUTIONS

Conceptualization: Chul Woo Ahn. Data curation: Jung Hye Kim and Arim Choi. Formal analysis: Jung Hye Kim and Hye-Kyung Chung. Funding acquisition: Chul Woo Ahn. Investigation: Yu-Sik Kim and Ji Sun Nam. Methodology: Jung Hye Kim and Yu-Sik Kim. Project administration: Yu-Sik Kim and Ji Sun Nam. Resources: Jung Hye Kim and Arim Choi. Software: Jung Hye Kim. Supervision: Yu-Sik Kim and Ji Sun Nam. Validation: Ji Sun Nam. Visualization: Hye-Kyung Chung and Ji Sun Nam. Writing—original draft: Jung Hye Kim. Writing—review & editing: Hye-Kyung Chung and Ji Sun Nam. Approval of final manuscript: all authors.

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