Original Article

Association between the Circulating Total Osteocalcin Level and the Development of Cardiovascular Disease in Middle-aged Men: A Mean 8.7-year Longitudinal Follow-up Study

You-Cheol Hwang[1], Mira Kang[2], In-Jin Cho[1], In-Kyung Jeong[1], Kyu Jeung Ahn[1], Ho Yeon Chung[1] and Moon-Kyu Lee[3]

You-Cheol Hwang and Mira Kang contributed equally to this work.

[1]Division of Endocrinology and Metabolism, Department of Medicine, Kyung Hee University Hospital at Gangdong, Kyung Hee University School of Medicine, Seoul, Korea
[2]Center for Health Promotion, Samsung Medical Center, Seoul, Korea
[3]Division of Endocrinology and Metabolism, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

***Aim*: Recent studies have suggested that the serum osteocalcin level is associated with various cardiovascular risk factors. The aim of this study was to determine whether the serum total osteocalcin level is associated with the development of cardiovascular disease (CVD).**
***Methods*: A total of 1,290 men 40-78 years of age were enrolled. The subjects were followed regularly at the Health Promotion Center on an outpatient basis and during hospitalization for a mean of 8.7 years, and the incidence of CVD (coronary heart disease [CHD] and stroke) was determined.**
***Results*: At baseline, the body mass index, body fat percentage, fasting glucose, homeostasis model assessment-insulin resistance, triglyceride and non-high density lipoprotein (HDL) cholesterol levels were inversely and the HDL cholesterol levels were positively associated with the serum osteocalcin levels. In addition, the prevalence of diabetes or metabolic syndrome decreased as the osteocalcin tertile increased. However, no differences were observed in the prevalence of hypertension across the osteocalcin tertiles. Incident CVD occurred in 74 (5.7%) of the study subjects (29 patients with CHD and 47 patients with stroke). According to the Cox proportional hazards models, however, there were no statistical differences in the development of stroke, CHD or CVD across the osteocalcin tertiles after adjusting for other risk factors for CVD, including age, body mass index, current smoking, low-density lipoprotein cholesterol, diabetes, hypertension and the serum creatinine level.**
***Conclusion*: In conclusion, the serum total osteocalcin level was not associated with the development of CVD after adjusting for other risk factors for CVD in this cohort.**

J Atheroscler Thromb, 2015; 22:136-143.

Key words: Osteocalcin, Cardiovascular disease, Coronary heart disease, Stroke

Introduction

Mounting evidence supports the existence of a close relationship between CVD and bone metabolism. In women, the loss of hip bone mineral density (BMD) has been shown to be associated with an increase in total mortality, particularly that due to coronary heart disease (CHD), after adjusting for other confounders during an average follow-up of 3.2 years[1)]. In addition, an increase in the total hip BMD has been demonstrated to be independently associated with a decreased age-adjusted relative risk for all-cause and CVD mortality in older men[2)]. In agreement with these findings, recent studies have reported an inverse

Address for correspondence: Moon-Kyu Lee, Division of Endocrinology and Metabolism, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Ilwon-dong, Kangnam-ku, Seoul 135-710, Korea
E-mail: leemk@skku.edu
Received: April 18, 2014
Accepted for publication: July 8, 2014

association between calcification in the abdominal aorta and the bone mass at the lumbar and femoral levels[3, 4]. Furthermore, a significant negative correlation has been reported between the pulse wave velocity (PWV), a surrogate marker of CVD, and the hip BMD, which persists after adjusting for age, mean arterial pressure and serum lipids, in post-menopausal women[5].

As a plausible explanation of the relationship between CVD and bone metabolism, these two conditions share common risk factors, including age, estrogen depletion, a sedentary lifestyle, smoking, alcohol consumption and dietary factors, such as the level of calcium intake and deficiencies in vitamins C and K[6-9]. However, it is difficult to explain all aspects of the relationship based only on these common risk factors, and there may be a missing link between CVD and bone metabolism.

Recently, the osteoblast-specific protein, osteocalcin, was suggested to be a central factor involved in the cross-talk between bone and energy metabolism[10, 11], and accumulating human evidence has demonstrated that the serum osteocalcin level is inversely associated with impaired glucose tolerance[12-14]. In addition, the serum osteocalcin level has been shown to be inversely correlated with the body mass index (BMI), homeostatic model assessment-insulin resistance (HOMA-IR), triglycerides, leptin and number of metabolic syndrome traits, positively correlated with adiponectin[15] and significantly and negatively correlated with the brachial artery PWV and intima-media thickness in men following additional adjustments for other cardiovascular risk factors[16]. Moreover, the serum total osteocalcin level was found to predict all-cause and CVD-related mortality in a prospective cohort study of community-dwelling older men 70-89 years of age[17].

However, little is still known about whether the aforementioned findings are merely correlations or if osteocalcin directly affects the development of CVD. Therefore, we performed this study in order to determine whether the serum osteocalcin level is independently associated with the development of CVD (CHD and stroke) over an 8.7-year mean follow-up period.

Methods

Study Population

A detailed description of the study design and methods has been published previously[18]. Briefly, the study subjects were recruited from among patients who visited the Health Promotion Center of the Samsung Medical Center between January 1997 and December 1997. A total of 2,435 subjects (1,761 men and 674 women) 20-78 years of age were enrolled and followed up regularly at the Health Promotion Center on an outpatient basis and during hospitalization for a mean of 8.7 years. Of the 2,435 subjects, we limited the study participants to 1,290 men, 40-78 years of age, for whom the serum total osteocalcin levels had been determined. These subjects were subsequently followed up for a mean of 8.7 years. This study was approved by the Institutional Review Board and Ethics Committee of Samsung Medical Center and complied with the Declaration of Helsinki.

Definition of CVD and Other Measurements

In this study, CVD was defined as the occurrence of CHD and/or stroke. CHD included angina pectoris, myocardial infarction and congestive heart failure, as determined by the presence of typical symptoms, elevation of cardiac enzymes and the findings of electrocardiograms, echocardiography, stress tests and/or coronary angiography. Stroke included cerebral infarction, hemorrhagic stroke, transient ischemic attack, vertebrobasilar insufficiency and complete occlusion of the internal carotid arteries, as determined on carotid vascular Doppler, computed tomography or magnetic resonance imaging and/or the presence of accompanying neurologic signs or symptoms. Patients with incidental lacunar infarction on brain imaging were not considered to have incident stroke. Metabolic syndrome was defined using the modified National Cholesterol Education Program Adult Treatment Panel Ⅲ (NCEP-ATP Ⅲ) criteria[19]. Diabetes was defined according to the presence of one of the following items: a fasting glucose level of ≥126 mg/dL (≥7.0 mmol/L); the use of diabetes medication; a self-reported physician diagnosis; and a glycated hemoglobin (HbA1c) level of ≥6.5%[20]. Hypertension was defined as a systolic or diastolic blood pressure of ≥140/90 mmHg or the previous self-reported use of anti-hypertensive medications, and alcohol intake was defined as the consumption of more than three drinks per day (>30 g ethanol/day). The percentage of body fat was measured using a bioelectrical impedance analysis (InBody 3.0; Biospace, Seoul, Korea).

At baseline, the subjects were assessed for CHD, stroke, hypertension and diabetes, as determined according to a standardized questionnaire and their medical records and followed regularly for the new onset of CHD or stroke. In order to ascertain the incidence of CVD, all data were collected from the Health Promotion Center on an outpatient basis and during hospitalization.

Table 1. Baseline characteristics according to the serum total osteocalcin tertile

	Total (n=1290)	Low tertile (n=433)	Middle tertile (n=439)	Upper tertile (n=418)	p
Osteocalcin (nmol/L, range)	1.30±0.47 (0.27-3.69)	0.81±0.21 (0.27-1.08)	1.28±0.11 (1.09-1.45)	1.83±0.32 (1.47-3.69)	<0.001
Age (years)	49.6±6.2	49.7±6.2	49.8±6.4	49.2±5.9	NS
Body mass index (kg/m^2)	23.9±2.3	24.2±2.1	23.8±2.2	23.6±2.4	<0.001
Body fat (%)	22.4±4.5	22.9±4.1	22.1±4.5	22.1±4.7	0.011
Systolic blood pressure (mmHg)	121.6±14.3	122.0±13.9	121.3±14.4	121.5±14.6	NS
Diastolic blood pressure (mmHg)	83.5±10.2	83.9±10.0	83.1±10.1	83.5±10.5	NS
Fasting plasma glucose (mmol/L)	5.79±1.17	5.95±1.57	5.82±1.02	5.58±0.72	<0.001
Glycated hemoglobin (%)	5.4±0.7	5.5±0.9	5.4±0.6	5.3±0.5	0.001
Glycated hemoglobin (mmol/mol)	36±7	37±9	36±7	35±5	0.001
Log HOMA-IR	1.75 (1.25-2.40)	1.88 (1.34-2.59)	1.80 (1.23-2.45)	1.63 (1.20-2.23)	0.001
Log HOMA-B%	66.0 (46.8-90.5)	68.0 (47.0-93.2)	65.8 (45.0-87.7)	65.2 (49.5-92.4)	NS
Total cholesterol (mmol/L)	5.17±0.82	5.24±0.82	5.14±0.81	5.13±0.82	0.089
Log triglycerides (mmol/L)	1.49 (1.07-2.06)	1.59 (1.14-2.22)	1.42 (1.07-2.01)	1.44 (1.02-1.95)	0.002
HDL cholesterol (mmol/L)	1.27±0.30	1.24±0.29	1.27±0.29	1.30±0.31	0.032
LDL cholesterol (mmol/L)	3.13±0.74	3.17±0.76	3.11±0.73	3.10±0.74	NS
Non-HDL cholesterol (mmol/L)	3.90±0.83	4.00±0.82	3.87±0.83	3.83±0.83	0.008
Serum creatinine (μmol/L)	100.4±10.1	100.3±10.1	99.9±10.4	100.9±9.9	NS
Log γGT (U/L)	30.0 (22.0-45.0)	34.0 (24.0-52.0)	29.0 (22.0-47.0)	27.5 (21.0-39.0)	<0.001
Uric acid (μmol/L)	344.2±64.4	345.4±64.6	347.3±64.9	339.8±63.6	NS
Fibrinogen (μmol/L)	9.16±1.75	9.28±1.78	9.05±1.69	9.15±1.77	NS
Log lipoprotein (a) (μmol/L)	0.46 (0.25-0.89)	0.41 (0.22-0.81)	0.46 (0.26-0.90)	0.49 (0.28-0.93)	0.055
Log plasminogen activator inhibitor-1 (μg/L)	26.0 (16.0-39.0)	29.0 (18.0-43.5)	26.0 (17.0-38.0)	23.0 (15.0-37.0)	0.001
Alcohol intake (%)	399 (30.9)	146 (33.7)	141 (32.1)	112 (26.8)	0.029
Current smoking (%)	583 (45.2)	191 (44.1)	199 (45.3)	193 (46.2)	NS
Hypertension (%)	373 (28.9)	131 (30.3)	116 (26.4)	126 (30.1)	NS
Diabetes (%)	118 (9.1)	57 (13.2)	41 (9.3)	20 (4.8)	<0.001
Metabolic syndrome (%)	427 (33.1)	171 (39.5)	153 (34.9)	103 (24.6)	<0.001

The data are expressed as the mean±SD, median (interquartile range) or frequency.
NS, not significant

The serum total osteocalcin level was measured at the time of sampling in 1997 and analyzed according to the Metra TM Osteocalcin system (Quidel Corp., Santa Clara, USA) using a Dade Behring ELISA-Processor Ⅲ (Behring Diagnostics, Inc., Somerville, USA) with intra- and inter-assay CVs of 4.8-10.0% and 4.8-9.8%, respectively.

Statistical Analysis

All data are presented as the mean±SD or proportion, except for skewed variables, which are presented as the median (interquartile range, 25%-75%). Because the distributions of the serum levels of triglycerides, gamma-glutamyl transpeptidase (γGT), lipoprotein (a), plasminogen activator inhibitor (PAI)-1 and HOMA were skewed, natural logarithmic transformation was applied in the statistical analyses. In the interest of simplicity, non-transformed median values are presented in the tables and text. A one-way ANOVA was used to compare the means between the tertiles of the osteocalcin level. The linear-by-linear association χ^2-test was used to conduct a trend analysis between the tertiles. Pearson correlation coefficients were calculated to evaluate the associations between the osteocalcin levels and the clinical and laboratory measurements. A Kaplan-Meier survival curve was used to compare the development of CVD according to the osteocalcin tertile. A Cox proportional hazards regression with backward selection model was used to calculate the adjusted hazard ratio (HR) for incident cases of cardiovascular disease among the subjects based on the osteocalcin tertile. The lowest osteocalcin tertile was used as a reference, and the results for the analyses are presented as the HR with the 95% confi-

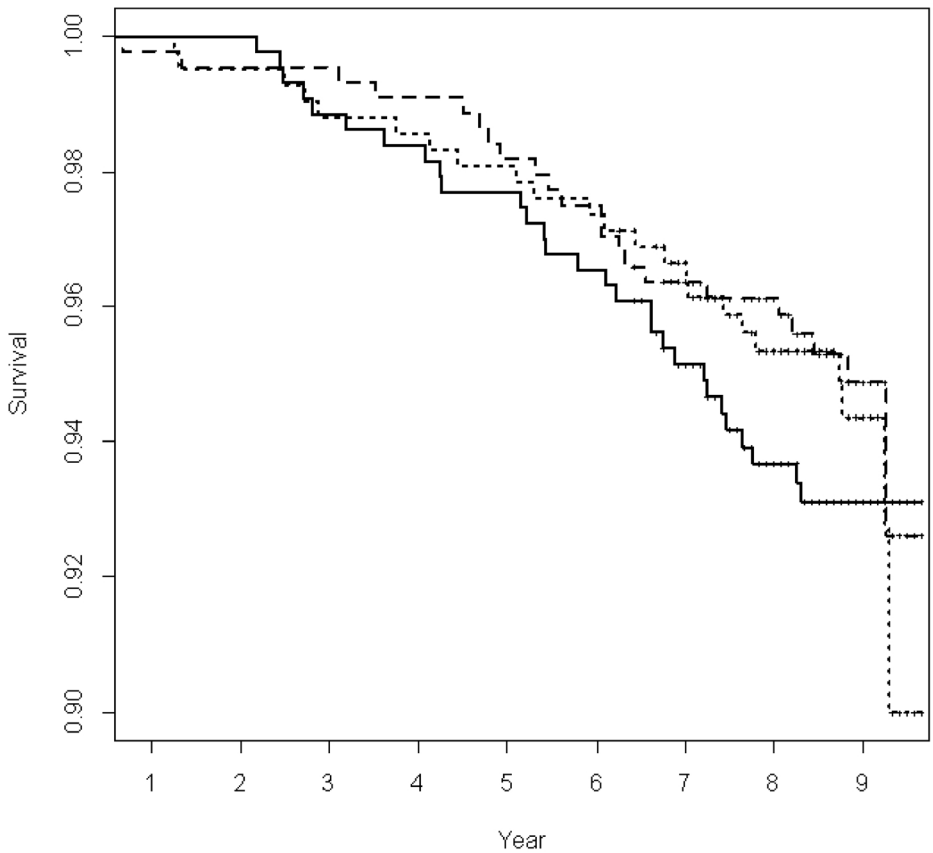

Fig. 1. Kaplan-Meier survival curve for the development of cardiovascular disease according to the serum total osteocalcin tertile (dotted line, upper tertile; dashed line, middle tertile; and solid line, lower tertile) ($p>0.05$ across the tertiles)

dence interval (CI). All analyses were performed using the PASW version 18.0 software program (SPSS, Chicago, IL, USA). *P* values of <0.05 were considered to be statistically significant.

Results

The baseline characteristics of the study subjects according to the serum total osteocalcin tertile are shown in **Table 1**. The serum total osteocalcin level was not correlated with aging in our study population (data not shown). However, BMI, body fat percentage and the fasting glucose, glycated hemoglobin, HOMA-IR, triglyceride, non-HDL cholesterol, γGT and PAI-1 levels decreased as the osteocalcin tertile increased ($p<0.05$), and the prevalence of diabetes and NCEP-defined metabolic syndrome showed a decreasing tendency across the osteocalcin tertiles ($p<0.05$). However, there were no differences in blood pressure, the rate of hypertension or the proportion of current smokers across the osteocalcin tertiles.

Incident CVD occurred in 74 (5.7%) of the study subjects (29 cases of CHD and 47 cases of stroke) during the mean 8.7-year follow-up period. Compared with the lowest osteocalcin tertile (reference group), no differences were observed in the middle or upper osteocalcin tertiles for the development of CVD according to the Kaplan-Meier survival curve (**Fig. 1**). Separately, the development of CHD and stroke was not associated with the baseline serum total osteocalcin level (both $p>0.05$). In addition, the serum total osteocalcin level was not associated with the development of CHD, stroke or CVD in women only or both men and women (data not shown).

The results of the Cox proportional hazard model analysis are shown in **Table 2**. The independent variables included in the models comprised age, BMI, current smoking, low-density lipoprotein (LDL) cholesterol, diabetes, hypertension, the serum creatinine and serum osteocalcin levels according to the tertile. Consequently, age (HR, 1.05; 95% CI, 1.02-1.09), current smoking (HR, 2.18; 95% CI, 1.36-

Table 2. Serum total osteocalcin and the development of cardiovascular disease

	Stroke	CHD	CVD
Osteocalcin lower tertile (reference)	1	1	1
middle tertile	0.71 (0.36-1.41)	0.79 (0.32-1.96)	0.78 (0.44-1.35)
upper tertile	0.69 (0.34-1.39)	1.05 (0.44-2.50)	0.83 (0.48-1.44)
Age	1.06 (1.01-1.10)	NS	1.05 (1.02-1.09)
Current smoking	1.99 (1.11-3.58)	NS	2.18 (1.36-3.50)
Hypertension	2.33 (1.29-4.24)	2.54 (1.20-5.38)	2.60 (1.62-4.17)

NS, not significant; CHD, coronary heart disease; CVD, cardiovascular disease
The data are adjusted for age, BMI, current smoking, LDL-cholesterol, diabetes, hypertension and serum creatinine.

3.50) and hypertension (HR, 2.60; 95% CI, 1.62-4.17) were found to independently predict the future development of CVD. However, the serum total osteocalcin level was not associated with incident CVD across the tertiles. The results for stroke and CHD were similar to those for CVD.

Discussion

Accumulating evidence has indicated an association between the serum osteocalcin level and favorable metabolic phenotypes and atherosclerosis parameters[12-16]. First, it has been reported that an elevated osteocalcin level is associated with better glycemic control, improved insulin sensitivity and/or insulin secretory capacity[12-14]. In addition, some small longitudinal studies have shown the serum total osteocalcin concentration to be associated with changes in the fasting plasma glucose level and risk of incident diabetes[21]. Second, the osteocalcin level has been reported to be associated with a decreased fat mass and low prevalence of metabolic syndrome and its traits[7, 15]. The serum osteocalcin level is also reported inversely associated with waist circumference, fasting glucose, triglycerides, insulin resistance defined according to the HOMA-IR and leptin and positively associated with adiponectin[15]. In addition, in the same study, the subjects in the highest osteocalcin quartile exhibited a lower risk of metabolic syndrome after adjusting for age, gender, smoking, serum creatinine and statin and estrogen use[15]. Third, an inverse relationship was recently reported between the serum osteocalcin level and parameters of atherosclerosis, including the brachial artery PWV and intima-media thickness, in 179 men following adjustment for age, duration of diabetes, BMI and serum creatinine[16]. More directly, the serum osteocalcin levels were significantly lower in the angiographically-proven CHD group than in the non-CHD group and decreased in association with the number of diseased vessels involved. Furthermore, the odds ratios for CHD have been reported to decrease as the serum osteocalcin quartile increases after adjusting for other conventional risk factors for CHD in Chinese adults[22]. Another cell-based study demonstrated that treatment with uncarboxylated osteocalcin prevents free fatty acid-induced apoptosis in insulin-stimulated endothelial cells in a phosphatidylinositol 3-kinase/Akt-dependent manner[23].

Despite the association between an increased serum osteocalcin level and a decreased cardiometabolic risk, to date only a few longitudinal follow-up studies have investigated the effects of osteocalcin on the development of CVD. In a longitudinal observational cohort study conducted among 126 hemodialysis patients, a lower median level of serum osteocalcin was significantly associated with the development of CVD after adjusting for age, sex, time of hemodialysis, previous history of CVD, presence of diabetes and the serum calcium X phosphate product and parathyroid hormone levels over a period of five years (HR, 2.925; 95% CI, 1048-9.066; $p=0.04$)[24]. However, another longitudinal study reported somewhat contrary results; all-cause and cardiovascular mortality was highest in the subjects in the highest total osteocalcin quintile among elderly men during a median 5.2-year follow-up[17]. However, the relationship was U-shaped, with both the lowest and highest osteocalcin quintiles associated with an increased risk following adjustment for age and the waist/hip ratio and further adjustment for other risk factors for CVD and medical comorbidities. Therefore, the authors argued that there may exist an optimal range of serum total osteocalcin that identifies better health outcomes. In addition, they speculated that the increased mortality observed among patients with lower osteocalcin levels may be related to insulin resistance, although other mechanisms, such as increased bone turnover, play a role in men with higher osteocalcin levels[17].

In the present study, the subjects were followed for at least six years and, of the 1,290 subjects, 578

(44.8%) received an annual medical check-up at the Health Promotion Center during the entire follow-up period, with the remaining subjects also followed up regularly at least biannually. Consequently, the serum total osteocalcin level was found to be inversely associated with obesity, abnormal glucose and lipid metabolism, insulin resistance, inflammatory markers and the prevalence of diabetes and metabolic syndrome at baseline. However, the serum total osteocalcin level was not associated with the development of any of cardiovascular events, including stroke, CHD and CVD, after adjusting for other risk factors for CVD during the 8.7-year follow-up period. In addition to the total study population, we investigated the impact of the osteocalcin level on the development of CVD in other groups, including women and both men and women. Similarly, no effects of osteocalcin on the development of CVD were evident (data not shown).

The reason for the conflicting data observed between our results and the findings of previous studies is unclear. However, there are several possible explanations. First, the effects of osteocalcin on the development of cardiovascular disease may vary according to the γ-carboxylation status; however, we only measured the total form of osteocalcin. Second, elevated serum osteocalcin is a marker of bone turnover and the serum undercarboxylated osteocalcin levels in particular are elevated in elderly women in association with a reduced bone mineral density and elevated hip fracture risk[25, 26]. In addition, osteoporosis and CVD share common risk factors, including aging, smoking, physical inactivity, diabetes, hypertension and inflammation[6], and patients with osteoporosis have an increased incidence of CVD[27]. Therefore, it is possible that an elevated serum osteocalcin level is a predictor for the development of CVD. As a plausible mechanism, it has been suggested that the actions of osteocalcin are closely linked to atherosclerosis. The percentage of CD34+/KDR+ and CD34+/CD133+/KDR+ endothelial progenitor cells costaining for osteocalcin has been reported to be significantly increased in patients with severe multi-vessel coronary artery disease as well as those with normal coronary arteries with endothelial dysfunction compared to that observed in control patients[28]. Furthermore, another study reported that the undercarboxylated osteocalcin levels were higher in the common carotid artery calcification group than in non-calcification group among 92 patients with essential hypertension[29]. Collectively, there may be a link between osteocalcin and atherosclerosis and/or vascular calcification.

There are several limitations associated with this study, and interpretations should be made with caution. For example, this study was primarily conducted based on data obtained from the medical records of a retrospective cohort, which may have resulted in bias, especially in the assessment of cardiovascular events. However, we made every effort to ascertain the true incidence of cardiovascular events; the rate of CVD events was determined using a standardized questionnaire distributed by trained nurses at the Health Promotion Center and we determined the frequency of CVD on an outpatient basis and during hospitalization based on the detection of signs or symptoms and the findings of biochemical tests and imaging workups. In addition, because our institute is one of the largest tertiary centers in Korea, cases lost to follow-up were relatively few in number. Second, according to the initial observations of Lee *et al.*[10], only the undercarboxylated form of osteocalcin has biologic effects. However, we did not differentiate serum osteocalcin with respect to the gamma-carboxylation status; that is, carboxylated and undercarboxylated osteocalcin. Third, we did not fully consider the effects of altered bone turnover, osteoporosis or medications on the serum osteocalcin levels. However, we limited the study subjects to 1,290 men, 40-78 years of age, who are less likely to have osteoporosis or receive calcium, vitamin D or osteoporosis medications compared with women. In agreement with our expectations, the serum total osteocalcin levels were not correlated with aging in our study population (data not shown) and thus the effects of altered bone turnover on the serum osteocalcin level may have been minimal.

Despite these limitations, this study differed from previous studies in that it investigated the causal relationship between the serum osteocalcin level and the development of CVD with regard to the following issues. First, this study was conducted using a relatively large number of study subjects with a long-term follow-up period. Second, we determined the incidence of CVD based on signs or symptoms in addition to validated methods, including biochemical tests and imaging, whereas other studies identified cardiovascular events using only self-reported questionnaire data and medical diagnosis codes. Third, we divided CVD into the categories of stroke and coronary heart disease and determined the association between the serum total osteocalcin level and the occurrence of stroke and coronary heart disease separately.

Conclusion

In the present study, the serum total osteocalcin level was not associated with the development of stroke, CHD or CVD in a relatively large sample size

with a long-term follow-up period. Recently, we reported that the circulating osteocalcin level was not associated with incident type 2 diabetes in the same cohort[30]. Therefore, contrary to the results of animal studies, it appears that the serum total osteocalcin level has minimal cardiovascular and glycemic effects and its clinical relevance may be limited in humans.

Acknowledgements

The authors are grateful to Hong-Yup Ahn from the Department of Statistics, Dongguk University-Seoul, Seoul, Korea for helping with the statistical analysis.

Sources of Funding

This study was supported by the Samsung Medical Center Clinical Research Development Program grant #CRS-110-52-11.

Conflicts of Interest

The authors have no conflicts of interest to declare.

References

1) Kado DM, Browner WS, Blackwell T, Gore R, Cummings SR: Rate of bone loss is associated with mortality in older women: a prospective study. J Bone Miner Res, 2000; 15: 1974-1980
2) Trivedi DP, Khaw KT: Bone mineral density at the hip predicts mortality in elderly men. Osteoporos Int, 2001; 12: 259-265
3) Kiel DP, Kauppila LI, Cupples LA, Hannan MT, O'Donnell CJ, Wilson PW: Bone loss and the progression of abdominal aortic calcification over a 25 year period: the Framingham Heart Study. Calcif Tissue Int, 2001; 68: 271-276
4) Hyder JA, Allison MA, Wong N, Papa A, Lang TF, Sirlin C, Gapstur SM, Ouyang P, Carr JJ, Criqui MH: Association of coronary artery and aortic calcium with lumbar bone density: the MESA Abdominal Aortic Calcium Study. Am J Epidemiol, 2009; 169: 186-194
5) Frost ML, Grella R, Millasseau SC, Jiang BY, Hampson G, Fogelman I, Chowienczyk PJ: Relationship of calcification of atherosclerotic plaque and arterial stiffness to bone mineral density and osteoprotegerin in postmenopausal women referred for osteoporosis screening. Calcif Tissue Int, 2008; 83: 112-120
6) Eastell R, Newman C, Crossman DC: Cardiovascular disease and bone. Arch Biochem Biophys, 2010; 503: 78-83
7) Bolland MJ, Avenell A, Baron JA, Grey A, MacLennan GS, Gamble GD, Reid IR: Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. BMJ, 2010; 341: c3691
8) Bolland MJ, Grey A, Avenell A, Gamble GD, Reid IR: Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. BMJ, 2011; 342: d2040
9) Elamin MB, Abu Elnour NO, Elamin KB, Fatourechi MM, Alkatib AA, Almandoz JP, Liu H, Lane MA, Mullan RJ, Hazem A, Erwin PJ, Hensrud DD, Murad MH, Montori VM: Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis. J Clin Endocrinol Metab, 2011; 96: 1931-1942
10) Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Dacquin R, Mee PJ, McKee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P, Karsenty G: Endocrine regulation of energy metabolism by the skeleton. Cell, 2007; 130: 456-469
11) Ducy P: The role of osteocalcin in the endocrine cross-talk between bone remodelling and energy metabolism. Diabetologia, 2011; 54: 1291-1297
12) Fernandez-Real JM, Izquierdo M, Ortega F, Gorostiaga E, Gomez-Ambrosi J, Moreno-Navarrete JM, Fruhbeck G, Martinez C, Idoate F, Salvador J, Forga L, Ricart W, Ibanez J: The relationship of serum osteocalcin concentration to insulin secretion, sensitivity, and disposal with hypocaloric diet and resistance training. J Clin Endocrinol Metab, 2009; 94: 237-245
13) Hwang YC, Jeong IK, Ahn KJ, Chung HY: The uncarboxylated form of osteocalcin is associated with improved glucose tolerance and enhanced beta-cell function in middle-aged male subjects. Diabetes Metab Res Rev, 2009; 25: 768-772
14) Hwang YC, Jeong IK, Ahn KJ, Chung HY: Circulating osteocalcin level is associated with improved glucose tolerance, insulin secretion and sensitivity independent of the plasma adiponectin level. Osteoporos Int, 2012; 23: 1337-1342
15) Saleem U, Mosley TH Jr, Kullo IJ: Serum osteocalcin is associated with measures of insulin resistance, adipokine levels, and the presence of metabolic syndrome. Arterioscler Thromb Vasc Biol, 2010; 30: 1474-1478
16) Kanazawa I, Yamaguchi T, Yamamoto M, Yamauchi M, Kurioka S, Yano S, Sugimoto T: Serum osteocalcin level is associated with glucose metabolism and atherosclerosis parameters in type 2 diabetes mellitus. J Clin Endocrinol Metab, 2009; 94: 45-49
17) Yeap BB, Chubb SA, Flicker L, McCaul KA, Ebeling PR, Hankey GJ, Beilby JP, Norman PE: Associations of total osteocalcin with all-cause and cardiovascular mortality in older men. The Health In Men Study. Osteoporos Int, 2012; 23: 599-606
18) Hwang YC, Jee JH, Oh EY, Choi YH, Lee MS, Kim KW, Lee MK: Metabolic syndrome as a predictor of cardiovascular diseases and type 2 diabetes in Koreans. Int J Cardiol, 2009; 134: 313-321
19) Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C; American Heart Association; National Heart, Lung, and Blood Institute: Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on sci-

entific issues related to definition. Circulation, 2004; 109: 433-438
20) American Diabetes Association: Diagnosis and classification of diabetes mellitus. Diabetes Care, 2009; 32 Suppl 1: S62-67
21) Ngarmukos C, Chailurkit LO, Chanprasertyothin S, Hengprasith B, Sritara P, Ongphiphadhanakul B: A reduced serum level of total osteocalcin in men predicts development of diabetes in a long-term follow-up cohort. Clin Endocrinol, 2012; 77: 42-46
22) Zhang Y, Qi L, Gu W, Yan Q, Dai M, Shi J, Zhai Y, Chen Y, Liu J, Wang W, Ning G, Hong J: Relation of serum osteocalcin level to risk of coronary heart disease in Chinese adults. Am J Cardiol, 2010; 106: 1461-1465
23) Jung CH, Lee WJ, Hwang JY, Lee MJ, Seol SM, Kim YM, Lee YL, Park JY: The preventive effect of uncarboxylated osteocalcin against free fatty acid-induced endothelial apoptosis through the activation of phosphatidylinositol 3-kinase/Akt signaling pathway. Metabolism, 2013; 62: 1250-1257
24) Yamashita T, Okano K, Tsuruta Y, Akiba T, Nitta K: Serum osteocalcin levels are useful as a predictor of cardiovascular events in maintenance hemodialysis patients. Int Urol Nephrol, 2013; 45: 207-214
25) Szulc P, Arlot M, Chapuy MC, Duboeuf F, Meunier PJ, Delmas PD: Serum undercarboxylated osteocalcin correlates with hip bone mineral density in elderly women. J Bone Miner Res, 1994; 9: 1591-1595
26) Szulc P, Chapuy MC, Meunier PJ, Delmas PD: Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. J Clin Invest, 1993; 91: 1769-1774
27) Tanko LB, Christiansen C, Cox DA, Geiger MJ, McNabb MA, Cummings SR: Relationship between osteoporosis and cardiovascular disease in postmenopausal women. J Bone Miner Res, 2005; 20: 1912-1920
28) Gossl M, Modder UI, Atkinson EJ, Lerman A, Khosla S: Osteocalcin expression by circulating endothelial progenitor cells in patients with coronary atherosclerosis. J Am Coll Cardiol, 2008; 52: 1314-1325
29) Okura T, Kurata M, Enomoto D, Jotoku M, Nagao T, Desilva VR, Irita J, Miyoshi K, Higaki J: Undercarboxylated osteocalcin is a biomarker of carotid calcification in patients with essential hypertension. Kidney Blood Press Res, 2010; 33: 66-71
30) Hwang YC, Jee JH, Jeong IK, Ahn KJ, Chung HY, Lee MK: Circulating osteocalcin level is not associated with incident type 2 diabetes in middle-aged male subjects: mean 8.4-year retrospective follow-up study. Diabetes Care, 2012; 35: 1919-1924