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Follow-Ups of Metabolic, Inflammatory and Oxidative Stress Markers, and Brachial–Ankle Pulse Wave Velocity in Middle-Aged Subjects without Metabolic Syndrome

Oh Yoen Kim,[1] Jean Kyung Paik,[2] Ju Young Lee,[3] Sang-Hyun Lee,[4] Jong Ho Lee[3,5]

[1]*Department of Food Science and Nutrition, Dong-A University, Busan, Republic of Korea,* [2]*Department of Food and Nutrition, Eulji University, Gyeonggi-do, Republic of Korea,* [3]*National Leading Research Laboratory of Clinical Nutrigenetics/ Nutrigenomics, Department of Food and Nutrition, Yonsei University, Seoul, Republic of Korea,* [4]*Department of Family Practice, National Health Insurance Corporation Ilsan Hospital, Goyang-si, Republic of Korea,* [5]*Yonsei University Research Institute of Science for Aging, Yonsei University, Seoul, Republic of Korea*

Abstract

This study investigates the association among metabolic risk factors, inflammatory and oxidative stress markers, and brachial–ankle pulse wave velocity (ba-PWV). We conducted a 3-year longitudinal, observational study of 288 middle-aged adults not meeting the criteria for metabolic syndrome (MetS) at the initial screening. We measured metabolic risk factors, inflammatory and oxidative stress markers, and ba-PWV. Within the 3-year study period, 15.6% (45 out of 288) of participants developed MetS. At the 3-year follow-up, patients were categorized as those with MetS ($n = 45$) and those without MetS ($n = 243$). Patients with MetS had significantly unfavorable initial measurements of baseline body mass index (BMI), waist circumference (WC), blood pressure (BP), triglyceride (TG), high-density lipoprotein (HDL)-cholesterol, glucose, insulin, homeostasis model assessment of insulin resistance (HOMA-IR) index, and ba-PWV. After 3 years, participants without MetS showed significant increases in WC, diastolic BP (DBP), total- and low-density lipoprotein (LDL)-cholesterol, malondialdehyde (MDA), oxidized-LDL (ox-LDL), and ba-PWV and a significant decrease in HDL-cholesterol and free fatty acids (FFA). Subjects who developed MetS showed significant increases in BMI, WC, BP, TG, glucose, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), MDA, ox-LDL, and ba-PWV and a significant decrease in HDL-cholesterol. Changes in BMI, WC, BP, TG, HDL-cholesterol, glucose, HOMA-IR index, FFA, C-reactive protein ($P = .022$), IL-6 ($P = .004$), leukocyte count ($P < .001$), MDA ($P = .002$), ox-LDL ($P = .015$), and ba-PWV ($P = .001$) differed significantly between the two groups after adjustment for baseline values. Changes in ba-PWV were positively correlated with the changes in systolic and DBP, total-cholesterol, glucose, leukocyte count, and MDA. The age-related increase in arterial stiffness is greater in the presence of MetS with higher levels of inflammatory and oxidative stress markers.

Keywords: MetS, inflammatory, oxidative stress markers, brachial–ankle pulse wave velocity

INTRODUCTION

Metabolic syndrome (MetS) has been linked to accelerated central arterial aging (i.e., increased arterial stiffness and thickness) (1,2). Some prospective studies show that the progression of arterial stiffness was associated with the changes in MetS risk and was significantly more pronounced in MetS subjects (3,4). A growing body of literature suggests that diverse inflammatory and oxidative stress markers correlate with arterial damage (1,5–7) and, thus, with increased arterial stiffness and thickness (8–11). Inflammatory markers have been reported to be elevated in patients with diabetes mellitus (12), and a proinflammatory state accompanies the presence of altered MetS components. MetS treatment and management programs conventionally focus on patients with the condition; however, careful observation for changes in inflammatory and oxidative stress markers and arterial stiffness is also important for patients at risk for developing MetS.

Therefore, our follow-up study included apparently healthy subjects who do not meet the MetS criteria at baseline, and then after 3 years, subdivided the subjects into non-MetS and MetS, and this study included inflammatory and oxidative stress markers and brachial-ankle pulse wave velocity (ba-PWV) together with metabolic risk factors, and investigated the association of these parameters.

Address correspondence to Jong Ho Lee, PhD, Department of Food and Nutrition, College of Human Ecology, Yonsei University, 134 Shinchon-Dong, Sudaemun-Gu, Seoul 120-749, Republic of Korea. E-mail: jhleeb@yonsei.ac.kr
Oh Yoen Kim and Jean Kyung Paik contributed equally to this work.

METHODS AND MATERIALS

Study Participants

A total of 288 participants between the ages of 30 and 69 years who visited a health promotion center of the National Health Insurance Corporation of Ilsan Hospital in Korea between October 2006 and October 2007 were enrolled in this study. Participants also completed a personal health and medical history questionnaire, which served as a screening tool. The Institutional Review Board of the National Health Insurance Corporation of Ilsan Hospital approved the study protocol, which was conducted in accordance with the Helsinki Declaration. At the end of the 3-year study, participants were subdivided into groups according to the criteria for MetS (non-MetS and MetS).

MetS was diagnosed if the participants showed at least three of the following components: central obesity (i.e., waist circumference [WC] >90 cm for males and >80 cm for females); high blood pressure (BP) (i.e., systolic BP [SBP] $\geq$130 mm Hg or diastolic BP [DBP] $\geq$85 mm Hg); hyperglycemia (i.e., fasting glucose $\geq$100 mg/dL); hypertriglyceridemia (i.e., fasting triglycerides [TGs] $\geq$150 mg/dL); and low high-density lipoprotein (HDL)-cholesterol (i.e., HDL-cholesterol <40 mg/dL for males and <50 for females) (13,14).

Anthropometric Parameters, Blood Pressure, Blood Collection, and Energy Intake

Body mass index (BMI) was calculated as body weight in kilograms divided by the square of the height in meters (kg/m^2). Waist circumference was measured at the umbilicus with the subjects standing after normal expiration. The SBP and DBP were obtained from the left arms of seated patients with an automatic BP monitor (TM-2654; A&D, Tokyo, Japan) after 20 minutes of rest. After overnight fasting, venous blood samples were collected in ethylenediaminetetraacetic acid-treated or plain tubes, separated into plasma and serum, and then stored at $-70°$C until analysis. Participants were interviewed about their smoking and drinking behaviors. Dietary intake was assessed using a semiquantitative food frequency questionnaire and a 24-hour recall method. Nutrient intake was determined and calculated based on the 3-day food records using the computer-aided nutritional analysis program (CAN-pro 2.0; Korean Nutrition Society, Seoul, Korea). Total energy expenditure (TEE) (kcal/d) was calculated based on the activity patterns of the study participants, such as basal metabolic rate, 24-hour physical activity, and specific dynamic actions of food.

Measurement of Serum Lipid Profiles and Fasting Glucose, Insulin, and Homeostasis and Model Assessment of Insulin Resistance and Free Fatty Acid

Fasting total-cholesterol and TG levels were measured using commercially available kits and a Hitachi 7150 autoanalyzer (Hitachi Ltd., Tokyo, Japan). After precipitation of serum chylomicrons using dextran sulfate magnesium, HDL-cholesterol concentrations in the supernatants were enzymatically measured. For participants with serum TG levels <400 mg/dL, low-density lipoprotein (LDL)-cholesterol levels were estimated directly using the Friedewald formula: LDL-cholesterol = total-cholesterol $-$ (HDL-cholesterol + [TG/5]). Fasting glucose levels were measured by the glucose oxidase method using a Beckman glucose analyzer (Beckman Instruments, Irvine, CA, USA). Insulin levels were measured by a radioimmunoassay using a commercial kit (Immuno Nucleo Corporation, Stillwater, MN, USA). Insulin resistance (IR) was calculated based on the homeostasis model assessment (HOMA) using the following equation: HOMA-IR = (fasting insulin [μIU/mL] $\times$ fasting glucose [mmol/L])/22.5. Free fatty acids (FFA) were analyzed with a Hitachi 7150 autoanalyzer.

Measurement of Serum Interleukin-6, Tumor Necrosis Factor-α, High-Sensitivity C-Reactive Protein Levels, and White Blood Cell Counts

Serum interleukin (IL)-6 and tumor necrosis factor (TNF)-α concentrations were measured using Bio-Plex™ reagent kits and a Bio-Plexz™ system (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. High-sensitivity C-reactive protein (hs-CRP) levels were measured on an Express Plus™ autoanalyzer (Chiron Diagnostics Co., Walpole, MA, USA) using commercially available hs-CRP-Latex (II) X2 kits (Seiken Laboratories Ltd., Tokyo, Japan). White blood cell (WBC) counts were determined using a hematology analyzer from HORIBA ABX Diagnostic (HORIBA ABX SAS, Parc Euromedicine, France).

Plasma Oxidized-LDL, Malondialdehyde, and ba-PWV Measurements

Plasma oxidized (ox)-LDL levels were measured using an enzyme immunoassay (Mercodia, Uppsala, Sweden). The absorbances of the resulting color reactions were measured at a wavelength of 450 nm using a Wallac Victor^2 multilabel counter (Perkin Elmer Life Sciences, Turku, Finland). The wavelength correction was set to 540 nm. Plasma malondialdehyde (MDA) concentrations were measured based on the production of thiobarbituric acid-reactive substances (TBARS Assay Kit, Zepto-Metrix Co., Buffalo, NY, USA). Branchial–ankle pulse wave velocities were measured using an automatic waveform analyzer (model VP-1000; Nippon Colin Ltd., Komaki, Japan).

Data Analysis

Statistical analyses were performed using SPSS version 12.0 for Windows (SPSS Inc., Chicago, IL, USA). The skewed variables were logarithmically transformed for statistical analysis. For descriptive purposes, mean values were presented using untransformed values. Results are expressed as means $\pm$ standard error. A two-tailed P value of <.05 was considered statistically significant.

Frequency was tested with the chi-square test. Paired t tests were used to evaluate differences between baseline and 3-year follow-up levels. Differences in the clinical variables between the without MetS and with MetS outcome groups were tested by independent t tests. A general linear model test was applied to adjust for baseline values. Pearson's correlation coefficients were used to examine the relationships between variables. Multiple logistic regression analyses were also performed.

RESULTS

Clinical Characteristics, Metabolic Risk Factors, Inflammatory and Oxidative Stress Markers, ba-PWV, and Nutrient Intake at Baseline and 3-Year Follow-Up

After 3 years, participants showed a decrease in HDL-cholesterol ($P < .001$), insulin ($P = .039$), and FFA ($P < .001$), and an increase in WC ($P < .001$), DBP ($P = .001$), total-cholesterol ($P = .002$), LDL-cholesterol ($P < .001$), serum TNF-α ($P = .033$), MDA ($P < .001$), ox-LDL ($P < .001$), and ba-PWV ($P < .001$) (Table 1). The estimated total calorie intake was reported at baseline (2439 ± 19 kcal/d) and at the

Table 1. Clinical characteristics, metabolic risk factors, inflammatory and oxidative stress markers, and ba-PWV at baseline and after 3 years

| | Non-MetS ($n = 288$) | | |
	Baseline	Follow-up	P
Age (y)	46.0 ± 0.56	49.0 ± 0.57	<.001
BMI (kg/m^2)	23.1 ± 0.15	23.2 ± 0.15	.081
Waist (cm)	81.9 ± 0.40	85.2 ± 0.42	<.001
SBP (mm Hg)	117.0 ± 0.80	118.3 ± 0.85	.084
DBP (mm Hg)	71.6 ± 0.62	73.6 ± 0.66	.001
TGs (mg/dL)[a]	100.3 ± 3.39	104.6 ± 3.82	.347
Total-cholesterol (mg/dL)[a]	188.3 ± 1.93	193.4 ± 1.91	.002
HDL-cholesterol (mg/dL)[a]	55.3 ± 0.87	51.9 ± 0.84	<.001
LDL-cholesterol (mg/dL)[a]	113.3 ± 1.85	121.2 ± 1.71	<.001
Glucose (mg/dL)[a]	90.6 ± 0.59	92.3 ± 0.56	.006
Insulin (IU/mL)[a]	8.40 ± 0.20	8.05 ± 0.23	.039
[b]HOMA-IR[a]	1.89 ± 0.05	1.85 ± 0.06	.196
FFA (Eq/L)[a]	513.5 ± 14.5	437.2 ± 12.3	<.001
hs-CRP (mg/dL)[a]	1.17 ± 0.14	1.12 ± 0.13	.943
Serum IL-6 (pg/mL)[a]	3.59 ± 0.19	3.34 ± 0.14	.964
Serum TNF-α (pg/mL)[a]	8.75 ± 0.48	9.36 ± 0.48	.033
WBCs ($\times 10^9$/L)[a]	5.45 ± 0.10	5.35 ± 0.09	.538
[c]ba-PWV (cm/s)[a]	1293.6 ± 10.9	1334.2 ± 11.4	<.001
MDA (nmol/mL)[a]	9.75 ± 0.16	11.1 ± 0.16	<.001
ox-LDL (U/L)[a]	33.2 ± 0.67	44.1 ± 0.66	<.001

[a]Means ± SE tested by logarithmic transformation, P values derived from paired t test.
[b]HOMA-IR = (fasting insulin (µIU/mL) × fasting glucose (mmol/L))/22.5.
[c]ba-PWV = brachial–ankle pulse wave velocity.
Abbreviations: BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; TGs – triglycerides; FFA – free fatty acids; WBCs – white blood cells; MDA – malondialdehyde ; ox-LDL – oxidized-LDL.

3-year follow-up (2428 ± 18 kcal/d). No significant differences in macronutrient intake, TEE, smoking, or drinking between the baseline and the 3-year follow-up were noted in whole subjects (data not shown). Additionally, we observed significant decreases in TEE, and significant increases in total calorie intake (TCI)/TEE in the MetS outcome groups, which were not observed in the non-MetS outcome groups (data not shown).

Metabolic Risk Factors and Lipid Profiles According to MetS Outcome at 3 Years

Within a 3-year interval, 15.6% (45 out of 288) of the participants met the criteria for MetS. At the 3-year follow-up, participants were divided into two groups according to MetS outcome: those who developed MetS ($n = 45$) and those who did not develop MetS ($n = 243$). At baseline, participants who later developed MetS showed significantly unfavorable measurements for baseline BMI, WC, BP, TG, HDL-cholesterol, glucose, insulin, and HOMA-IR index (Table 2). After 3 years, participants who did not develop MetS showed a significant increase in WC, DBP, total- and LDL-cholesterol, and a significant decrease in HDL-cholesterol and FFA. Participants who developed MetS showed a significant increase in BMI, WC, BP, TG, and glucose, and a significant decrease in HDL-cholesterol. Changes in BMI, WC, BP, TG, HDL-cholesterol, glucose, HOMA-IR index, and FFA were significantly different between the non-MetS and MetS groups after adjustment for baseline values. At the 3-year follow-up, participants who developed MetS had significantly unfavorable measurements for BMI, WC, BP, TG, HDL-cholesterol, glucose, insulin, HOMA-IR index, and FFA (Table 2).

Inflammatory and Oxidative Stress Markers and ba-PWV According to MetS Outcome at 3 Years

At baseline, participants who later developed MetS had significantly higher ba-PWV than those who did not develop MetS ($P < .001$) (Table 3). However, there were no significant differences in inflammatory and oxidative stress markers between the non-MetS and MetS groups at baseline. After 3 years, participants without MetS showed a significant increase in MDA ($P < .001$), ox-LDL ($P < .001$), and ba-PWV ($P < .001$). Participants with MetS showed a significant increase in IL-6 ($P = .011$), TNF-α ($P = .017$), MDA ($P < .001$), ox-LDL ($P < .001$), and ba-PWV ($P < .001$). Changes in hs-CRP ($P = .022$), IL-6 ($P = .004$), leukocyte count ($P < .001$), MDA ($P = .002$), ox-LDL ($P = .015$), and ba-PWV ($P = .001$) were significantly different between the non-MetS and MetS groups after adjustment for baseline values. Additionally, changes in ba-PWV were significantly different between the two groups after further adjustment for baseline ba-PWV, SBP, DBP, and heart rate ($P = .002$). At the 3-year follow-up,

Table 2. Clinical characteristics, metabolic risk factors, and lipid profiles according to the MetS outcome at 3 years

| | Baseline: non-MetS (n = 288) | | | | | |
	Without MetS outcome (n = 243)	P^b	With MetS outcome (n = 45)	P^b	P^c	P^d
Male/female, n (%)	130 (53.5)/113 (46.5)		30 (66.7)/15 (33.3)		.102	
Cigarette smoker, n (%)	61 (25.1)		16 (35.6)		.146	
Alcohol drinker, n (%)	166 (68.3)		31 (68.9)		.939	
BMI (kg/m²)						
Baseline	22.8 ± 0.16	.521	24.7 ± 0.35	.006	<.001	
Follow-up	22.8 ± 0.16		25.2 ± 0.32		<.001	
Change	0.05 ± 0.07		0.49 ± 0.17		.013	<.001
WC (cm)						
Baseline	81.1 ± 0.42	<.001	85.9 ± 0.99	<.001	<.001	
Follow-up	84.1 ± 0.44		91.4 ± 0.81		<.001	
Change	2.96 ± 0.37		5.54 ± 0.80		.006	<.001
SBP (mm Hg)						
Baseline	115.8 ± 0.86	.942	123.4 ± 1.94	.001	<.001	
Follow-up	115.8 ± 0.83		131.7 ± 2.13		<.001	
Change	0.06 ± 0.79		8.27 ± 2.27		<.001	<.001
DBP (mm Hg)						
Baseline	70.5 ± 0.67	.036	77.4 ± 1.44	<.001	<.001	
Follow-up	71.9 ± 0.69		82.8 ± 1.43		<.001	
Change	1.37 ± 0.65		5.42 ± 1.36		.013	<.001
TGs (mg/dL)						
Baseline[a]	93.0 ± 3.10	.589	140.2 ± 12.4	<.001	<.001	
Follow-up[a]	91.9 ± 3.37		173.1 ± 12.0		<.001	
Change	−1.05 ± 3.03		32.9 ± 9.25		.001	<.001
Total-cholesterol (mg/dL)						
Baseline[a]	187.5 ± 2.04	.001	192.6 ± 5.66	.668	.446	
Follow-up[a]	193.3 ± 2.06		194.0 ± 5.04		.926	
Change	5.81 ± 1.76		1.42 ± 5.10		.345	.500
HDL-cholesterol (mg/dL)						
Baseline[a]	56.5 ± 0.96	.002	48.6 ± 1.67	<.001	.001	
Follow-up[a]	53.8 ± 0.92		41.5 ± 1.26		<.001	
Change	−2.70 ± 0.84		−7.13 ± 1.40		.008	<.001
LDL-cholesterol (mg/dL)						
Baseline[a]	112.4 ± 1.90	<.001	118.2 ± 5.98	.368	.688	
Follow-up[a]	121.4 ± 1.84		120.0 ± 4.63		.752	
Change	8.91 ± 1.77		1.87 ± 5.36		.141	.157
Glucose (mg/dL)						
Baseline[a]	90.0 ± 0.62	.093	93.5 ± 1.73	.001	.041	
Follow-up[a]	91.1 ± 0.57		98.4 ± 1.52		<.001	
Change	1.12 ± 0.66		4.84 ± 1.31		.023	<.001
Insulin (IU/mL)						
Baseline[a]	8.15 ± 0.21	.055	9.74 ± 0.59	.443	.008	
Follow-up[a]	7.78 ± 0.23		9.52 ± 0.70		.006	
Change	−0.37 ± 0.26		−0.22 ± 0.59		.819	.093
HOMA-IR						
Baseline[a]	1.81 ± 0.05	.160	2.28 ± 0.17	.870	.002	
Follow-up[a]	1.76 ± 0.06		2.32 ± 0.19		<.001	
Change	−0.05 ± 0.06		0.04 ± 0.15		.587	.032
FFA (Eq/L)						
Baseline[a]	520.8 ± 16.2	<.001	466.9 ± 25.8	.238	.431	
Follow-up[a]	427.4 ± 13.5		500.0 ± 27.2		.001	
Change	−93.5 ± 18.6		33.1 ± 25.6		<.001	.004

[a]Means ± SE tested by logarithmic transformation.
[b]P values derived from paired t test.
[c]P values derived from independent t test.
[d]P values derived after adjustment for baseline value.
Abbreviations: BMI – body mass index; WC – waist circumference; SBP – systolic blood pressure; DBP – diastolic blood pressure; TGs – triglycerides; FFA – free fatty acids.

participants with MetS had significantly unfavorable inflammatory and oxidative stress measurements for IL-6 (P = .002), leukocyte count (P < .001), MDA (P = .004), and ba-PWV (P < .001).

Relationship between Changes in ba-PWV and Changes in Metabolic, Inflammatory, and Oxidative Stress Markers

Changes in ba-PWV were positively correlated with changes in SBP (r = 0.287, P < .001), DBP (r = 0.155,

Table 3. Inflammatory and oxidative stress markers and ba-PWV according to MetS outcome at 3 years

	Baseline: non-MetS ($n = 288$)					
	Without MetS outcome ($n = 243$)	P^b	With MetS outcome ($n = 45$)	P^b	P^c	P^d
hs-CRP (mg/dL)						
Baseline[a]	1.23 ± 0.16	.733	0.87 ± 0.08	.442	.153	
Follow-up[a]	1.06 ± 0.14		1.44 ± 0.35		.061	
Change	-0.16 ± 0.21		0.57 ± 0.36		.147	.022
Serum IL-6 (pg/mL)						
Baseline[a]	3.66 ± 0.22	.270	3.24 ± 0.33	.011	.483	
Follow-up[a]	3.14 ± 0.14		4.35 ± 0.47		.002	
Change	-0.53 ± 0.26		1.11 ± 0.49		.009	.004
Serum TNF-α (pg/mL)						
Baseline[a]	8.94 ± 0.55	.147	7.77 ± 0.77	.017	.743	
Follow-up[a]	9.33 ± 0.56		9.49 ± 0.64		.196	
Change	0.40 ± 0.75		1.73 ± 1.01		.451	.493
WBCs ($\times 10^9$/L)						
Baseline[a]	5.41 ± 0.11	.143	5.67 ± 0.22	.137	.235	
Follow-up[a]	5.21 ± 0.09		6.13 ± 0.27		<.001	
Change	-0.20 ± 0.11		0.46 ± 0.33		.027	<.001
MDA (nmol/mL)						
Baseline[a]	9.69 ± 0.18	<.001	10.1 ± 0.39	<.001	.327	
Follow-up[a]	10.9 ± 0.18		12.2 ± 0.41		.004	
Change	1.21 ± 0.15		2.15 ± 0.41		.018	.002
ox-LDL (U/L)						
Baseline[a]	33.3 ± 0.76	<.001	32.7 ± 1.34	<.001	.910	
Follow-up[a]	43.6 ± 0.67		46.9 ± 2.04		.249	
Change	10.2 ± 0.70		14.3 ± 1.97		.061	.015
ba-PWV (cm/s)						
Baseline[a]	1274.0 ± 11.1	<.001	1396.7 ± 31.8	<.001	<.001	
Follow-up[a]	1308.4 ± 11.6		1469.1 ± 29.3		<.001	
Change	34.5 ± 7.35		72.4 ± 19.7		.046	.001

[a]Mean $\pm$ SE tested by logarithmic transformation.
[b]P values derived from paired t test.
[c]P values derived from independent t test.
[d]P values derived after adjustment for baseline value.
Abbreviations: hs-CRP – high-sensitivity C-reactive protein; IL-6 – interleukin-6; TNF-α – tumor necrosis factor-α; WBCs – white blood cells; MDA – malondialdehyde ; ox-LDL – oxidized-LDL; ba-PWV – branchial–ankle pulse wave velocity.

$P = .010$), total-cholesterol ($r = 0.137$, $P = .024$), glucose ($r = 0.152$, $P = .012$), leukocyte count ($r = 0.121$, $P = .046$), and MDA ($r = 0.131$, $P = .046$). Changes in leukocyte count were positively correlated with changes in BMI ($r = 0.159$, $P = .007$), WC ($r = 0.135$, $P = .023$), CRP ($r = 0.299$, $P < .001$), and IL-6 ($r = 0.314$, $P < .001$). Changes in MDA were positively correlated with changes in ox-LDL ($r = 0.139$, $P = .039$), DBP ($r = 0.137$, $P = .033$), and total-cholesterol ($r = 0.176$, $P = .006$). Furthermore, changes in IL-6 were positively correlated with changes in CRP ($r = 0.388$, $P < .001$).

DISCUSSION

Major strengths of this report include the longitudinal, observational study of middle-aged individuals not meeting the MetS criteria, the inclusion of ba-PWV (a marker of arterial stiffness) (15), and the inclusion of several inflammatory and oxidative stress markers and MetS components. The results of this study suggested that age-associated changes in ba-PWV and inflammatory and oxidative stress markers differ between individuals with and without MetS outcomes. Additionally, the age-

related increase in ba-PWV was associated with changes in inflammatory and oxidative stress markers. The age-related increase in arterial stiffness was greater in patients who developed MetS and showed higher levels of inflammatory and oxidative stress markers.

Pulse wave velocity is an established index of arterial stiffness (16), and ba-PWV shows characteristics similar to those of central aortic PWV (17). Arterial stiffness, one of the most significant manifestations of vascular aging (18,19), can increase SBP (20,21), and increases with age, even in healthy individuals without clinical cardiovascular disease (CVD) (22). The underlying mechanisms responsible for arterial stiffness remain unknown; however, oxidative stress, such as the age-related increase in ox-LDL and MDA in middle-aged subjects, could play an important role given that there were positive correlations between changes in ba-PWV and MDA and between MDA and ox-LDL. In our study, multiple logistic regression analysis revealed that change of ba-PWV increased by 0.135 cm/s (95% CI: 0.058, 11.588, $P = .04$) per unit change of MDA levels after adjustment for age and change of BMI, SBP, DBP, total-cholesterol, glucose, and WBCs. Tso et al. (23) also reported that age

and SBP of studied patients were independently associated with ba-PWV. Recently, Brinkley et al. (11) also suggested that oxidative stress, including ox-LDL, may be related to the pathogenesis of arterial stiffness, independent of other CVD risk factors.

The presence of CVD risk factors, including obesity, has been shown to accelerate the vascular changes that result in arterial stiffening (22). In this study, age-related changes in BMI, ba-PWV, and inflammatory and oxidative stress markers were greater in individuals who developed MetS than in those who did not. Increased evidence suggests that obesity and being overweight constitute low-grade inflammatory states (24,25). A chronic inflammatory response might increase the blood concentration of acute-phase reactants, including leukocytes (25). Thus, detecting a high leukocyte count or a change in the count has been linked to predicting specific diseases, such as MetS (26), subclinical atherosclerosis (25), and CVD (27), as well as all-cause mortality (28). Meanwhile, cytokines, such as IL-6 (a potent leukocyte differentiation factor), have been associated with IR (29). Interlukin-6, which is produced by immune cells and adipose tissue (30), increases hepatic synthesis of CRP (31). In this study, changes in IL-6 were associated with changes in leukocyte count and hs-CRP. In addition, changes in leukocyte count were positively correlated with changes in BMI and ba-PWV. This result supports the previous suggestion of a possible synergistic effect between MetS and inflammation on the arterial wall (1).

Within 3 years, a considerable number (15.6%; 45 out of 288) of the middle-aged participants of this study fulfilled the criteria for MetS. The incidence of MetS in our middle-aged study population was as high as reported in a survey for Korean middle-aged adults (2005 Korea National Health and Nutrition Examination Survey Report III). Participants with MetS outcomes had significantly unfavorable initial measurements, including baseline BMI, WC, BP, TG, HDL-cholesterol, glucose, insulin, HOMA-IR index, and ba-PWV. These results are consistent with a previous finding of a significant correlation between the baseline MetS component count and MetS development (14). In fact, Lin et al. (14) showed that middle-aged adults having one or two MetS components had a 2.8-fold and 7.3-fold increased risk for developing MetS within 5 years, respectively, versus adults having no MetS components.

Additionally in our study, significant decreases in TEE and significant increases in TCI/TEE were observed in the MetS outcome groups after the 3-year follow-up, but which were not observed in the non-MetS outcome groups. Our result is partly in accordance with the work of Esmaillzadeh and Azadbakht (32), which shows that higher dietary energy density was significantly associated with a greater risk of MetS and most of its components. It may suggest that usual lifestyle is closely associated with the risk of MetS and important in the prevention of MetS.

In conclusion, this study showed that the simultaneous occurrence of MetS and higher proinflammatory and oxidative stress markers increase the likelihood for greater age-related increases in arterial stiffness. Therefore, lifestyle modifications, such as caloric restriction and exercise, are recommended to the middle-aged individuals at risk for MetS criteria as an efficacious therapeutic intervention for preventing MetS and the progression of arterial stiffness.

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