The effect of dietary intervention on reduction of oxidative stress of patients with metabolic syndrome

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Directed by Professor Chul Woo Ahn

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ABSTRACT

The effect of dietary intervention on reduction of oxidative stress of patients with metabolic syndrome

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Metabolic syndrome (MetS) is a common metabolic disorder characterized by abnormal and cardiovascular risk factors. Insulin resistance is known to be the biggest cause of MetS. However, oxidative stress often causes MetS.

Oxidative stress is a condition imbalanced between the production and inactivation or reactive oxygen species (ROS). In our body, ROS causes damage to DNA, lipids and proteins. In addition, oxidative stress can lead to cell injury and death. Therefore, controlling oxidative stress is important in MetS and also play a crucial role in atherosclerosis. There are researches reporting the patients with MetS have high risk of cardiovascular disease (CVD).

Therefore, we used diet intervention to reduce oxidative stress. The intervention groups ingested foods which have a lot of antioxidants. As a results, in intervention group, lipid peroxidation which is oxidative stress index was decreased and tumor necrosis factor-α (inflammatory marker) was decreased. The oxidized low density lipoprotein and advanced glycation end-product (risk factors of atherosclerosis) was also decreased. In addition, blood pressure and pulse wave velocity decreased. Thus, it suggests that intake foods which have a lot of antioxidants reduce oxidative stress and promote vascular health in patients with MetS.

Key words: metabolic syndrome, oxidative stress, antioxidants, diet interventions

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I. INTRODUCTION

The metabolic syndrome (MetS) is clusters of metabolic abnormalities and cardiovascular risk factors, such as insulin resistance, hypertension, central obesity, and atherogenic dyslipidemia.[1-3] The patient with MetS has two or more of the following conditions: high blood pressure, high plasma insulin, high plasma triglyceride, low HDL or impaired blood glucose regulation.[3] The criteria for MetS diagnosis is based on waist circumference (WC), blood pressure (BP), blood glucose, triglyceride and HDL-cholesterol. Diagnostic criteria have been developed by the World Health Organization (WHO) in 1998, and then subsequently have been developed by the European Group for Study of Insulin Resistance (EGIR) and the National Cholesterol Education Program Adult Treatment Panel III (NCEP: ATP III). Many organs have formulated definitions that agree on the essential components, such as glucose intolerance, obesity, hypertension, and dyslipidemia.[4]

Since the prevalence of MetS is dependent on the definitions and criteria, it is difficult to estimate the prevalence of MetS. Although, many researches have reported the number of

patients with MetS has increased worldwide. The prevalence of MetS adults has varied depending on the study designs and different criteria[5], but using IDF criteria, the incidence of the MetS among adults was 13.5% for men and 15.0% for women in Korea.[6] The prevalence of MetS is increasing at an annual rate of 0.4% in Korea.[7] In the United States (US), the incidence of type II diabetes mellitus (T2DM) has increased to 25.2% with age.[1] Mohammad suggested that one billion people world are MetS patients because MetS occurs about three times more than diabetes.[1]

Previous researches have reported that the patients with MetS have a high risk of cardiovascular disease (CVD)[8-11] and T2DM[8,12-14]. Peter *et al*. reported that the MetS has increased, and the relative risks of T2DM and CVD have also increased in both sexes with MetS.[8] Kim et al. was shown that MetS was associated with CVD in Korea.[15] Isomaa et al. the Patients with MetS had three-fold higher an incidence of CVD than in patients without in Finland and Sweden.[10] Alexander *et al.* reported that in the population aged >50 years in US, over 80% of participants with diabetes also have MetS.[14]

Metabolic syndrome has several causes such as insulin resistance, hyperinsulinemia, visceral adioposity, dyslipidemia, hypothalamic-pituitary axis dysregulation. Insulin resistance is strongly associated with MetS.[2-4] Which is defined as a failure of target organs to responding normally to the action of insulin. Insulin resistance promotes organ dysfunction and disease. In insulin resistance, impaired muscle glycogen synthesis results in reducing intracellular glucose translocation.[16] In adipocyte, insulin resistance induces and increases lipolysis, an increase adipocyte size and inflammation.[17] In liver, insulin resistance results in increased glucose output via gluconeogenesis, accumulation of triglyceride (TG), increased very low density lipoprotein (VLDL) production and decreased insulin clearance.[18] In pancreas β-cells, insulin secretion is impaired. In addition, since insulin plays crucial role in nitric oxide production as an aspect of endothelial function, impaired insulin response can be expected to be associated with endothelial dysfunction.[17] The cluster of abnormalities which occur more frequently in insulin resistant individuals includes glucose intolerance, dyslipidemia, endothelial dysfunction and elevated inflammatory markers.

MetS is often characterized by oxidative stress.[19] Oxidative stress resulted from imbalance between the production and inactivation of reactive oxygen species (ROS).[19,20]

ROS are produced by aerobic metabolism and belongs to free radical which is chemical species that containing unpaired electrons. The examples of ROS are the superoxide radical (O_2^-), the hydroxyl radical (·OH), and hydrogen peroxide (H_2O_2) along with reactive nitrogen species, such as nitric oxide (NO ·) and the peroxynitrite radical ($ONOO^-$).[19] Normally, ROS is present at a very low concentration, but increasing intracellular level of ROS causes damage to DNA, lipids and proteins.[21] Oxidative stress can lead to cell injury and death.[22] When ROS balance is broken, various biological molecules are formed by ROS. They are oxidized proteins (i.e. protein carbonyls), oxidized lipids (i.e. lipid hydro peroxides, oxidized low-density lipoproteins (oxLDL), 4-hydroxynonenal (4-HNE)) and oxidized carbohydrates (glycated products).

Oxidative stress is controlled by endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione peroxidase (GPX) etc., in our body.[19,23] The exogenous antioxidants are also used. Exogenous antioxidants, including hydrophilic as vitamin C, flavonoids, lipophilic as vitamin E and carotenoids are included in fruits and vegetables.[24] Therefore, fruits and vegetables which possess antioxidant can lead to reduce oxidative stress in the body. You-Jin et al suggested that antioxidant from nutrients and phytochemicals in healthy subjects may be beneficial providing resistance to oxidative damage to DNA and LDL.[25] Oxidative stress is associated with the pathogenesis of several chronic disorders such as CVD, diabetes, hypercholesterolemia, and atherosclerosis. Therefore, it is important to reduce oxidative stress in patients with MetS.

Dietary intake of antioxidants reduces the risk factors for CVD, diabetes and its complications. For example, more than five servings of fruit and vegetables per day is associated with 17% reduction in coronary heart disease (CHD) risk.[26,27] The researches have shown that higher consumption of fruits and vegetables is associated with a lower risk of all-cause mortality, particularly cardiovascular mortality excluding cancer mortality.[28,29]

Several studies on antioxidants as protective agents have been conducted. Coleman suggested that triple antioxidant therapy (vitamin E, lipoic acid and vitamin C) in diabetic volunteers attenuates the experimental oxidative stress of methemoglobin formation in vitro and reduces hemoglobin glycation in vivo.[30] In diabetic BALB/c mice model, the beneficial effect of vitamin E was observed to retard coronary atherosclerosis accelerated which was by

diabetes mellitus (DM).[31] The antioxidant vitamin E can attenuate macrophage oxidative stress which exists in diabetes mellitus (DM) and lead to accelerated atherosclerosis development.[32] Taken together, antioxidant therapy is beneficial for health.

In this study, we studied whether dietary control with foods known to reduce oxidative stress could alleviate the increased oxidative stress in the MetS.

II. MATERIALS AND METHODS

1. Types of participants

The subjects who met three or more items of IDF criteria for metabolic syndrome were selected. A total of 32 patients were assessed at baseline of criteria which are shown in Table 1 and 2. Figure 1 displays a flow chart of study. The subjects were randomly assigned to either the intervention (experimental) group or the control group. Finally, study was conducted with 15 control subjects and 16 experimental subjects, except for one person from the control group. All participants were informed about the study and agreed upon.

Table 1. Definition of Metabolic syndrome from IDF

List	Standard
Central obesity (South Asians)	Male: ≥90 cm (35.4 inch) Female: ≥80 cm (33.5 inch)
TG	≥150 mg/dL
HDL-C	Male: ≤40 mg/dL Female: ≤50 mg/dL
BP	Systolic BP: ≥130 mmHg Diastolic BP: ≥85 mmHg
FPG	≥100 mg/dL

TG, Triglycerides; HDL-C, high-density lipid cholesterol; BP, Blood pressure; FPG, fasting plasma glucose

Table 2. Exclusion criteria of participants

List	Standard
Smoking	Smoker
Drinking	More than 1 glass per day (beer 355 ml, wine 5 oz, soju 1.3 glasses)
Exercise	Exercise regularly
Weight	Weight changes over ±5 % of the original weight during the past month
Medication	Taking two or more medicines to treat chronic diseases
Surgery	A person who had surgery within past year
etc.	A person who is allergic or sensitive to certain foods. People who are difficult to participate in the experiment at the doctor's discretion

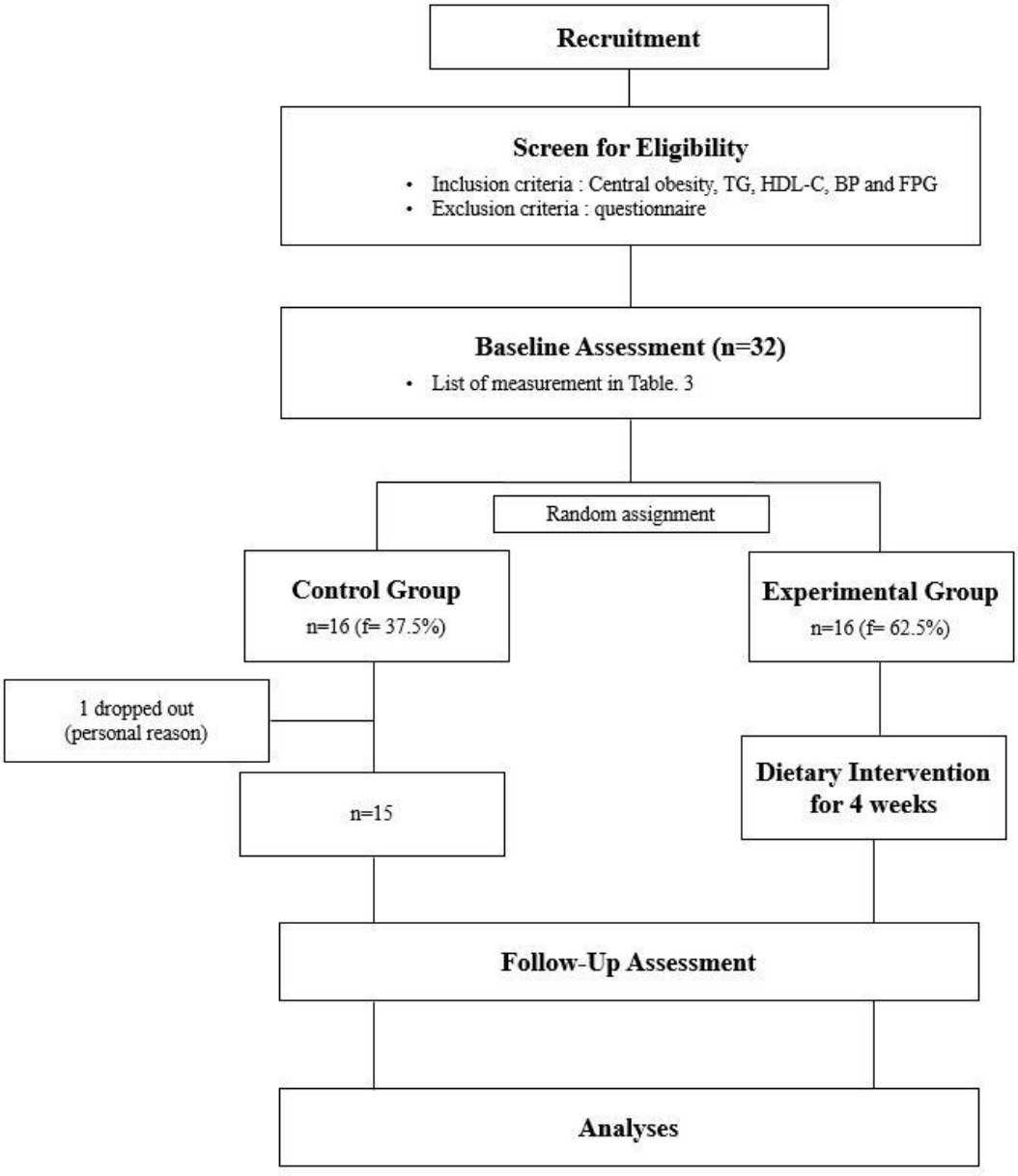

Figure 1. Participants flow

2. Dietary intervention

For dietary intervention, lunch were provided and those were designed to reduce oxidative stress. It consisted of large fruits and vegetables which contained more antioxidants.[33] Therefore, we expected high antioxidant capacity. We analyzed information of cooking methods, functional ingredients, nutrients and harmful substances. Information analysis, selected materials and cooking methods were experimented by food nutritionist and cook from central laboratory of corporation Our-Home (Korea). The lunch box provided for intervention group consists of three meals and snacks per day. According to Korean Diabetes Association (KDA), caloric intake of a person who is 60kg is 1800kcal, therefore 1800kcal were offered per day. To control any effect of certain foods, intakes of foods were restricted for the duration of study. Instead the control group maintained usual diet. All participants were trained to maintain the usual lifestyle such as exercises except diet. The dietary control was carried out for 4 weeks.

3. Measurements

The lists we measured are shown in Table. 3.

A. Blood sample

After fasting overnight (minimum 8 hours), blood samples from all study participants were collected in 5 ml of serum separating tube (SST) and ethylenediaminetetraacetic acid (EDTA) tube. To separate serum, SST was centrifuged for 10 min at 3,000 rpm at 4℃.

B. Physical examination and medical interview

We measured weight (kg), height (m^2), waist circumference (WC, cm) and Body Mass Index (BMI). BMI calculated weight (kg) divided by the square of the height (m^2). It has the highest correlation with body fat mass and is the most widely used index. We checked on the lifestyle, smoking, drinking, the medicine being taken and personal medical history through questionnaires.

C. ELISA assay kit

We measured oxidized low density lipoprotein (oxLDL), advanced glycation end-products (AGEs), tumor necrosis factor α (TNFα) and protein carbonylation. We used ELISA assay kit oxLDL (10-1143-01, Mercodia, Uppsala, Sewden), AGEs (CSB-E09412h, Cusabio Biotech, USA), sdLDL (MBS700740, MyBioSource, San Diego, California, USA), TNF-α (DTA00D, R&D systems, Minneapolis, Minnesota, USA) and protein carbonylation (STA-310, Cell Biolabs, San Diego, California, USA) respectively. All measurements were performed according to the respective ELISA manuals and absorbance was measured using VersaMax™ microplate reader (Molecular Devices, Sunnyvale, California, USA).

D. Lipid peroxidation assay kit

We used lipid peroxidation colorimetric assay kit (MBS480415, MyBioSource, San Diego, California, USA) to measure level of lipid peroxidation.

E. Oxidative Radical Antioxidant Capacity (ORAC) assay

We measured antioxidant capacity of blood through ORAC (STA-345, Cell Biolabs, San Diego, California, USA) kit.

F. Blood pressure (BP)

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by an experienced technician by placing the arm at heart level. TM-2655P (HANLIM technology, Seoul, Korea) was used to estimate BP.

G. Fasting plasma glucose (FPG), total cholesterol (TC), high-density lipid cholesterol (HDL-C), low-density lipid cholesterol (LDL-C), triglycerides (TG), fasting C-peptide, fasting insulin, apolipoprotein-B (Apo-B), C-reactive protein (CRP), Cystatin-C and estimated glomerular filtration rate (eGFR)

We used the blood sample to measure the above. FPG, TC, HDL-C, LDL-C, TG, Apo-B, CRP, and Cystatin-C were measured using AU5800 (Beckman Coulter, Brea, California, USA). Fasting C-peptide and Fasting insulin were measured using Cobas e602 (Roche, Basel, Switzerland). eGFR was estimated using the Jaffe IDMS-traceable. Creatinine was measured using AU5800 (Beckman Coulter, Brea, California, USA).

H. Insulin resistance

We used a homeostasis model assessment of insulin resistance (HOMA-IR) and homeostatic model assessment-beta cell function (HOMA-%B) to quantify insulin resistance and beta-cell function. Insulin resistance was estimated using following formula.

HOMA-IR (Homeostasis model assessment of insulin resistance) = Fasting blood insulin (uU/mL) x fasting plasma glucose (mg/dL) / 405

HOMA-%B (Homeostatic model assessment-beta cell function)

= (360 x fasting blood insulin (uU/mL)) / (fasting plasma glucose (mg/dL) - 63)

I. Pulse wave velocity (PWV)

PWV was a marker of arterial stiffness and was measured using instrument VP-1000 (Colin, Komaki, Japan). After taking a rest in supping position for 5 minutes, we measured of blood pressure and wave forms in all four limbs. The brachium-ankle PWV (baPWV) was estimated using following formula. *L*a-*L*b means the distance between the brachium and the ankle. It was automatically estimated according to the subject's height. And Δ*T*ba means the time interval between the brachial and the ankle wave forms.

baPWV = (*L*a− *L*b) / Δ*T*ba (in m/s).

Table 3. List of measurements

Related list	Method	Factors
Metabolism	blood test	FPG, fasting insulins, fasting C-peptide, HOMA-IR, HOMA-%B, Lipid Blood Test (TC, HDL-C, LDL-C, TG, sdLDL)
Obesity	Physical measurement	weight, height, waist circumference, BMI, PWV
Inflammation	blood test	CRP, TNFα
Kidney function	urinalysis, blood test	creatinine (+eGFR), cystatin C
Oxidative	blood test	ORAC, oxLDL, AGEs, lipid peroxidation,

stress	protein carbonylation

FPG, fasting plasma glucose; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HOMA-%B, Homeostatic model assessment-beta cell function; TC, total cholesterol; HDL-C, high density lipid-cholesterol; LDL-C, low density lipid-cholesterol; TG, Triglycerides; Apo-B, Apolipoprotein-B; sdLDL, small dense low density lipid-cholesterol; BMI, body mass index; PWV, pulse wave velocity; CRP, C-reactive protein; TNFα, tumor necrosis factor α; eGFR, estimated glomerular filtration rate; ORAC, Oxygen radical antioxidant capacity; oxLDL, oxidized low density lipoprotein; AGEs, Advanced glycation end-products

4. Statistical analysis

All analyses were performed using SPSS software Version 18.0 for windows (IBM, Armonk, New York, USA). The statistical significance of the values was compared by measuring the clinical variables and the oxidative index after the first and fourth weeks (±1 weeks) respectively. Pre- and post- data of each groups (control and intervention) were assessed by paired *t-test*. $P \leq 0.05$ was considered statistically significant.

III. RESULTS

1. Measurements of metabolism data before and after each group

The results of measurements of metabolism data are shown in Table. 5. We measured weight, BMI, WC, FPG, HbA1c, fasting insulin and fasting C-peptide. Only WC decreased significantly of intervention group ($p=0.001$).

HOMA-IR and HOMA-%B were measured for insulin resistance and β-cell function of pancreas respectively.[34] There was no statistically significant difference in HOMA-IR and HOMA-%B in all groups. We also measured TC ($p<0.001$), HDL ($p=0.001$) and LDL ($p<0.001$). Intervention group was the only group which decreased significantly.

TG, sdLDL, CRP, cystatin C and eGFR were also measured. Only sdLDL decreased significantly in intervention group ($p<0.001$).

Table 4. The results of measurements of metabolism data

	Control			Experiment		
Variable	**Baseline**	**Follow-up**	**p-value**	**Baseline**	**Follow-up**	**p-value**
Age	72.93 ± 4.13			70.69 ± 3.82		
Weight (kg)	63.63 ± 7.47	63.36 ± 7.14	0.595	64.14 ± 9.69	63.79 ± 9.40	0.448
BMI (kg/m^2)	24.46 ± 1.78	24.49 ± 1.82	0.925	25.02 ± 1.69	24.84 ± 1.51	0.340
WC (cm)	92.27 ± 4.06	92.67 ± 3.89	0.458	91.81 ± 6.18	88.19 ± 7.71	0.001
FPG (mg/dL)	102.27 ± 8.57	101.93 ±10.26	0.846	101.31 ± 8.55	98.88 ± 7.52	0.088
HbA1c (%)	5.79 ± 0.36	5.73 ± 0.35	0.056	5.68 ± 0.36	5.67 ± 0.35	0.544
Fasting insulin (mcIU/mL)	8.49 ± 4.26	7.71 ± 5.49	0.664	7.66 ± 3.8	10.86 ± 14.69	0.402
Fasting C-peptide (ng/mL)	2.23 ± 0.66	1.93 ± 0.60	0.188	1.96 ± 0.47	2.12 ± 1.44	0.659
HOMA-IR	1.71 ± 0.53	1.48 ± 0.5.	0.207	1.50 ± 0.38	1.59 ± 1.04	0.708
HOMA-%B	103.99 ± 14.15	94.78 ± 13.22	0.064	98.01 ± 17.79	107.61 ± 57.70	0.479
TC (mg/dL)	193.47 ± 35.64	189.67 ± 30.61	0.435	193.75 ± 39.94	170.75 ± 38.14	<0.001
HDL-C (mg/dL)	53.8 ± 13.46	53.87 ± 13.16	0.968	55.50 ± 10.01	51.69 ± 9.05	0.001
LDL-C (mg/dL)	112.93 ± 24.04	112.40 ± 20.20	0.886	112.31 ± 29.20	99.88 ± 28.27	<0.001
TG (mg/dL)	117.00 ± 36.05	107.73 ± 39.77	0.406	115.19 ± 55.01	93.38 ± 29.92	0.084
sdLDL(μM/ml)	2.19 ± 0.36	1.76 ± 0.63	0.024	2.44 ± 0.48	1.52 ± 0.44	<0.001
CRP (mg/L)	2.59 ± 6.86	1.03 ± 1.31	0.301	0.88 ± 0.84	0.61 ± 0.33	0.180
Cystatin C (mg/L)	0.89 ± 0.12	0.91 ± 0.09	0.418	0.91 ± 0.10	0.91 ± 0.13	0.901
eGFR (mL/min)	91.51 ± 15.97	88.35 ± 10.64	0.326	88.55 ± 11.70	89.14 ± 14.81	0.767

BMI, Body mass index; WC, Waist circumference; FPG, fasting plasma glucose; HbA1c, Glycosylated Hemoglobin, Type A1C. HOMA-IR, Homeostatic model assessment-insulin

resistance; HOMA-%B, Homeostatic model assessment-beta cell function; TC, Total cholesterol; HDL-C, High density lipid-cholesterol; LDL-C, Low density lipid-cholesterol; TG, Triglycerides; sdLDL, small dense low lipoprotein; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate.

2. The effect of dietary intervention on parameter of vascular health

We chose BP and PWV as parameter of vascular health. In control group, SBP changed 137.20 ± 8.84 mmHg to 136.73 ± 7.27 mmHg. It was not statistically significant. The intervention group decreased significantly 140.31 ± 12.76 mmHg to 130.63 ± 13.04 mmHg (p=0.004). In control group, DBP changed 83.53 ± 8.32 mmHg to 83.07 ± 5.82 mmHg. It was not statistically significant. The intervention group decreased significantly 95.69 ± 15.61 mmHg to 81.00 ± 7.99 mmHg (p=0.001).

We also measured PWV. Rt. PWV which changed 16.23 ± 2.76 m/s before and 16.89 ± 2.90 m/s after experiment in control group. But in intervention group, it was changed 17.24 ± 3.74 m/s before and 16.26 ± 3.26 m/s after (p=0.031). Lt. PWV was changed 16.49 ± 2.76 m/s before and 16.86 ± 2.92 m/s after experiment in control group. But in intervention group, it was 17.43 ± 3.85 m/s before and 16.34 ± 2.92 m/s after. (p=0.031)

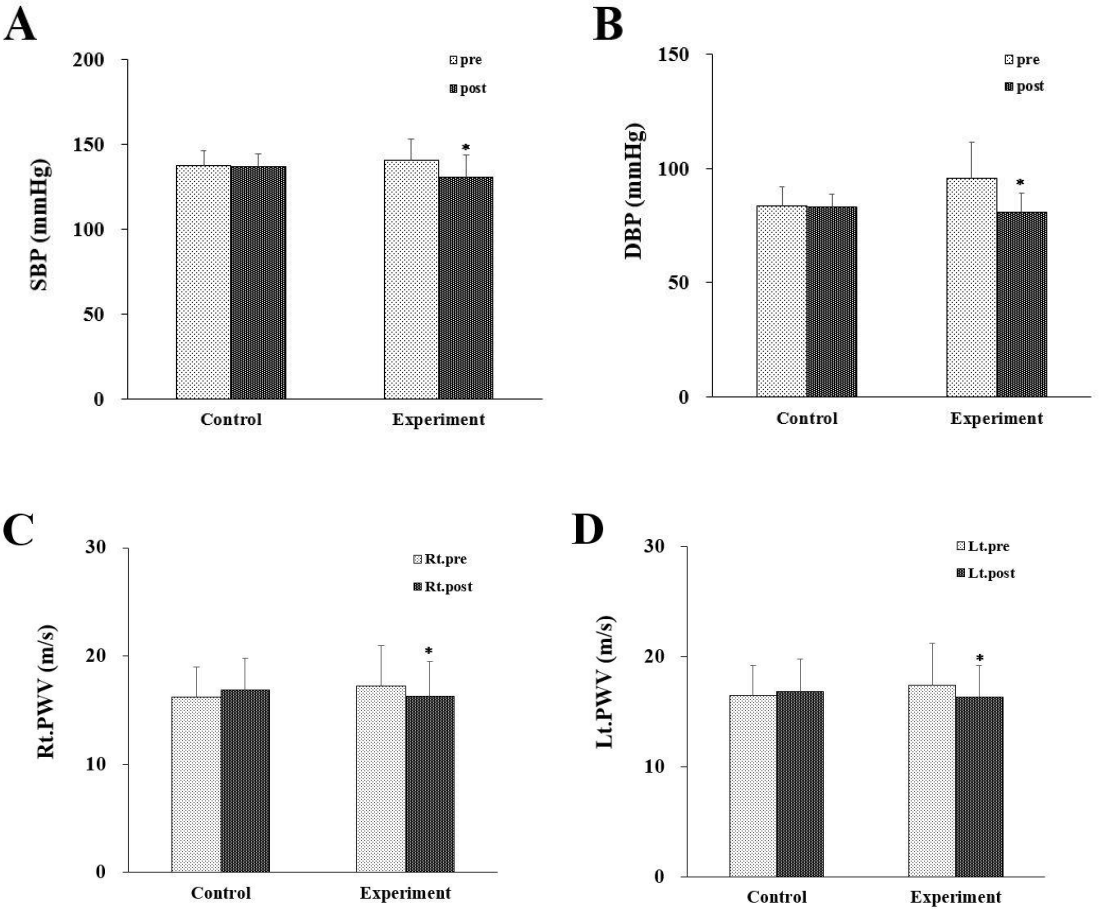

Figure 2. The effect of dietary intervention on vascular health. (A) SBP. (B) DBP. SBP and DBP were measured by an experienced technician by placing the arm at heart level. (C) Rt. PWV. (D) Lt. PWV. PWV was a marker of arterial stiffness and was measured using instrument. SBP, systolic blood pressure; DBP, diastolic blood pressure; Rt. PWV, Right. pulse wave velocity; Lt. PWV, Left. Pulse wave velocity. * $p<0.05$ vs pre-date of each group

3. The effect of dietary intervention on antioxidant capacity of blood

The ORAC of the control group was changed from 6.486 ± 1.014mM to 6.897 ± 0.649mM, the intervention was from 7.085 ± 0.881mM to 6.994 ± 0.649mM. Both groups were not statistically significant.

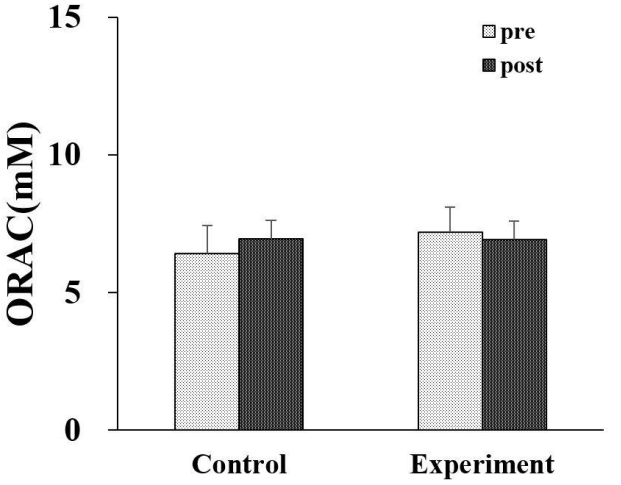

Figure 3. The effect of dietary intervention on antioxidant capacity of blood. We measured ORAC by assay kit to investigate antioxidant capacity of blood. ORAC, oxygen radical absorbance capacity

4. The effect of dietary intervention on oxidative stress index

We investigated lipid peroxidation and protein carbonylation as index of oxidative stress.

Control group was changed from 0.68 ± 0.14mM to 0.65 ± 0.13mM, intervention group was changed from 0.75 ± 0.12mM to 0.46 ± 0.14mM. The p-value was <0.001 in intervention group. Control group was changed from 7.07 ± 0.54nmol/mg to 7.38 ± 0.85nmol/mg and intervention group was changed from 6.92 ± 0.52nmol/mg to 6.60 ± 0.68nmol/mg.

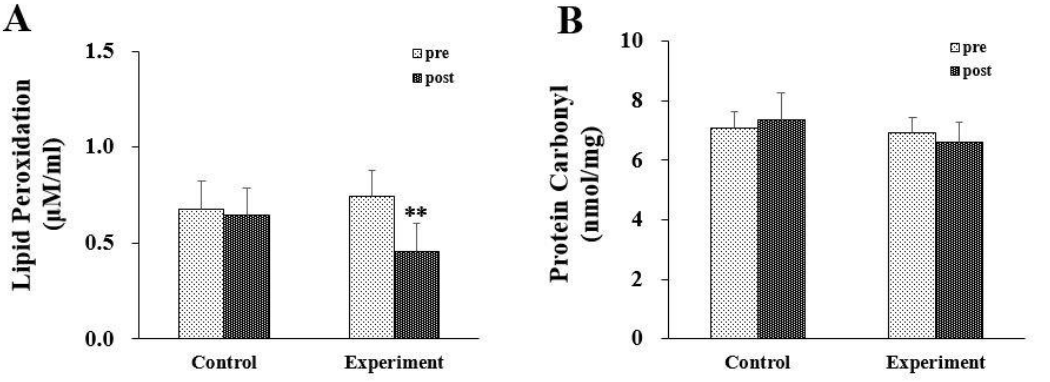

Figure 4. The effect of dietary intervention on oxidative stress index (A) Lipid peroxidation. (B) Protein Carbonylation. Lipid peroxidation and protein carbonylation which are oxidative stress indexes were measured by kit. ***p<0.001* vs pre-date of each group

5. Dietary intervention reduced risk factors of arteriosclerosis

We investigated whether diet intervention reduces risk factors of arteriosclerosis. We used oxLDL and AGEs that are considered as major factors of arteriosclerosis.

The oxLDL of the control group changed from 39.91 ± 11.65U/L to 40.64 ± 7.37U/L, the intervention was from 41.63 ± 10.29U/L to 33.36 ± 9.09U/L. The decrease in intervention group was statistically significant ($p<0.001$).

The AGEs of the control group was changed from 26.57 ± 7.26ng/mL to 21.87 ± 6.41ng/ml, the intervention was from 32.08 ± 14.91ng/ml to 22.20 ± 14.47ng/ml. The decrease in intervention group was statistically significant ($p=0.002$).

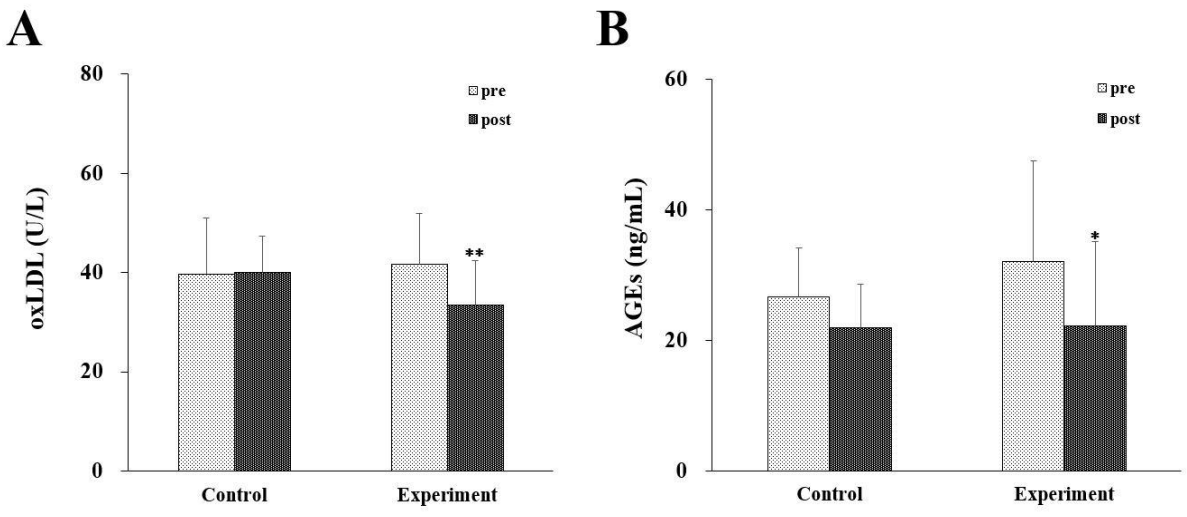

Figure 5. The effect of dietary intervention on risk factors of arteriosclerosis (A) oxLDL. (B) AGEs. oxLDL and AGES which are risk factors of atherosclerosis were measured by ELISA kit. oxLDL, oxidized low density lipoprotein; AGEs, Advanced glycated end products. ***p<0.05* vs pre-date of each group, ****p<0.001* vs pre-date of each group

6. The effect of dietary intervention on tumor necrosis factor-α (TNF-α) which is inflammatory factors

We evaluated change of in TNF-α. It decreased in both groups, but not statistically significant. The TNF-α of the control group changed from 4.0 ± 1.745pg/ml to 3.78 ± 1.72pg/mL, the intervention was from 4.53 ± 1.56pg/mL to 3.45 ± 2.15pg/mL

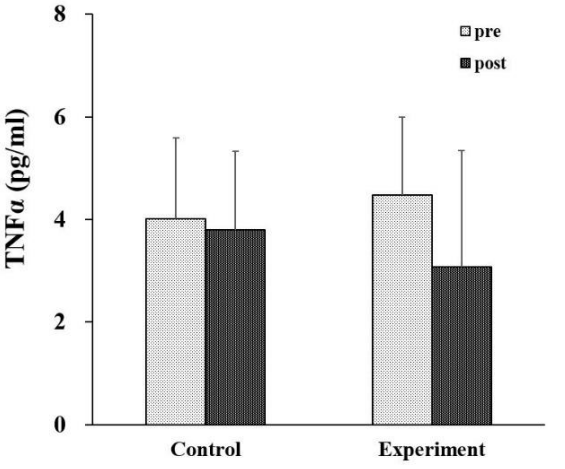

Figure 6. The effect of dietary intervention on tumor necrosis factor-α TNF-α which is inflammatory marker was measured by ELISA kit. TNF-α, tumor necrosis factor-α

IV. DISCUSSION

We investigated the effect of dietary intervention on reduction of oxidative stress on patients with MetS. Dietary intervention had carried out by the lunch boxes which contained a lot of fruits and vegetables. Because it had high distribution ratio of fruits and vegetables, we expected high antioxidants capacity. It is difficult for a patient to follow the standard long-term diet and exercise method that can alleviate or improve the MetS. Therefore, examining food components dealing with MetS features is an important factor for attenuating MetS dietary-based therapies. Recently, antioxidants are interesting because they are associated with obesity, cardiovascular alterations, and oxidative stress. In recent years, foods and certain nutritional components have attracted attention as a treatment for patients with MetS.[3] Researches have reported that the patients with MetS have high risk of cardiovascular disease in world including Korea.[8-10,15,35] One of the causes of MetS is accepted as oxidative stress.[19] In addition, oxidative stress is also associated with CVD.[36] Thus, we have shown that dietary intervention which reduces oxidative stress can decrease the risk of CVD in patients with MetS.

We reduced oxidative stress by taking foods which have high antioxidant capacity. In

particular, antioxidants were obtained by increasing the intake of fruits and vegetables. In the previous paper, eating a lot of fruits and vegetables increased the plasma concentrations of antioxidants.[37] Although antioxidants can be taken from foods as well as supplementation, we chose taking from foods like fruits and vegetables. First of all, the reason why we chose to take antioxidants as foods is the bioactivity and bioavailability of food's antioxidants is better than supplements. Separated compounds may lose biological activity or not behaving the way as whole food compounds.[38] A tablet simply cannot mimic the balanced natural combination of plant chemicals from fruits and vegetables.[38] Second, long-term intake supplements can induce increase of mortality [39,40] or have no benefits. Bjelakovic et al. reported that mortality increased when taking β-carotene, and vitamin E alone or in combination with other antioxidant supplements.[39] Fan et al. reported that dietary supplement does not benefit mortality, and that nutrient intake from food may contribute reducing the risk of death, furthermore overdose of supplements may increase mortality.[40] Edgar et al. reported that although vitamin E reduces oxidative stress, intaking vitamin E supplement may increase mortality.[41] Mayne has also recommended increasing the intake of carotenoid-rich fruits and vegetables, rather than taking beta-carotene supplements prevents CVD.[42] Taken together, high-level antioxidant supplements could potentially upset an important physiological balance.[43] Lastly, taking vegetables or fruits has low toxicity and synergistic effects of consuming fibers and phytochemical. Taking phytochemicals plays an important role in disease prevention and management, such as antioxidant effects.[44] By intaking increased-fiber decreased blood pressure and total cholesterol in children and adults of USA, indeed, prevalence of coronary heart disease significantly low (29%) in individuals.[45] Intaking quercetin, a phytochemical, was inversely associated with TC and LDL-C in Japan.[46] Plant-based therapies have been proved to be less toxic and have a significant effect preventing oxidative stress.[47] Thus, we suggest that taking antioxidants from foods including fruits and vegetables have greater benefits than taking supplements.

To examine the effects of reduction of oxidative stress, we measured various physical and metabolism indexes. Among physical markers related to obesity, only WC was significantly decreased in intervention group ($p=0.001$). The eating habit was probably changed because three meals and snacks were provided to the intervention group. Recently,

Ibrahim et al. reported that when eating regularly, WC decreased statistically significantly.[48] Despite the fact that the lunchboxes according to the average calorie of the adult men and women were provided, it seems that WC had also decreased due to improvement of eating habit.

In the metabolism index, TC ($p<0.001$), HDL-C ($p=0.001$), LDL-C ($p<0.001$) and sdLDL ($p<0.001$) were significantly decreased only in intervention group. TC, HDL-C and LDL-C were both decreased. LDL-C and HDL-C, which are subfractions of lipoprotein, have been reported to be inversely correlated with cardiovascular risk factors.[49] An increase in LDL-C, induces risk of CVD, while decreased in HDL-C.[49] Thus, we wanted a reduction in LDL-C excepted TC and HDL-C, but we thought that both HDL-C and LDL-C were reduced by TC reduction through dietary intervention. Moreover, Catherin et al. reported that in rat, the intake of lettuce contributes to cholesterol emissions and increases antioxidant status by intaking a lot of antioxidants which are included in vegetables.[50] Thus, we suggest that taking antioxidants may increase the excretion of TC, which may reduce both HDL-C and LDL-C. sdLDL is considered as a risk factor of coronary heart disease by the NCEPIII.[51] sdLDL is one of subfractions of LDL-C known to be a better index for cardiovascular biomarker than whole LDL-C.[52] In paper, the patient with acute coronary syndrome and MetS had higher concentration of sdLDL than without MetS.[53] Another paper also suggests serum level of sdLDL was higher in who have MetS.[54] sdLDL has a longer circulation time than other large LDLs and penetrate well into the arterial wall. Therefore, the level of sdLDL in blood is a risk factor of CVD. In our results, sdLDL was decreased in both the control and intervention groups. However, the reduction of the intervention group was greater than of the control group. It suggests that dietary intervention reduced the risk of arteriosclerosis.

We confirmed the effect of dietary intervention on parameter of vascular health. Only in intervention group, SBP, DBP, Rt, PWV and Lt. PWV were significantly decreased. Arterial stiffness reflects the stiffness of the arterial wall.[55] Therefore, arterial stiffness is considered as a strong predictor of cardiovascular risk and myocardial infarction. PWV is proportional to the degree of stiffness of the coronary artery wall and is known to be inversely proportional to vessel diameter.[55] PWV is closely related to the incidence and mortality of cardiovascular disease and can be measured using noninvasive machines. It is based on the fact that arterial

stiffness decreases arterial elasticity and stiffness increases and blood flow and pulse wave conduction speed. K.M et al. reported that baPWV level was higher in women with metabolic syndrome (1540 ± 281 cm/s) than men (1312 ± 223 cm/s).[56]

The long term hypertension increases the risk of various diseases such as heart disease and kidney failure. NO· increased form endothelial nitric oxide synthase (eNOS) is important in maintaining vascular health, such as vascular tone, vasoreactivity, vasodilation, and maintenance of platelet aggregation and smooth muscle cell growth and differentiation.[57] The increase of ROS, such as O_2^-, forms $ONOO^-$ which remove NO· reducing the bioavailability of NO·, thereby reducing endothelium-dependent vasodilation and causes hypertension.[57] In our results, SBP, DBP, Rt.PWV and Lt.PWV were significantly decreased in intervention group. It suggests that consumption of antioxidants through foods can reduce oxidative stress and promotes vascular health.

To compare pre- and post- antioxidant capacity, we investigated oxygen radical absorbance capacity (ORAC). ORAC assay is a method for quantifying antioxidant capacity by analyzing the ability of the antioxidant to block the peroxyl radical chain which is the ability to remove ROS.[58] Peroxyl radical are produced by the oxidation of lipids in food and biological systems. Trolox™ is used as reference in ORAC assay.[58] We expected ORAC to improve the intervention group with dietary control. However, effect of dietary control was not found. ORAC assay has a disadvantage that only the antioxidant ability against peroxyl radical can be measured. In the body, along with peroxyl radicals, we have superoxide radical (O_2^-), hydroxyl radical (·OH) nitric oxide (NO·) and the peroxynitrite radical ($ONOO^-$). Thus, ORAC assay not regarded to reflect antioxidant capacity for all these radicals. And there was a difference between the starting and ending time of the intake lunch box and the sampling time of all the subjects, so all the subjects' samples were stored in the freezing in -80˚C and then examined at once. Some of the antioxidants may have been lost during this process, and ORAC levels of the sample may have limited antioxidant capacity. In future research, we think that we have to quantify ORAC by collecting and measuring blood samples daily without freezing and thawing. For these reasons, the ORAC assay in this study seems to have limitations in reflecting antioxidant capacity through dietary intervention.

We identified lipid peroxidation and protein carbonlyation to determine whether oxidative stress was reduced or not. Both were measured by ELISA. An increase in oxidative stress, i.e. increased ROS and free radicals, directly affecting the lipid. In particular, lipid peroxidation begins when hydroxyl radical attacks carbon-carbon double bonds.[59] The product of lipid peroxidation is lipid hydroperoxides (LOOH).[59] Secondary products are aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE).[59] Thus, levels of MDA was measured by ELISA kit. MDA is the most prevalent byproduct of lipid peroxidation during oxidative stress and is considered an excellent measurement for use in clinical trials to evaluate oxidative stress. MDA was reported that they have biological activity such as destruction of cell membrane, mutagenicity and carcinogenicity. In the body, this is stopped by antioxidants. Therefore, long-term control of diet containing antioxidants is regarded to protect the cells by inhibiting the lipid peroxidation. Thus, we concluded that diet intervention which included antioxidants materials, give positive effects to health of patients with MetS.

Protein carbonylation is also protein oxidation promoted by ROS and decreased by antioxidatns.[60] They usually form reactive ketones or aldehydes, which are reacted by 2,4-dinitrophenylhydrazine (DNPH) to form the 2,4-dinitrophenylhydrazone (DNP hydrazone).[60] Thus, we measured DNP hydrazone with an ELISA kit to determine the degree of oxidative stress in the blood. Unfortunately, although lipid peroxidation of intervention group was significantly reduced, protein carbonylation was not significantly decreased in both groups. We suggest some reasons of it. First, protein carbonylation and lipid peroxidation are markers of oxidative stress, but protein carbonylation is also caused sequentially and indirectly by lipid peroxidation and it is irreversible oxidative protein modifications. If dietary interventions have been in progress for more than four weeks, protein carbonylation may be reduced due to reduced lipid peroxidation. Second, protein carbonyl groups are derived through two major mechanism; where amino acid side chains are oxidized directly (direct) or through conjugation by reactive species such as advanced lipoxidation end products and advanced glycation end products (indirect).[61] Although ELISA is the acceptable method for quantification of protein carbonyls, it is difficult to get any information about the molecules oxidized or the nature of carbonlyation i.e. primary or secondary.[62] Third, protein

carbonylation of plasma is significantly positive correlated with age.[63] Age of subjects in this study is more than 65 years, protein carbonlyation value is already high. Taken together, we have difficulty to estimate effect of dietary intervention on protein carbonlyation. In further study, we need to set up lower age of inclusion criteria and long-term dietary intervention.

In this study, oxLDL and AGEs decreased in intervention group only. It suggests that diet intervention which included antioxidants can reduce risk of CVD. OxLDL is an oxidation form of LDL and is converted to oxLDL by free radical such as ROS. Therefore, oxLDL is usually used **as** a marker of oxidative stress. In the arterial wall, formation of oxLDL is a key initiating step of atherosclerosis.[64] Because it leads to foam cell generation, endothelial dysfunction and inflammatory processes.[64] In previous paper, high levels of oxLDL in serum increase the incidence of MetS.[65] Research reported that increased level of oxLDL reduces arterial elasticity.[66] Ruel et al. reported that consumption of cranberry juice which is flavonoid-rich food is associated with significant reduction in circulating level of oxLDL.[67] In summary, oxLDL can contribute to increase risk factors of CVD.

AGEs are highly reactive and participate in the development of other components of MetS. Binding AGEs to apolipoprotein B (ApoB) occurs glycation ApoB and the clearance of LDL-C by receptor, decreases.[68] And AGE-LDL, a binding form of AGEs and LDL show better uptake in macrophages than native LDL-C.[69] Increased AGEs increase LDL-C uptake by human aortic intimal cells and macrophages, sequentially promoting foam cell formation and atherosclerosis. AGEs are generated by three pathways: glycation, oxidation and/or carbonylation. AGEs are produced from reactive carbonyl compounds made by the lipid peroxidation process in which lipid and ROS react.[70] Thus, the decrease of AGEs may reflect reduction effect of oxidative stress by dietary intervention. In conclusion, the decreased in oxLDL and AGEs as risk factors for arteriosclerosis shown that intake high level of antioxidants reduces oxidative stress, sequentially promotes vascular health.

Although diagnostic criteria of MetS from various organizations are not included as inflammatory factors,[71] MetS is often recognized in proinflammatory state.[72] Cells including white blood cells produce ROS during inflammation, thereby inflammation is a major source of oxidative stress. Intake of fruits and vegetables intake was associated with lower

concentrations of systemic oxidative stress and inflammation.[73] Inflammatory markers, including hs-CRP, TNF-α, fibrinogen, and IL-6(interleukin-6), are associated with MetS.[71] We evaluated changes of TNF-α. When AGEs bind to RAGE, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is activated.[74] NF-κB regulates the expression of TNF-α.[75] TNF-α was not decreased significantly in both groups, but the reduction was greater in the intervention group. We suggest that intake antioxidant may reduce NF-κB activity by AGEs, leading to decrease in TNF-α expression. A previous paper suggests that restriction of AGEs for 4 months results a decrease of TNF-α.[76] The decrease of TNF-α in the intervention group was resulted from the reduction of NF-κB activity by AGEs due to the intake of foods with high antioxidant capacity.

In summary, we studied the effects of dietary intervention on reduction of oxidative stress in patients with MetS. As a result, TC, HDL-C, LDL-C and sdLDL were significantly decreased in intervention group. SBP, DBP and PWV, the indicators of vascular health, significantly decreased. In addition, oxidative stress index lipid peroxidation and indicators oxLDL and AGEs were also significantly decreased in intervention group. Taken together, the study has to potential to promote health of vascular in patients with MetS, and it may have beneficial rather that supplementation.

V. CONCLUSION

In conclusion, dietary intervention which includes a high distribution ratio of fruits and vegetables may have beneficial effects, such as plasma lipids, blood pressure and plasma glucose, by reducing oxidative stress.

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APPENDICES

사진 (도시락 사진)

	1	2	3	4	5	6	7	8	9	10
아침	당근밥	현미밥	하와이안볶음밥	콩나물강된장비빔밥	검은콩밥	현미밥	볶음밥오믈렛	참나물감자밥	현미밥	뿌리채소밥
	돼지고기고추장볶음	죽순새우볶음	단호박불고기	들깨닭가슴살볶음	무나물소고기볶음	단호박불고기	강황닭갈비	소고기죽순볶음	닭가슴살채소볶음	소고기버섯샐러드
	닭가슴살오이샐러드	쑥갓두부무침	병아리콩샐러드		곤약잡채	느타리버섯무침	병아리콩샐러드	브로콜리토마토무침	도라지구이	브로콜리토마토무침
		새송이양파볶음			케일무침	쑥갓두부무침			쑥갓두부무침	
		계란말이			브로콜리샐러드	구운야채샐러드			풋고추들깨된장무침	
		도라지구이			단호박구이	컬리플라워들깨무침			죽순유부조림	

	1	2	3	4	5	6	7	8	9	10
점심	흑미밥	수수밥	검은콩밥	차조밥	현미밥	검은콩밥	현미밥	차조밥	퀴노아밥	흑미밥
	소고기죽순볶음	소고기숙주볶음	동태곤약조림	새우가지조림	임연수고추장구이	닭고기달걀야채구이	가자미구이	데리야끼닭다리구이	소고기청경채볶음	동태콩나물찜
	느타리버섯무침	시금치땅콩무침	파프리카깻잎무침	시금치땅콩무침	미니새송이볶음	팽이버섯오이무침	곤약잡채	새송이양파볶음	미니새송이볶음	무나물소고기볶음
	치커리무침	새송이들깨무침	죽순유부조림	모듬버섯들깨조림	비름나물된장무침	고사리들기름볶음	케일무침	무말랭이볶음	계란말이	시금치땅콩무침
	구운야채샐러드	채소스틱	미니새송이볶음	계란말이	병아리콩샐러드	무말랭이볶음	새송이양파볶음	브로콜리샐러드	구운야채샐러드	단호박구이
	컬리플라워들깨무침	단호박구이	브로콜리토마토무침	브로콜리토마토무침		브로콜리토마토무침	톳어묵야채조림	계란말이	컬리플라워들깨무침	새송이들깨무침

	1	2	3	4	5	6	7	8	9	10
저녁	검은콩밥	차조밥	현미밥	퀴노아밥	수수밥	차조밥	퀴노아밥	흑미밥	수수밥	검은콩밥
	닭고기된장구이	마파두부	닭갈비	돼지고기고추장볶음	닭안심아몬드볶음	돼지고기된장숙주볶음	소고기버섯샐러드	소고기두부불고기	깻잎제육볶음	들깨닭가슴살볶음
	케일무침	소고기청경채볶음	참나물무침	실곤약미나리무침	열무된장무침	치커리무침	알배추들깨무침	매콤알배추볶음	열무된장무침	무말랭이볶음
	톳어묵야채조림	고사리들기름볶음	느타리버섯무침	청경채유부무침	도라지구이	도라지구이	도라지구이	들깨버섯조림	고사리들기름볶음	참나물겉절이
	팽이버섯오이무침	무말랭이볶음	구운야채샐러드	무말랭이볶음	채소스틱	닭가슴살오이샐러드	구운야채샐러드	닭가슴살오이샐러드	톳어묵야채조림	병아리콩샐러드
	비름나물된장무침	구운야채샐러드	컬리플라워들깨무침	구운야채샐러드	새송이들깨무침		시금치땅콩무침		채소스틱	

ABSTRACT(IN KOREAN)

식이 조절을 통한 산화스트레스 감소가 대사증후군 환자에 미치는 효과

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박혜준

대사 증후군은 흔한 대사 장애이며, 인슐린 저항성, 고혈압, 복부 지방 및 죽상경화성 이상 지혈증과 같은 이상 및 심혈관 질환 위험 요소의 집합으로 알려져 있다. 대사 증후군의 원인으로 인슐린 저항성이 가장 널리 받아들여지지만, 산화스트레스도 대사 증후군의 원인으로 여겨진다.

산화 스트레스는 활성 산소의 생산과 비활성의 균형이 깨지면서 발생한다. 산화 스트레스는 우리 몸에서 세포 손상과 죽음을 일으키므로, 산화 스트레스를 조절하는 것은 대사 증후군에서 매우 중요하다. 산화스트레스는 동맥경화와 같은 심혈관계 질환에서도 중요한 역할을 하며, 몇몇의 연구들은 대사증후군 환자의 경우 심혈관계 질환의 위험성이 증가한다고 보고 하고 있다.

따라서 우리는 식이 조절을 통해 산화스트레스를 줄였다. 실험군의 경우 항산화제가 많이 포함된 과일과 야채를 섭취하였다. 그 결과, 실험군에서 염증인자인 tumor necrosis factor-α와 동맥경화 위험인자인 oxidized low density lipoprotein 과 advanced glycation end-product 가 감소되었다. 또한 산화스트레스 지표인 지질 괴산화도 줄어들었다. 따라서, 우리는 항산화제가 많이 포함된 음식을 섭취하면 대사 증후군 환자의 혈관 건강을 향상 시킬 수 있음을 시사한다.

핵심되는 말 : 대사증후군, 산화 스트레스, 항산화제, 식이 조절