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Association between atherosclerosis and bone loss in the Korean population: results from a large community-based cohort study

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Major in Translational Medicine

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ABSTRACT

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Many clinicians have demonstrated the frequent presence of atherosclerosis in osteoporosis patients. Although the number of proposals for the co-pathways related to atherosclerosis and osteoporosis are on the rise, no studies have evaluated the relationship between changes in carotid intima-media thickness

(CIMT) and changes in bone density, in a longitudinal cohort. We aimed to evaluate the association between changes in CIMT and changes in bone mineral density (BMD) by using dual-energy X-ray absorptiometry (DXA).

Data of 3,403 participants who underwent both CIMT measurement and DXA were performed in 2011-2012 and 2013-2014 were analyzed, as a part of the Ansung cohort study. Carotid atherosclerosis was classified according to the CIMT quartiles, into the group with a CIMT of 0.1 cm or higher and the group with a CIMT lower than 0.1 cm. Based on changes in the CIMT, patients were classified into the accelerating progressor group and non-progressor group, by mean + 1 standard deviation (0.0915 cm/2 years), and the new plaque group or disappeared plaque group, by changes in the plaque status. By various carotid atherosclerosis criteria, adipokine levels were additionally compared.

As the CIMT quartiles increased, the total hip and femur neck BMD, in men, and the lumbar, total hip and femur neck BMD, in women, decreased. The risk of the CIMT being greater than 0.1

cm increased as the lumbar BMD decreased by 5%, in a longitudinal cohort analysis, with an odds ratio of 1.627 (95% confidence interval, 1.111–2.382) after adjusting for age, hypertension, diabetes, dyslipidemia and postmenopausal duration. Significant decreases were observed in the femur neck BMD in the accelerating progressor group compared to the non-progressor group ($P < 0.001$); this significance persisted after adjusting for age (0.012±0.047 mm vs. −0.008±0.050 mm, $P < 0.001$). On comparing between the new plaque and disappeared plaque groups, the trabecular bone score (TBS) values were significantly lower in the new plaque group than in the disappeared plaque group, at the 6-year follow-up, even after adjusting for age, sex, systolic blood pressure, levels of glycated hemoglobin, low-density lipoprotein cholesterol and serum creatinine, smoking status, and alcohol status. As for the biomarkers, the adiponectin level was lower in the new plaque group than in the disappeared plaque group. In contrast, the adiponectin levels were higher in those in the group with a CIMT of 0.1 cm or more than in the group with a CIMT lower than 0.1 cm.

For the postmenopausal women, at the 4-year follow-up, the risk of the CIMT being greater than 0.1 cm increased as the lumbar BMD decreased by 5%. The accelerating progressor group was associated with a much lower femur neck BMD than the non-progressor group, after adjusting for age. The new carotid plaque group had a much lower TBS than the disappeared plaque group. However, in terms of the adipokine levels, we did not obtain consistent results. In conclusion, in this large prospective Korean community-based cohort, we found that, in those with a CIMT higher than 0.1 cm (pathologic CIMT) or those in the accelerating progressor group or new plaque group, screening for bone health could be helpful.

Keywords: carotid intima media thickness, atherosclerosis, carotid plaque, osteoporosis, bone mineral density. trabecular bone score, adiponectin

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Introduction

With the increase in life-expectancy, the size of the elderly population has also risen; this has led to the increased prevalence of atherosclerosis and osteoporosis, which are associated with high morbidity and mortality [1, 2]. Several epidemiological studies have shown the relationship between atherosclerosis and osteoporosis [3-5], with some researchers considering the conditions common consequences of aging [6]. However, there is increasing evidence on the independent connection between atherosclerosis and osteoporosis, even after adjustment for age. It is because atherosclerosis and osteoporosis share common process of mineralization and inflammation [1, 7].

Some previously conducted studies have evaluated the relationship between bone loss and atherosclerosis [7-10]. A large population-based study in Norway, comprising 2,726 postmenopausal women and 2,543 men, reported the presence of a positive association between low bone mass and bone mineral

density (BMD) quartiles as well as plaque echogenicity, after adjusting for age, sex, and other cardiovascular risk factors [7]. Another large study—the Multi-Ethnic Study of Atherosclerosis—including 904 post-menopausal women and 929 men, reported that, even after adjusting for age, body mass index (BMI), diabetes, renal function, and other covariates, lower volumetric trabecular lumbar BMD was associated with greater internal carotid intima-media thickness (CIMT) in men (P <0.02), but not with common CIMT [8]. A study using data from the Korea National Health and Nutrition Examination Survey showed that, in the male population, femur neck BMD and lumbar spine BMD had an inverse correlation with the Framingham risk score after adjusting for covariates, and the first quartile of BMD had a greater 10-year risk than the fourth quartile, but not with the carotid parameters [10]. A recently conducted study investigated the associations between osteoporosis and atherosclerosis, by quantitative ultrasound (QUS) parameters and CIMT, carotid artery plaques, and the ankle-brachial index, with 5,680 men and women aged 20-90 years, from two large

cohorts. The QUS parameters were not significantly associated with the CIMT or ankle-brachial index in the adjusted models [9]. Therefore, there is still uncertainty surrounding the relationship between osteoporosis and atherosclerosis, and further investigation is required.

Several factors can be attributed to the correlations between atherosclerosis and bone loss. The possible candidates for the co-factors related to bone loss and the progression of atherosclerosis, with regards to bone and vascular mineralization, are osteoprotegerin, osteopontin, fetuin-A and matrix Gla protein, or inflammation factors such as adiponectin and plasminogen activator inhibitor-1 (PAI-1) [11]. However, there is a lack of evidence on the co-factors associated with atherosclerosis and bone loss, especially from a longitudinal study.

CIMT is known as a surrogate marker for subclinical atherosclerosis, and risk factor for myocardial infarction and stroke [12]. Some studies have shown that CIMT progression can predict future cardiovascular events [13]. However, there is

a lack of studies on the parameters related to atherosclerosis and longitudinal changes in BMD. Therefore, we hypothesized that atherosclerosis surrogate markers (CIMT, carotid plaque and adipokines, which reflect systemic inflammation) and bone-related parameters (BMD and trabecular bone score [TBS]) have a reciprocal negative relationship, using a large prospective cohort. For this, we first analyzed the cross-sectional relationship between CIMT, adipokines, base metabolic parameters and BMD. Next, we examined if there were longitudinal changes in BMD according to various CIMT criteria. Third, we analyzed the longitudinal change in BMD by a group with a CIMT of 0.1 cm or more (pathologic CIMT) or a group with a CIMT below 0.1 cm. Fourth, we examined the longitudinal changes in BMD according to the change in CIMT. Lastly, BMD, carotid plaque status, and the TBS were explored.

Materials and Methods

Ansung cohort study

In this study, data were derived from the Ansung cohort study, which is an on-going prospective study that began in 2001, with the support of the National Genome Research Institute (Korea Centers for Disease Control and Prevention, Cheongju, Korea). The study is a part of the Korean Genome and Epidemiology Study, a large community-based epidemiological survey to investigate the genome-wide association with chronic disease and the related risk factors, in Koreans. The initial samples included 5,018 participants, aged 40-69 years, who resided in the borders of the survey area at least 6 months prior to enrollment. Initially, a list of telephone numbers was obtained from the local telephone companies, and two-stage cluster sampling was performed, based on the information obtained from five of 11 governing districts (termed "myon"). Consequently, 7,192 eligible individuals were identified by mail, telephone calls or door-to-door visits. From the eligible individuals, a total of 5,018 participants were recruited and baseline examinations

were conducted in 2001–2002 at the Ajou University Medical Center. Baseline examinations included health check–ups, interviews, the collection of blood and urine samples, and dual–energy X–ray absorptiometry (DXA) and CIMT tests. Participants were surveyed for demographic data, medical history, family history of disease, and lifestyle habits, including smoking, alcohol and exercise, using an interview–based questionnaire. Biennial follow–ups were performed with similar examinations, interviews and collections. The last follow–up was conducted in 2015–2016. We predominantly used data from the 2011–2012 to 2013–2014 follow–up, as the CIMT test was conducted from the 2011–2012 follow–up, and the DXA test from 2008. We limited our analysis to men aged 65 years or older, and postmenopausal women. This cohort was approved by the respective institutional ethics review committees (IRB No. AJIRB–CRO–07–012 for the Ansung–Ansan cohort) and all the participants provided written informed consent. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Anthropometric measurements

Height and body weight were measured with participants dressed in light clothing. BMI was calculated as the weight divided by the height squared (kg/m^2). Anthropometric parameters and blood pressure were measured by standard methods. Levels of fasting plasma glucose (FPG), total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and glycated hemoglobin (HbA1c) were measured in a central laboratory after a 12-hour fast. The HbA1c level was measured using high-performance liquid chromatography (Variant II; BioRad Laboratories, Hercules, CA). The presence of diabetes was assessed by a 75-g oral glucose tolerance test and the HbA1c levels, and was defined according to the American Diabetes Association criteria: fasting blood glucose of 7.0 mmol/L or greater (126 mg/dL), 2-hour plasma glucose of 11.1 mmol/L or greater (200 mg/dL), HbA1c level of 6.5% or greater, or the current consumption of oral antidiabetic drugs or insulin. Hypertension was defined as a systolic blood pressure higher than 140 mmHg and diastolic blood pressure higher than 90 mmHg, or the consumption of anti-hypertensive medication.

Data on current alcohol intake, smoking status, exercise status, past medical history, and family history were collected by standardized questionnaires and face-to-face interviews. Past history of fragility fracture was defined as low-trauma hip, vertebral, proximal humerus, and radius fractures that occurred after the age of 40 years.

CIMT and plaque measurements

Participants' extracranial carotid arteries were examined by trained medical assistants using B-mode ultrasonography (GE Medical Systems, Philips). Longitudinal scans of the distal straight portion of the far wall of the common carotid artery (CCA) of both sides were recorded. CCA intima-media thickness was assessed using semi-automated tracking software, located directly proximal to the widening of the artery at the bifurcation, measuring the distance between the lumen-intima and media-adventitia interfaces at a segment length of 1 cm. The mean CIMT was used for statistical analyses, and calculated as the average of the mean values of the three points on both sides. If

at least one arterial segment was read as having a plaque, defined as a thickness of 0.9 mm or more, carotid plaque was classified as being "present". The presence of carotid plaque and CIMT were measured in every follow-up. The following CIMT criteria were used as cross-sectional values and changes: for cross-sectional values, we used the criteria of CIMT quartiles, and a group with a CIMT of 0.1 cm or higher and one with a CIMT lower than 0.1 cm were used; for changes of CIMT in carotid atherosclerosis, a CIMT accelerating progressor group and non-progressor group were divided by the mean + standard deviation (SD) criterion (0.0915 cm/2 years). Based on changes in the presence of carotid plaque, participants were classified into the new plaque group, unchanged plaque group or disappeared plaque group.

Bone DXA measurements

Lumbar spine, femoral neck, and total hip BMD (grams per square centimeter) measurements were taken from 2008, biennially, using DXA (Lunar Prodigy; GE Medical Systems), and

analyzed using Encore Software version 11.0. Through the measurements of the lowest lumbar spine, femoral neck or total hip score, having a T-score of -2.50 or lower was assessed as the presence of osteoporosis, T-score of -2.49 to -1.01 was assessed as the presence of osteopenia, and T-score of -1.00 or higher was assessed as being normal. The percentage of the coefficient of variation of BMD was 1.7% for the lumbar spine, 1.8% for the femoral neck, and 1.7% for the total hip. The 1^{st} to 4^{th} lumbar values of BMD were used for lumbar spine BMD analysis. TBS measurements were retrospectively performed using TBS iNsight Software, version 2.0.0.1 (MedImaps) from the spine DXA database. All the machines were calibrated using phantoms.

Adipokine measurements

We measured adipokine levels in the 2000-2001 period using participants' initial samples in the Ansung cohort. To measure the adipokine levels, plasma was obtained after centrifugation at 1500 × g for 10 min and stored at -70 ° C. Adipokines,

including PAI-1, resistin, interleukin 6 (IL-6), leptin, monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), retinol binding protein 4 (RBP4) and adiponectin, were measured through multiplex bead analysis using commercial immunoassays (Lincoplex, high-sensitivity human cytokine panel, Linco Research Inc., St. Charles, MO, USA).

Study design and statistical analyses

Data are presented as mean ± SD, or numbers and percentages. The constitutive variables were analyzed using Student *t* tests or one-way analysis of variance (ANOVA), followed by Bonferroni's post-hoc comparison tests. For qualitative variables, the results are expressed as percentages, and were compared using chi-square tests. Pearson's correlation analysis was used to determine the relationships between CIMT parameters and DXA measurements, adipokines and biochemical parameters. In addition, analysis of covariance models were used to adjust for age or BMI, for the comparison of CIMT and DXA parameters. Univariate and multivariate logistic regression

models were used to find the factors significantly associated with a CIMT of 0.1 cm or more, in postmenopausal women. Significance was defined as $P < 0.05$ for two-sided tests. All statistical analyses were performed using SPSS software for Windows (version 21.0; SPSS Inc, Chicago, IL).

Results

Ⅰ. Baseline cross-sectional analysis of the prospective community-based cohort

Clinical characteristics of the prospective community-based cohort

We included participants in whom both DXA and CIMT tests were performed, in 2011-2012 and 2013-2014. Of the 3,403 participants, 1,354 were men and 1,726 were women. The baseline characteristics of the participants in 2011-2012, by sex, are presented in Table 1. The mean ages for men and women were 64.5±8.4 and 65.7±8.3 years, respectively. The men were taller and heavier than the women, but the BMI and waist circumference were higher in the women. Fasting glucose levels were lower but HbA1c and fasting insulin levels were higher in the women (HbA1c 5.8±0.9 % in men and 5.9±0.9 % in women). In terms of the lipid profiles, the total cholesterol, HDL cholesterol, and LDL cholesterol levels were higher in the women, while the triglyceride levels were higher in the men. For renal and liver function, the men displayed higher values. As for the

bone markers, women had higher c-telopeptide and osteocalcin levels than the men. For comorbidities, men had a higher ratio of hypertension than the women [88 (6.5 %) vs. 72 (4.2 %); P = 0.004]. A higher proportion of women took medications for hypertension, dyslipidemia, anticoagulation, and osteoporosis. The percentage of smokers and those who regularly exercised was higher among men. The baseline CIMT and BMD values were higher in the men than women. In terms of adipokines, the men had higher PAI-1, TNF-α and RBP4 levels, while the women had higher leptin and adiponectin levels.

Table 1. Clinical characteristics of the prospective community-based cohort.

Characteristics	Male (n=1354)	Female (n=1726)	Total (n=3403)	P-value
Age (years)	64.5±8.4	65.7±8.3	65.2±8.3	<0.001
Height (cm^2)	165.8±6.0	151.9±6.1	158.0±9.2	<0.001
Weight (kg)	65.4±10.0	57.1±9.0	60.7±10.3	<0.001
BMI (kg/m^2)	23.7±3.1	24.7±3.4	24.3±3.3	<0.001
Waist circumference (cm)	86.6±8.7	87.3±9.2	87.0±9.0	0.021
SBP (mmHg)	118.9±15.4	118.7±16.7	118.8±16.2	0.802

Characteristics	Male	Female	Total	*P*-value
DBP (mmHg)	73.9±9.4	71.8±9.3	72.7±9.4	<0.001
HbA1c (%)	5.8±0.9	5.9±0.9	5.9±0.9	0.008
Fasting glucose (mg/dL)	103.2±28.4	99.5±25.6	101.1±26.9	<0.001
Fasting insulin (μIU/ml)	7.7±4.4	8.8±4.9	8.3±4.7	<0.001
Total cholesterol (mg/dL)	177.9±31.6	190.7±33.9	185.1±33.5	<0.001
Triglycerides (mg/dL)	146.4± 104.2	136.0± 80.6	140.6± 91.8	0.002
HDL-cholesterol (mg/dL)	45.0±12.5	46.8±11.2	46.0±11.8	<0.001
LDL-cholesterol (mg/dL)	107.6±32.6	118.1±31.4	113.5±32.3	<0.001
WBC ($10^3/\mu L$)	5.7±1.6	5.3±1.6	5.5±1.6	<0.001
BUN (mg/dL)	17.1±5.5	16.4±4.6	16.7±5.1	<0.001
Serum creatinine (mg/dL)	1.10±0.22	0.90±0.17	0.98±0.22	<0.001
AST (IU/L)	29.7±16.1	27.1±25.8	28.2±22.1	0.001
ALT (IU/L)	24.5±13.3	20.6±9.9	22.3±11.7	<0.001
hs-CRP (mg/dL)	2.2±10.8	1.8±4.5	2.0±7.9	0.159
After 2yr C-telopeptide (ng/ml)	0.33±0.17	0.38±0.22	0.36±0.20	<0.001
After 2yr Osteocalcin (ng/ml)	15.9±6.1	20.0±11.2	18.3±9.64	<0.001
Hypertension	88 (6.5%)	72 (4.2%)	160 (5.2%)	0.004
Diabetes mellitus	25 (1.9%)	34 (2.0%)	59 (1.9%)	0.895
Dyslipidemia	35 (2.6%)	58 (3.4%)	93 (3.0%)	0.244
Cerebrovascular disease	10 (0.7%)	11 (0.6%)	21 (0.7%)	0.826
Coronary artery disease	10 (0.7%)	12 (0.7%)	22 (0.7%)	1.000

Characteristics	Male	Female	Total	*P*-value
Compression fracture	101 (7.9%)	186 (11.3%)	287 (9.8%)	0.002
Hypertension medication	506 (37.5%)	778 (45.0%)	1284 (41.7%)	<0.001
Diabetes medication	172 (12.8%)	240 (13.9%)	412 (13.4%)	0.365
Insulin use	22 (1.6%)	15 (0.9%)	37 (1.2%)	0.066
Dyslipidemia medication	81 (6.0%)	160 (9.3%)	241 (7.8%)	0.001
Anticoagulation medication	5 (0.4%)	18 (1.0%)	23 (0.7%)	0.035
Osteoporosis medication	19 (1.4%)	250 (14.5%)	269 (8.7%)	<0.001
Steroid medication	3 (0.2%)	8 (0.5%)	11 (0.4%)	0.366
Past or current smoking	960 (64.2%)	42 (2.2%)	1002 (29.4%)	<0.001
Regular exercise	330 (24.5%)	352 (20.3%)	682 (22.2%)	0.007
Baseline mean CIMT (mm)	0.748± 0.020	0.715± 0.181	0.730± 0.190	<0.001
Baseline Lumbar BMD (g/cm^2)	1.127± 0.188	0.959± 0.170	1.034± 0.197	<0.001
Baseline Femur neck BMD (g/cm^2)	0.892± 0.138	0.767± 0.122	0.823± 0.144	<0.001
Baseline Total hip BMD (g/cm^2)	0.978± 0.142	0.852± 0.135	0.909± 0.152	<0.001

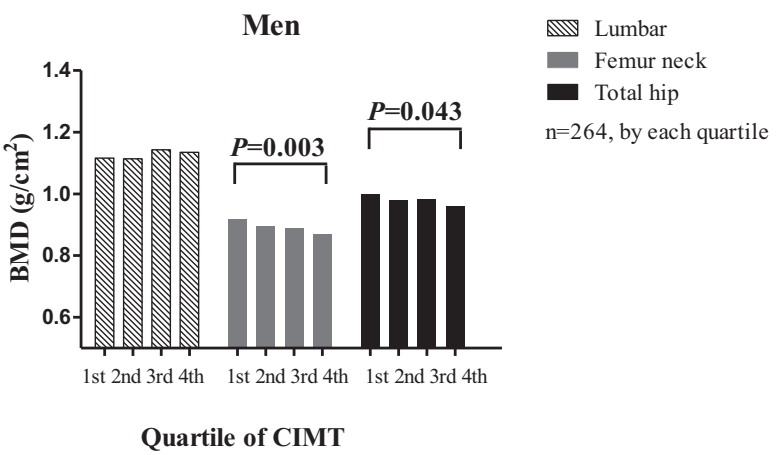
Characteristics	Male	Female	Total	*P*-value
Baseline TBS (unitless)	1.390± 0.094	1.347± 0.089	1.366± 0.093	<0.001
PAI-1 (ng/ml)	41.33± 12.62	37.43± 10.37	39.39± 11.71	<0.001
Resistin (ng/ml)	19.40± 10.80	19.40± 19.25	19.40± 15.58	0.999
IL-6 (pg/ml)	3.76±5.27	3.56±6.12	3.66±5.70	0.729
Leptin (ng/ml)	4.09± 3.92	15.90± 11.97	10.05± 10.71	<0.001
MCP-1 (pg/ml)	265.3±99.1	260.0±99.8	262.6±99.4	0.541
TNF-α (pg/ml)	7.71±5.65	6.62±3.90	7.16±4.88	0.010
RBP4 (mg/ml)	150.2±76.4	120.7±67.5	135.5±73.5	<0.001
Adiponectin (mg/ml)	2.23±2.16	4.09±3.17	3.16±2.86	<0.001

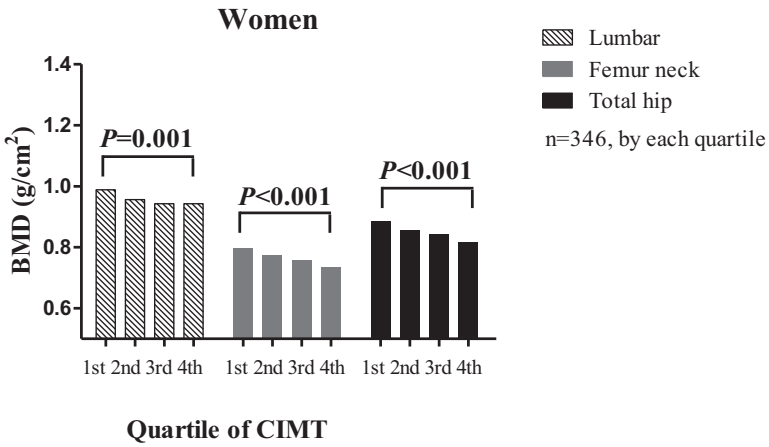
Data are means ± SD, or n (%), *P*-values are between male and female. Baseline data are from 2011-2012 Ansung data. Adipokine data are from 2000-2001 Ansung data (total n=528). BMI, body mass index; CIMT, carotid intima-media thickness; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; WBC, white blood cell count; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRP, C-reactive protein; PAI-1, plasminogen activator inhibitor-1; IL-6, Interleukin 6;MCP-1, Monocyte chemoattractant protein-1; TNF- α; tumor necrosis factor-α; RBP4, Retinol binding protein 4.

Cross-sectional comparison of the bone parameters, by CIMT quartiles

The cross-sectional comparison of the bone parameters, according to CIMT quartiles, by sex, are shown in Figure 1. Except for the lumbar BMD in men, all the other BMD values decreased significantly as the CIMT increased, according to the quartiles.

Figure 1. Cross-sectional comparison of bone parameters, by CIMT quartiles.





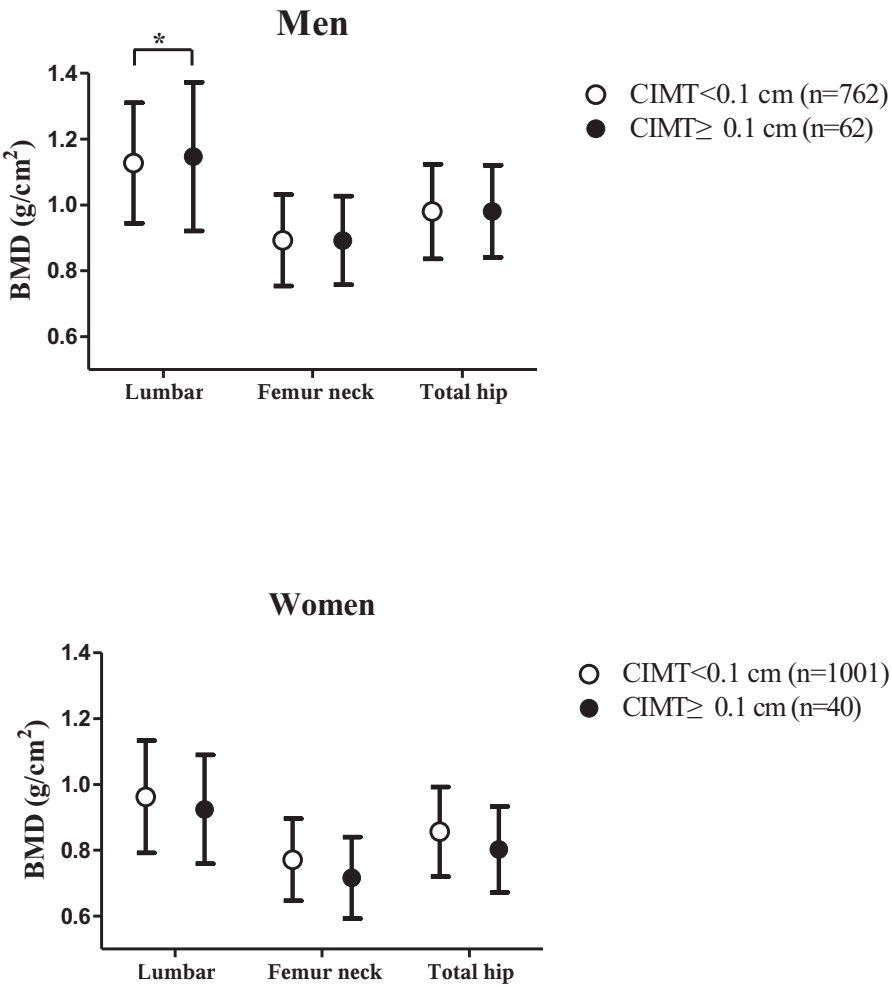
Data are means ± SD (g/cm^2), Baseline data are from 2011-2012 Ansung cohort. CIMT, carotid intima-media thickness; BMD, bone mineral density.

Cross-sectional comparison of the bone parameters, by CIMT < 0.1 cm vs. ≥0.1 cm

Since having a CIMT higher than 0.1 cm is known to increase the risk of stroke or cardiovascular disease by 10-15% [14,15], pathologic CIMT is often defined as a value of over 0.1 cm. Therefore, the cross-sectional comparison of the bone parameters, by CIMT < 0.1 cm vs. ≥0.1 cm, by sex, was performed, and is shown in **Figure 2**. We did not observe a significant downward trend in the BMD for the CIMT group with

values of 0.1 cm or higher, in comparison with the group with values lower than 0.1 cm, in the men. In women, although it was not statistically significant, CIMT and BMD showed a negative correlation; the CIMT group with values of 0.1 cm or higher showed a lower BMD than the group with values lower than 0.1 cm.

Figure 2. Cross-sectional comparison of bone parameters, by CIMT < 0.1 cm vs. ≥0.1 cm.



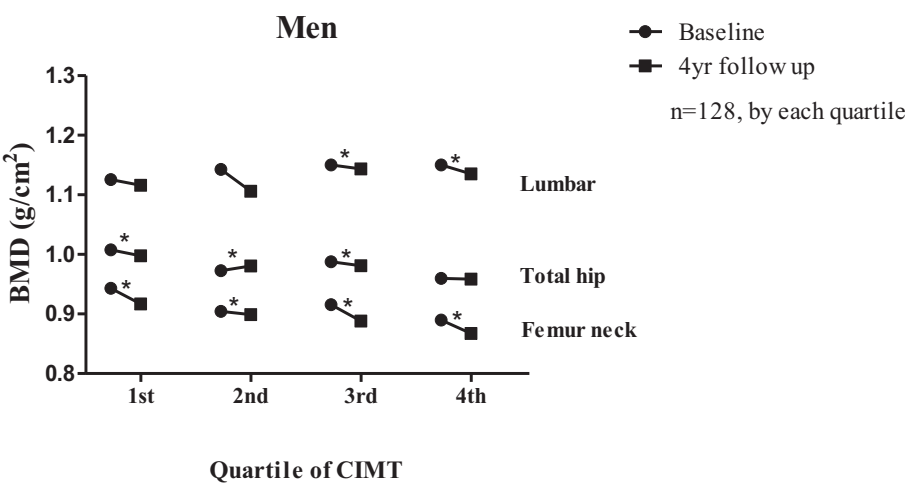
Data are means ± Standard deviation (g/cm^2), Baseline data are from 2011-2012 Ansung cohort. CIMT, carotid intima-media thickness; BMD, bone mineral density; *, $P < 0.05$.

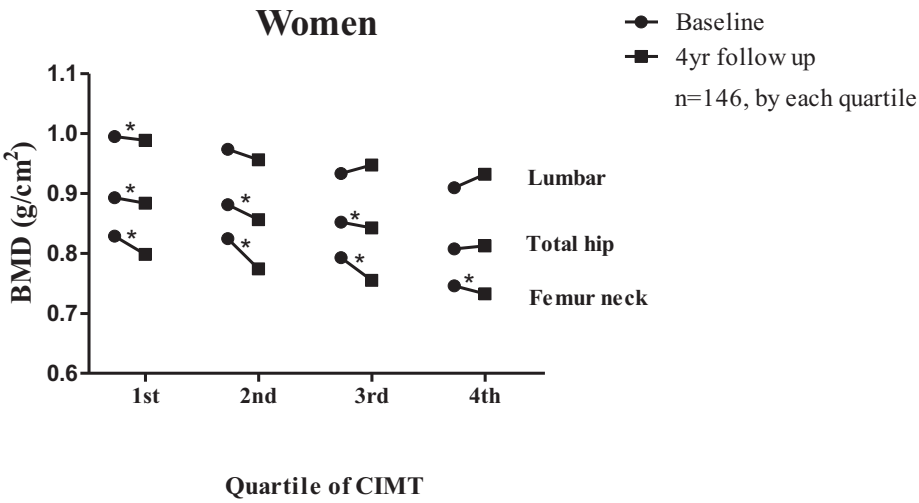
Ⅱ. Longitudinal changes in the BMD, according to the CIMT status

Longitudinal changes in BMD at 4 years, according to the CIMT quartiles

We compared the changes in BMD, at 4 years, according to the quartiles of CIMT. In the women, the higher the quartile, the lower the BMD. This tendency was not observed in the men.

Figure 3. Longitudinal changes in BMD at 4 years, according to the CIMT quartiles





Data are means (g/cm^2), Baseline data are from 2011−2012 Ansung cohort. BMD follow up data is from 2007−2008 to 2011−2012 CIMT, carotid intima-media thickness; BMD, bone mineral density; *, $P < 0.05$.

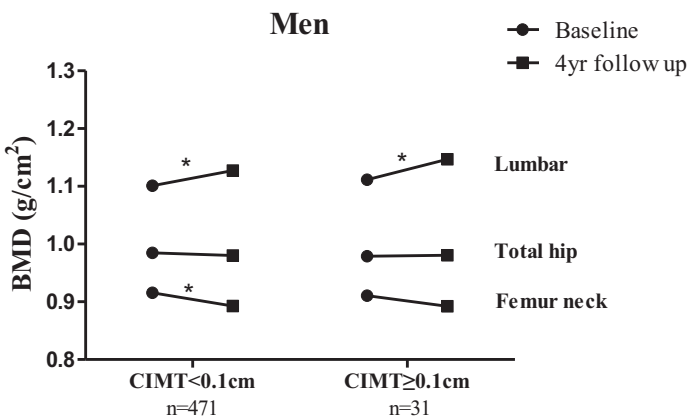
Ⅲ. Longitudinal changes in the BMD, by CIMT < 0.1 cm vs. ≥0.1 cm, at the baseline

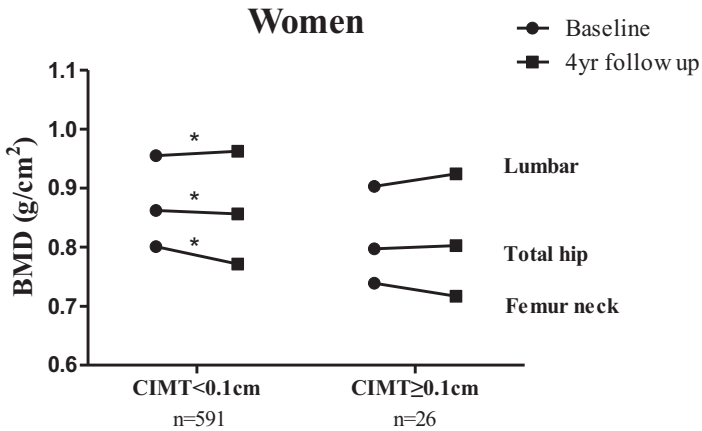
Longitudinal BMD changes for 4 years by CIMT < 0.1 cm vs. ≥0.1 cm at the baseline

For the longitudinal changes in the BMD, at 4 years, according to the presence of pathologic CIMT, at the baseline, we performed analyses between the CIMT < 0.1 cm and CIMT ≥0.1 cm groups.

Figure 4 shows the tendency of the BMD to decrease further in the group with a CIMT of 0.1 cm or higher, in women, than in the group with CIMT values lower than 0.1 cm, especially in the femur neck. This tendency was not observed in the lumbar spine. However, the differences in the BMD change, by pathologic CIMT, were not statistically significant across sexes.

Figure 4. Longitudinal BMD changes for 4 years by CIMT < 0.1 cm vs. ≥0.1 cm at the baseline.





Data are means (g/cm^2), Baseline data are from 2011–2012 Ansung cohort. BMD follow up data is from 2007–2008 to 2011–2012 CIMT, carotid intima–media thickness; BMD, bone mineral density; L, lumbar; FN, femur neck; TH, total hip; *, $P < 0.05$.

Clinical characteristics of the postmenopausal women, by CIMT < 0.1 cm vs. ≥0.1 cm, at the baseline

We additionally analyzed the cross–sectional relation between the criteria of the baseline CIMT thickness of 0.1 cm, and the changes in the bone parameters at the 4–year follow–up, only for the postmenopausal women. Women with a CIMT higher than 0.1 cm tended to be older, with a higher proportion of previous

or current smoking and a lower proportion of regular exercise, and have a longer postmenopausal duration. As for the laboratory parameters, those with CIMT values greater than 0.1 cm, in 2011–2012, had lower ALT levels and higher systolic blood pressure levels. For changes in BMD, all the BMD values at the baseline and at the 4–year follow–up were lower in the group with a CIMT value greater than 0.1 cm, than in the other group. In the 4–year follow–up, only the lumbar TBS values decreased; however, these changes were not statistically significant (Table 2).

Table 2. Clinical characteristics of the postmenopausal women, by CIMT < 0.1 cm vs. ≥0.1 cm at the baseline.

	CIMT ≤ 0.1 cm (n=1,227)	CIMT > 0.1 cm (n=42)	*P*–value
Age (years)	64.8±8.2	72.1±6.3	<0.001
Diabetes mellitus	296 (24.1%)	13 (31.0%)	0.360
Hyperlipidemia	40 (3.3%)	1 (2.4%)	1.000
Hypertension	53 (4.3%)	4 (9.5%)	0.115

	CIMT ≤ 0.1 cm	CIMT > 0.1 cm	*P*-value
Coronary artery disease	8 (0.7%)	1 (2.4%)	0.262
BMI (kg/m^2)	24.8±3.4	24.7±3.8	0.800
Previous or current Smoking	21 (1.7%)	3 (7.1%)	0.042
Regular exercise	256 (20.9%)	3 (7.1%)	0.031
Osteoporosis medication	161 (13.1%)	6 (14.3%)	0.816
DM medication	175 (14.3%)	8 (19.0%)	0.373
Dyslipidemia medication	115 (9.4%)	2 (4.8%)	0.422
Anti-coagulant medication	12 (1.0%)	0 (0.0%)	1.000
Steroid medication	7 (0.6%)	0 (0.0%)	1.000
Postmenopausal duration (years)	16.1±10.1	23.2±8.0	<0.001
Serum creatinine (mg/dL)	0.9±0.2	0.9±0.2	0.320
BUN (mg/dL)	16.4±4.5	17.7±5.7	0.063
Total cholesterol (mg/dL)	191.3±33.7	185.1±34.6	0.251
Triglyceride (mg/dL)	135.7±82.6	116.1±61.4	0.127
HDL-cholesterol (mg/dL)	46.9±11.4	47.2±10.4	0.886
LDL-cholesterol (mg/dL)	118.8±31.5	114.7±29.8	0.414
CRP (mg/dL)	1.8±4.8	1.2±1.6	0.449
AST (IU/L)	26.2±9.3	25.3±4.1	0.497
ALT (IU/L)	20.8±10.5	18.3±5.2	0.005
Systolic BP (mmHg)	118.1±16.7	126.9±16.8	0.001
Diastolic BP (mmHg)	71.8±9.4	70.5±9.2	0.383
Adiponectin (mg/ml)	3.84±3.01	6.37±4.55	0.046

	CIMT ≤ 0.1 cm	CIMT > 0.1 cm	*P*-value
Baseline Lumbar BMD (g/cm^2)	0.955±0.176	0.909±0.135	0.081
Baseline Total hip BMD (g/cm^2)	0.862±0.144	0.797±0.128	0.003
Baseline Femur neck BMD (g/cm^2)	0.801±0.135	0.741±0.118	0.004
Baseline Lumbar TBS (unitless)	1.364±0.093	1.353±0.091	0.453
4 yr f/u Lumbar BMD (g/cm^2)	0.963±0.171	0.924±0.162	0.120
4 yr f/u Total hip BMD (g/cm^2)	0.856±0.136	0.795±0.134	0.002
4 yr f/u Femur neck BMD (g/cm^2)	0.771±0.125	0.708±0.126	0.001
4 yr f/u Lumbar TBS (unitless)	1.349±0.089	1.313±0.102	0.013
Change between 4 years			
⊿ Lumbar BMD	0.007±0.072	0.012±0.113	0.824
⊿ Total hip BMD	0.015±0.051	0.020±0.061	0.629
⊿ Femur neck BMD	0.036±0.065	0.027±0.065	0.492
⊿ Lumbar TBS	−2.274±1.411	−2.254±1.189	0.946
PAI-1 (ng/ml)	37.46±10.74	34.46±11.60	0.517
Resistin (ng/ml)	18.44±19.34	18.15±14.83	0.971
IL-6 (pg/ml)	3.65±6.41	2.75±2.61	0.292

	CIMT ≤ 0.1 cm	CIMT > 0.1 cm	*P*-value
Leptin (ng/ml)	15.42±10.57	19.75±22.31	0.656
MCP-1 (pg/ml)	254.3±90.6	265.3±100.5	0.769
TNF-α (pg/ml)	6.46±3.50	9.97±9.80	0.313
RBP4 (mg/ml)	121.1±67.4	135.0±71.4	0.621
Adiponectin (mg/ml)	3.84±3.01	6.37±4.55	0.046

Values are means ± Standard deviation or n (%), Baseline data are from 2007-2008 Ansung cohort data. Adipokine data are from 2000-2001 Ansung cohort data (total n=528). CIMT, carotid intima-media thickness; BMD, bone mineral density; TBS, trabecular bone score; BMC, bone mineral content; BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; WBC, white blood cell count; BUN, blood urea nitrogen; CRP, C-reactive protein.

Risk factors associated with a CIMT higher than 0.1 cm in the univariate and multivariate logistic regression models, in postmenopausal women

Further analyses were performed for the factors associated with CIMT (Table 3). In the univariate analysis, the baseline total hip BMD per SD decrease, at the 4-year follow-up, elevated the risk for the CIMT to be higher than 0.1 cm by 1.623 times, and the baseline femur neck BMD per SD decrease elevated the risk for the CIMT to be higher than 0.1 cm by 1.605 times. Additionally, higher age, the presence of hypertension, and a longer postmenopausal duration increased the risk for the CIMT to be higher than 0.1 cm. Finally, a per 5% decrease in the lumbar spine BMD, over 4 years, was associated with an increased risk for the CIMT to be greater than 0.1 cm, with an odds ratio (OR) of 1.627 (95% confidence interval [CI], 1.111-2.382), after adjusting for age, hypertension, diabetes, dyslipidemia and postmenopausal duration.

Table 3. Risk factors associated with a CIMT higher than 0.1 cm in the univariate and multivariate logistic regression models, in postmenopausal women.

	Univariate OR		Adjusted OR*	
	OR	95% CI	OR	95% CI
Baseline Lumbar BMD per SD decrease	1.040	(0.772–1.400)	0.636	(0.367–1.102)
Baseline Total hip BMD per SD decrease	1.623	(1.138–2.315)		
Baseline Femur neck BMD per SD decrease	1.605	(1.123–2.293)		
Baseline Lumbar TBS per SD decrease	1.165	(0.817–1.661)		
⊿ Lumbar BMD per 5% loss	1.040	(0.772–1.400)	1.627	(1.111–2.382)
⊿ Total hip BMD per 5% loss	0.948	(0.576–1.558)		
⊿ Femur neck BMD per 5% loss	0.636	(0.418–0.967)		
⊿ Lumbar TBS per 5% loss	1.327	(0.808–2.179)		
Age	1.140	(1.095–1.187)	1.082	(0.966–1.211)
Hypertension	1.332	(1.162–1.526)	0.991	(0.753–1.304)
Diabetes	1.329	(0.787–2.244)	1.356	(0.518–3.550)

	Univariate OR		Adjusted OR*	
Dyslipidemia	1.290	(0.390–4.263)	1.686	(0.202–14.088)
Postmenopausal duration	1.093	(1.065–1.123)	1.075	(0.995–1.158)

CIMT, carotid intima-media thickness; BMD, bone mineral density; SD, standard deviation; TBS, trabecular bone score.

*Adjusted model included lumbar TBS 5% change for 4 years (from 2007–2008 to 2011–2012) adjusted for 2007–2008 lumbar TBS per SD decrease, age, hypertension, diabetes, dyslipidemia and postmenopausal duration. Analysis was done for CIMT over 0.1 cm (n=542), CIMT below 0.1 cm (n=25).

VI. Longitudinal changes in BMD, according to the longitudinal changes in CIMT

Baseline characteristics of the participants, categorized into the CIMT non-progressor and CIMT accelerating progressor groups

Furthermore, we divided the CIMT progression status from the baseline to the 2-year follow-up into the accelerating progressor group and non-progressor group. The baseline characteristics of the study participants from the 2011–2012 Ansung data, by CIMT progression status, are shown in Table 4.

Those in the accelerating progressor group had a CIMT greater than the mean + SD of the CIMT changes, while those with less than the mean + SD were categorized into the non-progressor group. The accelerating progressor group showed changes of 0.308±0.054 mm in the CIMT, at 2 years, compared to the change of 0.045±0.133 mm observed in the non-progressor group. Those in the accelerating progressor group were younger, and had a higher diastolic blood pressure and lower fasting glucose level than those in the non-progressor group. For renal and liver function, lower blood urea nitrogen and serum creatinine levels, and higher ALT levels were observed in the accelerating progressor group. There was no connection between the changes in the CIMT and carotid plaque status. The presence of carotid plaque was fewer in the accelerating progressor group than non-progressor group, at the 2-year follow-up. The c-telopeptide levels, measured after 2 years, were higher in the accelerating progressor group. In terms of the changes in the bone parameters, the change in the femur neck BMD was significantly lower in the accelerating progressor group than in the non-progressor group, even after adjusting for age;

however, the changes in the lumbar spine TBS were not significantly lower compared to the non-progressor group. Therefore, in the establishment of whether participants should be categorized into the accelerating progressor group, changes in the values of the femur neck BMD were meaningful (All, $P < 0.001$). For adipokines, when the levels were measured about 8 to 10 years before CIMT measurement, a significant decrease in the TNF-α level was observed in the accelerating progressor group.

Table 4. Baseline characteristics of the participants, categorized into the CIMT non-progressor and CIMT accelerating progressor groups

	CIMT Non-progressor (n=2,218)	CIMT Accelerating progressor (n=290)	*P*-value
Age (years)	65.5±8.2	62.1±8.3	<0.001
No. Men	964 (43.5%)	126 (43.4%)	1.000
Body weight (kg)	60.7±10.2	62.0±10.7	0.051

	CIMT Non-progressor (n=2,218)	CIMT Accelerating progressor (n=290)	*P*-value
Body mass index (kg/m^2)	24.3±3.3	24.6±3.2	0.267
Systolic blood pressure (mmHg)	118.9±16.2	117.2±15.6	0.083
Diastolic blood pressure (mmHg)	72.6±9.4	73.9±9.9	0.035
HbA1c (%)	5.9±1.0	5.8±0.7	0.064
Fasting glucose (mg/dL)	101.5±28.3	99.0±18.8	0.047
Fasting insulin	8.3±4.5	8.7±5.0	0.127
Total cholesterol (mg/dL)	185.5±33.1	184.4±32.5	0.604
Triglyceride (mg/dL)	140.3±88.6	147.6±122.8	0.324
HDL cholesterol (mg/dL)	45.9±11.8	46.5±12.1	0.426
LDL cholesterol (mg/dL)	113.9±32.0	113.3±33.3	0.756
BUN (mg/dL)	16.8±5.1	16.2±4.4	0.029
Serum creatinine (mg/dL)	0.99±0.23	0.94±0.19	<0.001
AST (IU/L)	27.6±10.9	28.7±17.3	0.139
ALT (IU/L)	22.3±11.8	23.8±13.7	0.042
hsCRP (mg/dL)	2.0±8.7	1.9±4.0	0.873
Baseline Mean CIMT (mm)	0.743±0.182	0.591±0.111	<0.001
After 2 yr Mean CIMT (mm)	0.790±0.140	0.899±0.108	<0.001
Mean CIMT change (mm)	0.045±0.133	0.308±0.054	<0.001
Baseline Carotid plaque	183 (8.3%)	22 (7.6%)	0.820

	CIMT Non-progressor (n=2,218)	CIMT Accelerating progressor (n=290)	*P*-value
After 2 yr Carotid plaque	182 (11.1%)	17 (5.9%)	0.006
After 2 yr Oseteocalcin (ng/ml)	18.0±7.9	19.2±8.6	0.066
After 2 yr C-telopeptide (ng/ml)	0.36±0.19	0.40±0.24	0.019
Smoking (>1 Pack per day)	716 (32.3%)	94 (32.4%)	1.000
Alcohol intake (≥60 kcal per day)	808 (36.5%)	117 (40.5%)	0.104
Exercise (≥2 per week)	484 (21.9%)	59 (20.4%)	0.649
Baseline L BMD (g/cm^2)	1.036±0.198	1.028±0.180	0.631
Baseline FN BMD (g/cm^2)	0.822±0.145	0.841±0.137	0.107
Baseline TH BMD (g/cm^2)	0.909±0.154	0.923±0.145	0.246
After 2yr L BMD (g/cm^2)	1.034±0.200	1.030±0.195	0.747
After 2yr FN BMD (g/cm^2)	0.829±0.149	0.833±0.171	0.723
After 2yr TH BMD (g/cm^2)	0.895±0.157	0.902±0.161	0.516
L BMD change	0.004±0.054	−0.001±0.050	0.221
FN BMD change	0.012±0.047	−0.008±0.050	<0.001
TH BMD change	−0.012±0.039	−0.016±0.046	0.230
L TBS change	−0.258±0.212	−0.089±0.184	<0.001
Age adjusted			
L BMD change	0.004±0.054	−0.001±0.050	0.407
FN BMD change	0.012±0.047	−0.008±0.050	<0.001

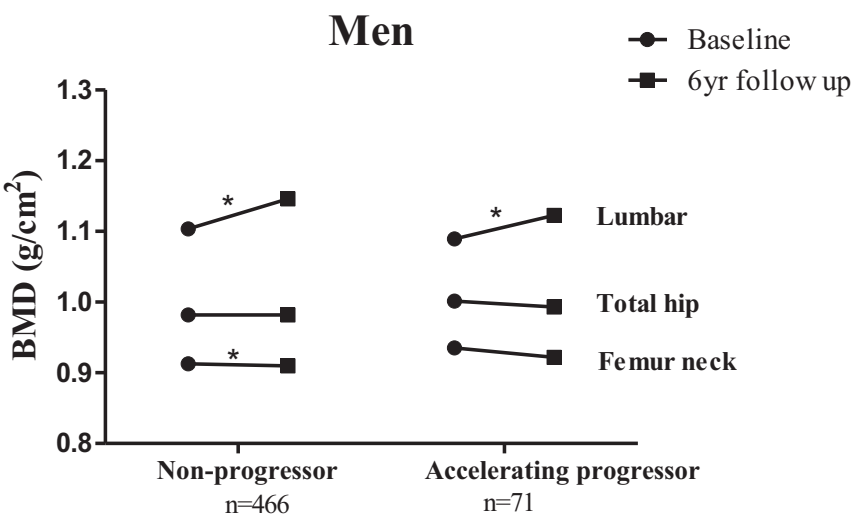
	CIMT Non-progressor	CIMT Accelerating progressor	*P*-value
TH BMD change	−0.012±0.039	−0.016±0.046	0.224
L TBS change	−0.258±0.212	−0.089±0.184	<0.001
PAI-1 (ng/ml)	39.46±11.65	38.87±12.79	0.736
Resistin (ng/ml)	19.70±16.32	17.16±10.29	0.278
IL-6 (pg/ml)	3.73±5.76	2.44±4.06	0.174
Leptin (ng/ml)	1.02±1.09	1.08±1.07	0.705
MCP-1 (pg/ml)	264.4±98.9	251.1±108.6	0.368
TNF-a (pg/ml)	7.30±5.01	5.62±2.98	<0.001
RBP4 (mg/ml)	137.0±73.1	126.0±80.7	0.314
Adiponectin (mg/ml)	3.05±2.75	3.40±3.36	0.401

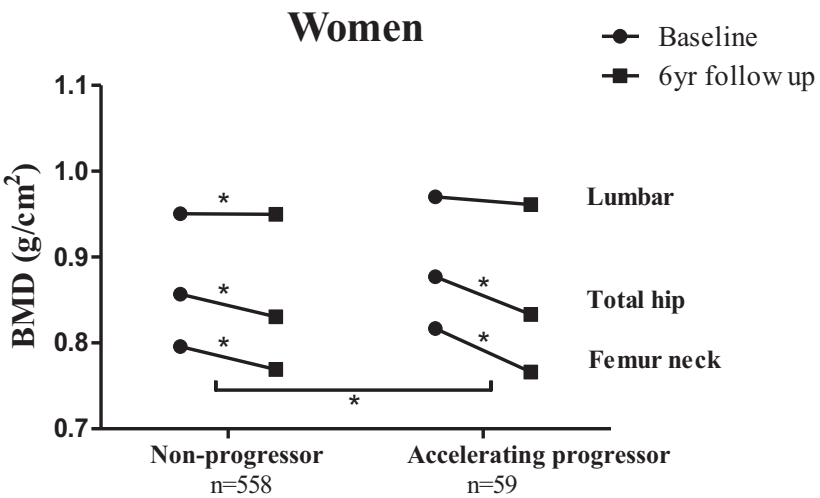
Data are means±SD by student t-test, N (%) by chi-square test. Baseline data are from 2011-2012 to 2013-2014 Ansung cohort data. Adipokine data are from 2000-2001 Ansung cohort data (total n=528). CIMT, carotid intima-media thickness; L, lumbar; FN, femur neck; TH, total hip; BMD, bone mineral density; TBS, trabecular bone score; BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; BUN, blood urea nitrogen; CRP, C-reactive protein. CIMT Non-progressor is categorized when CIMT change was below mean+SD value. CIMT accelerating progressor is defined when CIMT change was ≥ mean+SD value, which is 0.0915 cm.

BMD changes for 6-year follow-up, by CIMT accelerating progressor group vs. non-progressor group

The BMD changes over 6 years were analyzed by the changes in the CIMT accelerating progressor and non-progressor groups. For the women, there were significant differences in terms of the femur neck, which exhibited a steeper decline in the BMD in the accelerating progressor group than in the non-progressor group.

Figure 5. BMD changes for 6-year follow-up, by CIMT accelerating progressor group vs. non-progressor group.





Data are means (g/cm^2), Baseline data are from 2007−2009 Ansung cohort. BMD follow up data is from 2007−2008 to 2013−2014. CIMT changes are from 2011−2012 to 2013−2014. BMD, bone mineral density; *, $P < 0.05$.

V. Analysis of carotid plaque status

Baseline characteristics, by carotid plaque status

As carotid plaque is an important component of carotid atherosclerosis, we performed analyses, by carotid plaque status as well as CIMT. Based on the changes in the plaque status from the baseline to the 2−year follow−up, participants were categorized into the unchanged, new plaque and disappeared plaque groups. The baseline characteristics of the

study participants from the 2011–2012 Ansung data, by carotid plaque status, are as follows: the mean age was 64.3±8.1 years for those in the unchanged plaque group, 69.7±7.1 for those in the disappeared group, and 68.0±7.3 years for those in the new plaque group ($P < 0.001$). A higher proportion of men were in the new plaque group than in the unchanged or disappeared groups [unchanged plaque 690 (40.7%) vs. disappeared plaque 37 (38.5%) vs. new plaque 87 (64.9%); $P < 0.001$]. Those in the new plaque group had lower diastolic blood pressure, HbA1c, fasting glucose and HDL cholesterol levels, with the men displaying higher levels of total cholesterol and serum creatinine. Ironically, those in the new plaque group had a higher CIMT at the baseline than at the 2-year follow-up. The levels of the bone turnover markers (osteocalcin and c-telopeptide) and alkaline phosphatase in the new plaque group, as measured at the 2-year mark, were lower than those in the disappeared or unchanged plaque groups. The BMD was not significantly comparable at the baseline, and after 2 years, the new plaque group had higher BMD levels than the other groups. For comorbidities, the new plaque group had a higher proportion of

cerebrovascular disease patients, and a smaller proportion of patients who were taking hypertension and diabetes medications. The new plaque group had a higher proportion of smokers and alcohol consumers. As for the biomarkers measured 10 years before the CIMT and DXA measurements were taken, significant differences between the groups were only noted in terms of the adiponectin levels. The new plaque group had lower adiponectin levels than the disappeared plaque group.

Table 5. Baseline characteristics, by carotid plaque status.

	Unchanged plaque (n=1,705)	Disappeared plaque (n=98)	New plaque (n=140)	*P*-value
Age (years)	64.3±8.1	69.7±7.1	68.0±7.3	<0.001
No. of Men (%)	690 (40.7%)	37 (38.5%)	87 (64.9%)	<0.001
Body weight (kg)	61.2±10.3	59.6±10.0	61.6±10.0	0.271
Body mass index (kg/m^2)	24.5±3.3	24.4±3.3	24.0±3.2	0.182
Systolic blood pressure (mmHg)	118.2±16.0	125.2±16.7	119.9±16.5	<0.001

	Unchanged plaque	Disappeared plaque	New plaque	*P*-value
Diastolic blood pressure (mmHg)	72.8±9.4	74.1±11.0	70.8±8.3	0.021
HbA1c (%)	5.9±0.9	6.2±0.9	5.9±0.9	0.018
Fasting glucose (mg/dL)	100.9±25.3	108.0±30.5	102.0±27.9	0.030
Fasting Insulin (μIU/ml)	8.5±4.6	9.2±5.0	8.0±3.6	0.130
Total cholesterol (mg/dL)	186.4±32.5	176.9±31.3	183.8±32.3	0.016
Triglyceride (mg/dL)	142.0±97.7	138.0±93.9	152.8±105.6	0.424
HDL cholesterol (mg/dL)	46.2±11.5	44.8±11.1	43.5±10.8	0.024
LDL cholesterol (mg/dL)	114.8±32.5	107.2±29.2	114.0±32.3	0.078
hsCRP (mg/dL)	1.7±4.1	2.1±4.5	1.92.9	0.588
BUN (mg/dL)	16.6±4.5	16. 9±4.7	17.3±6.6	0.214
Serum creatinine (mg/dL)	0.97±0.19	0.99±0.25	1.06±0.33	<0.001
AST (IU/L)	27.6±11.6	27.5±16.2	28.0±8.3	0.937
ALT (IU/L)	22.6±12.1	21.2±12.3	23.1±11.7	0.445
Baseline Mean CIMT (mm)	0.071±0.017	0.080±0.017	0.081±0.020	<0.001

	Unchanged plaque	Disappeared plaque	New plaque	*P*-value
After 2yr Mean CIMT (mm)	0.080±0.014	0.089±0.014	0.082±0.015	<0.001
After 2yr Osteocalcin (ng/ml)	18.3±7.9	19.2±10.4	16.3±6.8	0.016
After 2yr C-telopeptide (ng/ml)	0.36±0.19	0.42±0.27	0.34±0.16	0.010
Serum Calcium (mg/dl)	9.3±0.4	9.4±0.4	9.3±0.4	0.228
Alkaline phosphatase (IU/L)	75.0±23.7	80.7±26.7	72.8±19.5	0.047
Baseline L BMD (g/cm^2)	1.039±0.195	0.993±0.237	1.063±0.196	0.131
Baseline FN BMD (g/cm^2)	0.830±0.143	0.782±0.134	0.823±0.142	0.077
Baseline TH BMD (g/cm^2)	0.915±0.152	0.865±0.151	0.921±0.162	0.078
After 2yr L BMD (g/cm^2)	1.032±0.198	0.991±0.226	1.070±0.205	0.016
After 2yr FN BMD (g/cm^2)	0.830±0.153	0.762±0.139	0.839±0.140	<0.001
After 2yr TH BMD (g/cm^2)	0.897±0.157	0.822±0.161	0.913±0.153	<0.001
Hypertension	95 (5.6%)	6 (6.3%)	9 (6.7%)	0.843
Diabetes	30 (1.8%)	2 (2.1%)	4 (3.0%)	0.598

	Unchanged plaque	Disappeared plaque	New plaque	*P*-value
Dyslipidemia	55 (3.2%)	2 (2.1%)	2 (1.5%)	0.448
Cerebrovascular disease	7 (0.4%)	1 (1.0%)	3 (2.2%)	0.021
Coronary artery disease	12 (0.7%)	1 (1.0%)	1 (0.7%)	0.932
Compression fracture history	150 (8.8%)	13 (13.5%)	12 (9.0%)	0.297
Hypertension medication	692 (40.8%)	53 (55.2%)	65 (48.5%)	0.006
DM medication	210 (12.4%)	19 (19.8%)	23 (17.2%)	0.039
Insulin therapy	14 (0.8%)	4 (4.2%)	2 (1.5%)	0.006
Dyslipidemia medication	128 (7.6%)	8 (8.3%)	10 (7.5%)	0.960
Steroid medication	7 (0.4%)	0 (0.0%)	0 (0.0%)	0.621
Anticoagulation medication	15 (0.9%)	1 (1.0%)	0 (0.0%)	0.540
Osteoporosis medication	153 (9.0%)	9 (9.4%)	5 (3.7%)	0.108
Smoker (>1Pack per day)	487 (28.7%)	36 (37.5%)	68 (50.7%)	<0.001
Alcohol intake (≥60 kcal per day)	623 (36.8%)	32 (35.4%)	57 (42.5%)	<0.001
Exercise (≥2 per week)	374 (22.1%)	16 (16.7%)	34 (25.4%)	0.289

	Unchanged plaque	Disappeared plaque	New plaque	*P*-value
PAI-1 (ng/ml)	40.27±11.81	35.16±11.64	37.80±10.13	0.106
Resistin (ng/ml)	19.29±17.52	18.91±9.71	18.53±9.36	0.970
IL-6 (pg/ml)	3.68±6.30	3.14±2.77	2.99±3.14	0.838
Leptin (ng/ml)	1.09±1.11	7.13±5.92	6.58±8.71	0.051
MCP-1 (pg/ml)	261.1±97.8	254.5±122.1	299.5±117.9	0.142
TNF-a (pg/ml)	7.03±4.68	6.39±3.00	9.21±6.36	0.051
RBP4 (mg/ml)	136.4±75.5	108.5±66.2	127.9±74.0	0.243
Adiponectin (mg/ml)	2.95±2.75	4.75±3.24	2.38±2.07	0.008

Mean (SD) by ANOVA, N(%) by chi-square test. Baseline data are from 2011-2012 Ansung-Ansan data. Adipokine data are from 2000-2001 Ansung-Ansan cohort data (total n=528). BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; WBC, white blood cell count; BUN, blood urea nitrogen; CRP, C-reactive protein; CIMT, carotid intima-media thickness; BMD, bone mineral density; L, lumbar; FN, femur neck; TH, total hip.

Subgroup analysis between two groups; disappeared plaque vs. new plaque

When comparing the bone density changes by the plaque status, at the 6-year follow-up, between the disappeared plaque group and new plaque group, there were no statistically significant results.

Table 6. Subgroup analysis between two groups; disappeared plaque vs. new plaque.

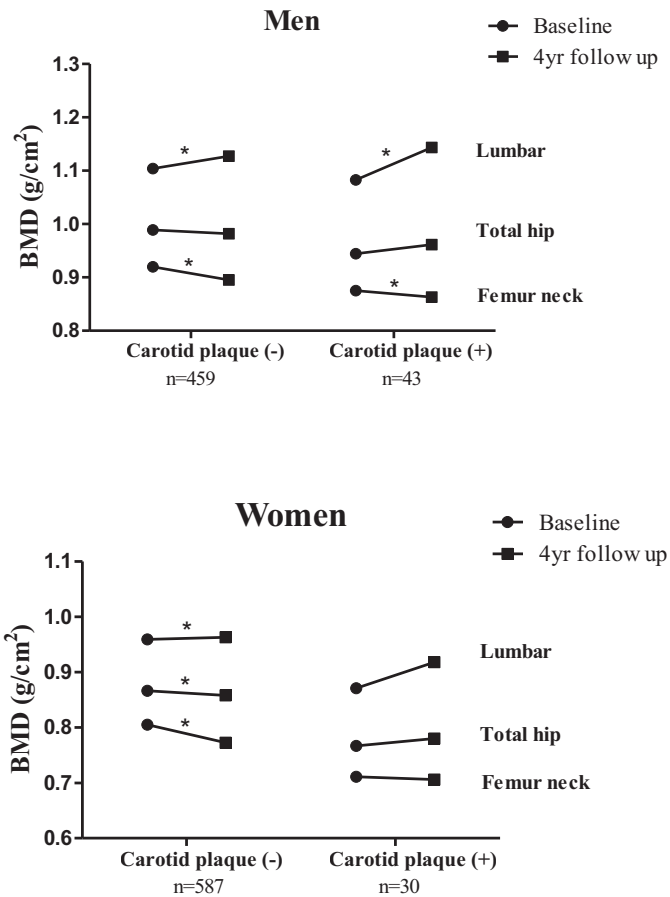
6 year follow up†	Disappeared (n=96)	New plaque (n=134)	*P*-value	*P*-value (1)	*P*-value (2)
L BMD change (g/cm^2)	0.042±0.088	0.020±0.089	0.140	0.127	0.027
FN BMD change (g/cm^2)	−0.021±0.042	−0.018±0.050	0.650	0.681	0.936
TH BMD change (g/cm^2)	−0.024±0.039	−0.018±0.042	0.446	0.650	0.768

Data are means±SD by student *t*-test. Plaque changes data are between 2011-2012 to 2013-2014 Ansung data. †, 6 year follow up data is from 2007-2008 to 2013-2014 data. BMD, bone mineral density; L, lumbar; FN, femur neck; TH, total hip. P-value (1) : Age adjusted, P-value (2) : P-value (1) + gender, systolic blood pressure, HbA1c, low-density lipoprotein cholesterol, serum creatinine, smoking, drinking

BMD changes for 4 years, by carotid plaque status

We analyzed the changes in BMD, by carotid plaque status at 4 years, by sex. The presence of carotid plaque did not significantly change the rate of BMD decline across sexes.

Figure 6. BMD changes for 4 years, by carotid plaque status



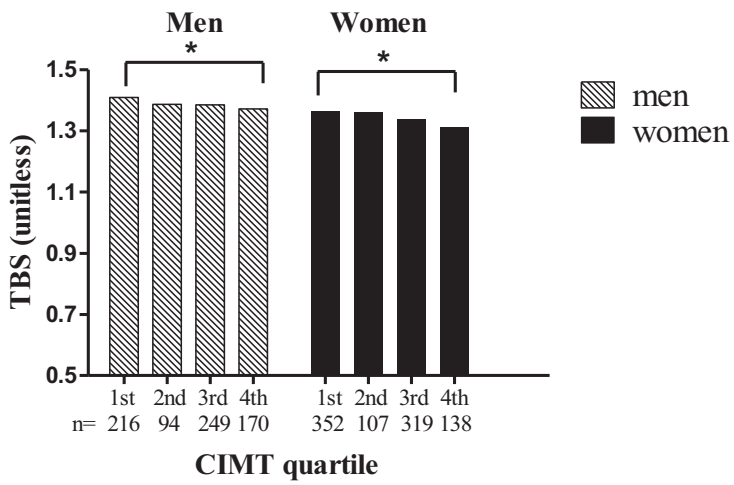
Data are means (g/cm^2), Baseline data are from 2007–2008 Ansung cohort. BMD follow up data is from 2007–2008 to 2011–2012. CIMT data is from 2011–2012 to 2013–2014. CIMT, carotid intima–media thickness; BMD, bone mineral density; *, $P < 0.05$.

VI. TBS analysis

Changes in the TBS, by sex, according to the CIMT quartiles

Next, the changes in the TBS were further analyzed. As shown in Figure 7, the TBS decreased significantly with increases in the CIMT quartile, in both sexes.

Figure 7. Changes in the TBS, by sex, according to the CIMT quartiles

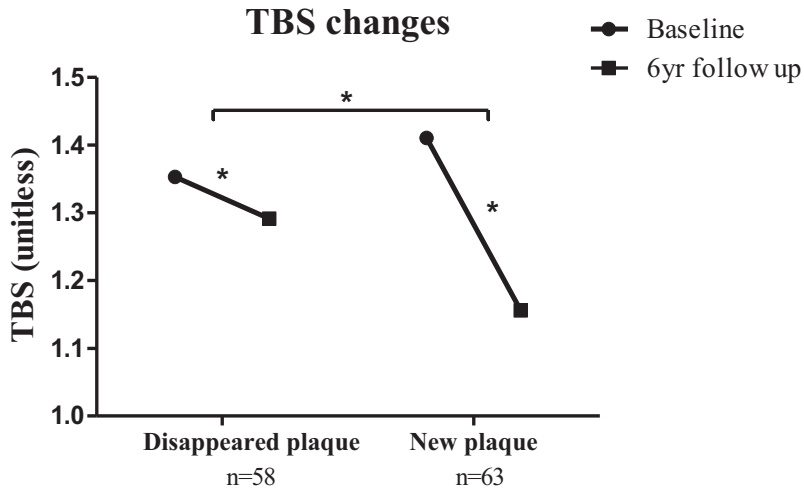


Data are means (unitless), Data are from 2011–2012 Ansung cohort. CIMT, carotid intima–media thickness; TBS, trabecular bone score; *, $P < 0.05$.

Changes in the TBS for 6 years, by plaque changes

Finally, we compared the degree of the decrease in the TBS, over 6 years, when participants were divided according to their plaque change status. As shown in **Figure 8**, the new plaque group had exhibited lower TBS values than the disappeared plaque group, up to the 6-year follow-up. The significance persisted even after adjustment for age, sex, systolic blood pressure, HbA1c level, LDL-cholesterol level, serum creatinine level, smoking status, and drinking status (−0.228±0.207(SD) vs. −0.056± 0.133; $P < 0.001$).

Figure 8. Changes in the TBS for 6 years, by plaque changes



Data are means (unitless), Baseline data are from 2011–2012 Ansung cohort. TBS follow up data is from 2007–2008 to 2013–2014. Carotid plaque changes are from 2011–2012 to 2013–2014. TBS, trabecular bone score; *, $P < 0.05$.

Discussion

In this study, we observed the presence of a negative relationship between the atherosclerosis surrogate markers such as CIMT and carotid plaque, and the bone-related parameters (BMD and TBS). For the postmenopausal women, the risk of the CIMT being greater than 0.1 cm increased as the lumbar BMD decreased by 5%, in a longitudinal cohort analysis, with an OR of 1.627 (95% CI, 1.111-2.382) after adjusting for age, hypertension, diabetes, dyslipidemia and postmenopausal duration. When we categorized participants based on their CIMT into the accelerating progressor and non-progressor groups, by mean + 1 SD (0.0915 cm/2 years), those in the accelerating progressor group had a lower femur neck BMD than those in the non-progressor group, after adjusting for age. Finally, those in the new carotid plaque group had a lower TBS than the disappeared plaque group, after adjusting for age.

It has been suggested by clinicians that a relationship exists between osteoporosis and atherosclerosis. From the 25-year follow-up of the Framingham Heart Study, it was found that the

abdominal aortic calcification index was significantly associated with changes in the the metacarpal relative cortical area, by 22.4%, in 364 older women, after controlling for potential confounders [16]. Recently conducted cell culture studies reported that bone mineralization and arterial plaque calcification share a common pathogenesis [17].

Several research studies focusing on the association between osteoporosis and atherosclerosis are underway. The San Antonio Family Osteoporosis Study, with Mexican Americans, demonstrated that decreased BMD had an inverse relationship with CIMT, in 535 women and 335 men older than 60 years of age [18]. The Japanese Population-based Osteoporosis Cohort study, with 609 women older than 50 years of age, demonstrated that the spine T-score had an inverse relationship with the CIMT of the carotid bifurcation, in a 10-year follow-up [19]. Another study showed comparable results in 54 postmenopausal women, suggesting that there was a negative correlation between pulse wave velocity and hip BMD, but not carotid plaque.

Carotid plaque was only significantly related to the TBS, and

not BMD. The TBS is a surrogate marker of trabecular microarchitecture, and recently conducted studies suggested that the TBS was associated with incident and clinical vertebral fractures [20]. Therefore, it is meaningful to confirm that the bone quality was lower in the new carotid plaque group, within 2 years, than in the disappeared plaque group.

Some studies have suggested that there is an independent association between BMD and the cortical or trabecular bone sites with vascular calcification. In our study, we confirmed that the accelerating progressor group had a significantly lower femur neck BMD than the non-progressor group. As a cross-sectional value of CIMT, the lumbar BMD significantly decreased when the CIMT was greater than 0.1 cm (rather than when it was lower than 0.1 cm). Therefore, this study suggests that the CIMT change velocity was associated with cortical bone health, and trabecular bone was associated with pathologic CIMT.

In terms of the biomarkers, the adiponectin values were lower in the new plaque group than in the disappeared plaque group (2.38±2.07 vs. 4.75±3.24 mg/ml, P = 0.008). However, the

adiponectin levels were higher in the group with a CIMT of 0.1 cm or higher, than in the group with CIMT values lower than 0.1 cm (6.37±4.55 vs. 3.84±3.01, P=0.046). In addition, in terms of CIMT changes, the accelerating progressor group showed lower TNF-α values than the non-progressor group (5.62± 2.98 vs. 7.30±5.01 pg/ml, P <0.001). Therefore, this study showed that lower adiponectin levels were associated with newly generating plaque, and high adiponectin values were associated with greater CIMT; these findings are inconsistent with those of previously conducted studies.

Previously conducted studies have suggested that adiponectin has a potential association with bone and vascular mineralization. In a cross-sectional study, elevated adiponectin levels increased the risk of fracture and decreased the femur neck BMD [21], while in another study in the Mexican-Mestizo population, it was shown that low adiponectin levels were associated with a higher CIMT (OR 1.505) [22]. However, the orientation of the association between CIMT and adiponectin in this study was contradictory to that observed in a previously conducted study in Mexico. This could be attributed to the different time points at

which measurements were performed in this study. The adiponectin measurement, in this study, was performed at 8 to 10 years before the CIMT measurement, which can weaken causality. Therefore, further studies need to be conducted to confirm the correlations.

Since changes in CIMT are known to be correlated with higher occurrence rates of cardiovascular events [23], one of the strengths of this study is that we analyzed the changes in the CIMT measurements within 2 years, in addition to the CIMT parameters in association with the changes in the bone parameters. Although the follow-up duration was relatively small, we were able to demonstrate the longitudinal changes in the CIMT and bone parameters. Contradictory to the findings of other studies, in our study, significance was observed in both sexes, in postmenopausal women and men aged over 50 years.

One limitation of our study is the short average follow-up duration of 2 years. Since the duration between the measurements of the changes in CIMT was relatively short in the present study, studies with longer follow-up periods may

strengthen our results. Additionally, we were unable to confirm if those in the accelerating progressor group or new plaque group had higher incidences of cardiovascular events, cerebrovascular accidents, or decreased survival, at the end. In terms of timing, since the biomarkers were measured from the baseline of the cohort samples, 8 to 10 years before the CIMT and DXA measurements were taken, it was difficult to establish a direct association between the adiponectin, TNF-α and the bone, atherosclerosis. However, this is the first study to evaluate the relationship between the changes in CIMT and bone parameters in a prospective Korean community-based cohort.

In conclusion, from our research, we identified that:

1. As the CIMT quartiles increased, the femur neck and total hip BMD in men, and the lumbar, femur neck and total hip BMD in women, at the baseline, tended to decrease.

2. The group with a CIMT of 0.1 cm or higher (pathologic CIMT) showed a tendency to have a lower BMD than the group with a CIMT lower than 0.1 cm, in postmenopausal women. At the 4-year follow-up of BMD, the highest

quintile of CIMT showed a lower femur neck BMD, in postmenopausal women. Moreover, the risk of the CIMT being greater than 0.1 cm increased as the lumbar BMD decreased by 5%, in a longitudinal cohort analysis, with an OR of 1.627.

3. When the CIMT changes were divided by over 0.0915 cm (mean + SD) for the accelerating progressor and less than 0.0915 cm as non-progressor group, the accelerating progressor group, in women, had a significantly lower femur neck BMD, at 6 years, than those in the non-progressor group.

4. For the correlation between the changes in the carotid plaque thickness and the TBS, the new plaque group had a significantly lower TBS at the 6-year follow-up compared to the disappeared plaque group.

Through this prospective Korean community-based cohort study, we suggest that, in postmenopausal women with a CIMT higher than 0.1 cm or those in the accelerating progressor (0.0915 cm/2 years) or newly generated plaque groups,

proactive screening for bone health may prevent the deterioration of bone health.

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국 문 초 록

많은 임상의들이 골다공증 환자에서 죽상동맥경화증이 자주 동반됨을 보고한 바가 있다. 죽상동맥경화증과 골다공증 간의 공통 기전에 대해서 여러 가지 가설이 제시되고 있지만 아직 장기간의 코호트에서 경동맥내막두께의 변화와 골밀도의 변화 사이의 관계를 평가한 연구는 없다. 본 연구에서는 경동맥 초음파를 이용하여 측정한 경동맥내막두께의 변화와 이중에너지 방사선흡수법을 이용하여 측정한 골밀도 변화의 관련성을 평가하였다.

한국의 안성 코호트 연구의 참여자들로 2011-2012년 및 2013-2014년에 경동맥내막두께와 골밀도를 모두 측정한 3,403명을 대상으로 데이터를 분석하였다. 경동맥 죽상경화증은 경동맥내막두께의 사분위수에 따라서 분류하였고 또한 경동맥내막두께가 0.1 cm 이상인 군과 0.1 cm 미만인 군으로 분류하였다. 경동맥내막두께의 변화는, 평균 +1 표준편차 (0.0915cm/2년)를 기준으로 그 이상이면 가속 진행형군, 그 미만이면 비진행형군으로 분류하였다. 플라크의 변화에 따라서는 새로운 플라크 생성군, 플라크가 사라진 군으로 분류하였다. 다양한 경동맥 죽상경화증 기준에 따라 adipokine 수치와의 관련성을 추가로 비교하였다.

경동맥내막두께 사분위수가 증가함에 따라서 남성의 경우 총 대퇴골 골밀도와 대퇴 경부 골밀도, 여성의 경우 요추, 총 대퇴골 및 대퇴 경부 골밀도가 감소했다. 장기간 코호트 분석에서 나이, 고혈압, 당뇨병, 고지혈증 여부, 폐경 후 기간을 보정한 이후에도 요추 골밀도가 5% 감소 할 때마다 경동맥내막두께가 0.1cm 이상으로 증가하는 위험이 1.627 (95% 신뢰 구간, 1.111−2.382)배 증가하였다. 비진행형군과 비교하여 가속 진행형군에서 대퇴 경부 골밀도에서 유의한 감소가 관찰되었다 (P <0.001); 이 유의성은 연령을 보정한 후에도 유지되었다 (0.012±0.047mm 대 −0.008±0.050mm, P <0.001). 새로운 플라크 생성군과 플라크가 사라진 군을 비교하였을 때, 연령, 성별, 수축기혈압, 당화혈색소, 저밀도 지단백 콜레스테롤, 혈청 크레아틴, 흡연 상태 그리고 음주 상태를 보정한 후에도 6년간 추적 관찰시 새로운 플라크 생성군에서 플라크가 사라진 군보다 Trabecular bone score가 유의하게 낮았다. Biomarker의 경우, adiponectin 값은 플라크가 사라진 군보다 새로운 플라크 생성군에서 낮았다. 이와 대조적으로 adiponectin 값은 경동맥내막두께가 0.1 cm 이상인 군에서 경동맥내막두께가 0.1 cm 미만인 군보다 높았다.

폐경기 여성의 경우 4년 추적시 요추 골밀도가 5% 감소함에 따라 경동맥내막두께가 0.1 cm 이상으로 증가하는 위험이 증가

했다. 가속 진행형군에서 나이를 보정한 후 비진행형군보다 더 낮은 대퇴 경부 골밀도와 관련이 있었다. 새로운 플라크 생성군은 플라크가 사라진 군보다 더 낮은 Trabecular bone score를 보였다. 하지만 adipokine 측면에서 우리는 일관된 결과를 얻지 못하였다. 결론적으로, 이 대규모 지역 사회 기반 코호트 연구에서 우리는 0.1 cm (병적인 경동맥내막두께) 이상의 경동맥내막두께를 가진 환자 또는 가속 진행형군 또는 새로운 플라크 생성군 환자에서 골밀도 검사가 도움이 될 수 있음을 확인하였다.

주요어 : 경동맥내막두께, 죽상동맥경화증, 플라크, 골다공증, 골밀도, trabecular bone score, adiponectin

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