Circulating Osteocalcin Level Is Not Associated With Incident Type 2 Diabetes in Middle-Aged Male Subjects

Mean 8.4-year retrospective follow-up study

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OBJECTIVE—Recent human studies suggested that serum osteocalcin is associated with the cross-talk between bone and energy metabolism. The aim of this study was to determine whether serum osteocalcin level is independently associated with the development of type 2 diabetes.

RESEARCH DESIGN AND METHODS—A retrospective cohort study was performed of 1,229 nondiabetic men, aged 25–60 years, who were recruited from the Health Promotion Center, Samsung Medical Center, between January 1997 and December 1997. They were followed regularly at the center on an out-patient basis and during hospitalization for a mean of 8.4 years, and the development of type 2 diabetes was determined.

RESULTS—In the baseline analysis, BMI, body fat percentage, triglyceride, homeostasis model assessment of insulin resistance value, and plasminogen activator inhibitor-1 levels varied inversely with the osteocalcin tertiles, and serum high-density lipoprotein cholesterol levels increased with the osteocalcin tertiles. However, no differences were observed in fasting glucose and glycated hemoglobin levels across the osteocalcin tertiles. Incident type 2 diabetes occurred in 90 (7.3%) of the study subjects. In Cox proportional hazards models, however, no statistical differences in the development of type 2 diabetes across the osteocalcin tertiles were evident after adjustment of other risk factors for incident diabetes.

CONCLUSIONS—Despite baseline associations with favorable metabolic parameters, the serum osteocalcin level was not associated with the development of type 2 diabetes in middle-aged males.

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R ecently, it has been suggested that the osteoblast-specific protein osteocalcin is central in the cross-talk between bone remodeling and energy metabolism (1–3). Osteocalcin signals to both pancreatic β-cells and adipose tissues have been demonstrated; they increase insulin secretion and β-cell proliferation and regulate body fat mass and adiponectin gene expression in a genetically modified mouse model (4). In addition, subsequent studies have shown that osteocalcin may be of value to prevent the development of type 2 diabetes in wild-type mice, which suggests a therapeutic potential for the treatment of metabolic diseases (5,6).

In agreement with these observations, accumulating human evidence has demonstrated that serum osteocalcin levels are inversely associated with blood glucose levels and positively associated with insulin secretion and insulin sensitivity (7–9). In addition, it has been suggested that serum osteocalcin levels are inversely associated with dysmetabolic phenotypes such as atherogenic dyslipidemia, abdominal obesity, metabolic syndrome, and increased brachial-ankle pulse wave velocity and carotid intima-media thickness (10–15). Moreover, in one study, serum osteocalcin levels were significantly lower in the angiographically proven coronary heart disease group than in the normal group and were decreased with the number of vessels involved (14).

However, almost all of the human studies were based on a cross-sectional analysis. Thus, it is still uncertain whether the aforementioned findings are merely correlations or if any osteocalcin actually directly affects energy metabolism in humans, as has been shown in animal- and cell-based studies. Therefore, we performed this study to determine whether serum osteocalcin levels are independently associated with the development of type 2 diabetes over a mean follow-up period of 8.4 years.

RESEARCH DESIGN AND METHODS

Study population
A detailed description of the study design has been published previously (16). In brief, the study subjects were recruited from among those who visited the Health Promotion Center of the Samsung Medical Center between January 1997 and December 1997. A total of 2,435 apparently healthy subjects (1,761 men and 674 women), aged 20–78 years, were enrolled in this study and were followed up regularly at the Health Promotion Center, on an out-patient basis and during hospitalization, for a mean of 8.7 years. Of the 2,435 subjects, we limited the subjects to 1,332 men, aged 25–60 years, who were expected to have minimal bone turnover, to minimize the effects of age, sex, and other factors on serum osteocalcin levels. Among

them, 103 subjects had diabetes at baseline and were excluded from the analysis. Finally, 1,229 men who had normal glucose tolerance (n = 622) or impaired fasting glucose (IFG; n = 607) were enrolled for the analyses and followed up for a mean of 8.4 years. This study was approved by the institutional review board and the ethics committee of Samsung Medical Center (IRB no. 2009-10-035) and complied with the Declaration of Helsinki.

Baseline examination

At baseline, a complete physical examination was performed, and personal medical history and lifestyle factors, including cigarette smoking and alcohol consumption, were determined using a standardized questionnaire. Smoking was classified into three groups (current smoker, ex-smoker, and never smoker), and alcohol intake was defined as more than three drinks per day (>30 g ethanol/day). Blood pressure was measured using a mercury sphygmomanometer on the right arm with subjects in a sitting position after a 5-min rest. Weight and height were measured in the morning with subjects wearing light clothing but no shoes, and the BMI was calculated as the weight in kilograms divided by the square of the height in meters (kg/m^2). Percentage of body fat was measured by bioelectrical impedance analysis (In Body 3.0; Biospace, Seoul, Korea). All blood samples were obtained during the morning after an overnight fast of 12–14 h. Plasma glucose was measured in duplicate by the hexokinase method using an autoanalyzer (Hitachi, Tokyo, Japan). The interassay coefficient of variation (CV) was 1.6%. Plasma insulin was measured in duplicate using an immunoradiometric assay method (Medgenix, Nivelles, Belgium). Intra- and interassay CV at serum insulin levels of <215 pmol/L were 2.2 and 5.8%, respectively, and at serum insulin levels of ≥215 pmol/L were 3.9 and 4.5%, respectively. Standard liver function tests, uric acid, γ-glutamyl transpeptidase (γGT), and lipid profiles, including serum total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol, were determined using an autoanalyzer (Hitachi), as were the white blood cell counts (Sysmex, Kobe, Japan). Fibrinogen level was measured by clotting method using a STA coagulation analyzer (Stago S.A., France). Plasminogen activator inhibitor-1 (PAI-1) level was measured by an enzyme-linked immunosorbent assay method using an Asserachrom PAI-1 kit reagent (Stago S.A., France) as specified by the manufacturer's protocols.

Lipoprotein (a) level was measured by the rate nephelometry method. Serum total osteocalcin level was analyzed by Metra TM Osteocalcin (Quidel, Santa Clara, CA) using a Dade Behring ELISA-Processor III (Behring Diagnostics, Somerville, NJ) with intra- and interassay CVs of 4.8–10.0 and 4.8–9.8%, respectively.

Definition of metabolic syndrome and diabetes

Metabolic syndrome was defined using the modified National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria (17). However, we used a BMI cutoff value of 25.0 kg/m^2 as the parameter for abdominal obesity instead of waist circumference. In this study, diabetes was defined by any one of the following: fasting plasma glucose (FPG) level ≥7.0 mmol/L, use of diabetes medication, or self-reported physician diagnosis. IFG was defined as glucose levels of 5.6–7.0 mmol/L. At baseline, the subjects were assessed for diabetes, as determined by a standardized questionnaire and the medical records, and followed regularly for the occurrence of new onset diabetes. To ascertain the incidence of type 2 diabetes, all data were collected from the Health Promotion Center on an out-patient basis and during hospitalization. Incident type 1 diabetes was excluded based on clinical parameters, including history of ketoacidosis at initial presentation, essentially requiring insulin therapy for blood glucose control, and low to undetectable serum insulin or C-peptide levels. The homeostasis model assessment (HOMA) was used to estimate the insulin sensitivity and β-cell function from FPG and fasting plasma insulin (FPI) concentrations. Insulin resistance (IR) was estimated using the HOMA-IR, defined as [FPI (μU/mL) × FPG (mmol/L)] ÷ 22.5. HOMA-%B was calculated using (20 × FPI) ÷ (FPG – 3.5) and was used to represent β-cell function.

Statistical methods

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 serum levels of triglyceride, γGT, lipoprotein (a), PAI-1, and HOMA values were skewed, natural logarithmic transformation was applied in the statistical analysis. For clarity, nontransformed median values are presented in the tables and text. One-way ANOVA, followed by Tukey's post hoc test, was used to compare the means between the

tertiles of osteocalcin levels. Linear-by-linear association χ^2 test was used for trend analysis between the tertiles. Pearson correlation coefficients were calculated to evaluate the associations between osteocalcin and clinical and laboratory measurements. Cox proportional hazards regression model was used to calculate an adjusted hazard ratio (HR) for incident type 2 diabetes among the subjects with osteocalcin tertiles. The upper osteocalcin tertile was used as a reference, and the results for the analyses are presented as HR with a 95% CI. Analysis was performed using PASW version 18.0 (SPSS, Chicago, IL). P values <0.05 were considered significant.

RESULTS—Baseline characteristics of the study subjects according to serum total osteocalcin tertiles are shown in Table 1. Serum total osteocalcin levels were associated with favorable metabolic profiles. BMI, body fat percentage, and triglyceride levels varied inversely with the osteocalcin tertiles (all P < 0.01), and serum HDL cholesterol levels increased with the osteocalcin tertiles (P = 0.014). In addition, inflammatory markers such as lipoprotein (a) (P = 0.049) and PAI-1 levels (P = 0.004) were different, and the prevalence of National Cholesterol Education Program–defined metabolic syndrome also showed a decreasing tendency across the osteocalcin tertiles (P < 0.001). However, although the levels of IR, represented by HOMA-IR value, tended to decrease across the osteocalcin tertiles (P = 0.025), no differences were observed in FPG and glycated hemoglobin (HbA$_{1c}$) levels with the osteocalcin tertiles. In line with the results of the Table 1, the correlation analysis showed similar results (Supplementary Table 1).

Incident type 2 diabetes occurred in 90 (7.3%) of the 1,229 total study subjects and 79 (13.0%) of the 607 IFG subjects during the 8.4 years of mean follow-up. When compared with the upper osteocalcin tertile (reference group), no differences were observed in the middle or lower osteocalcin tertiles for the development of type 2 diabetes in Kaplan-Meier survival curve (P = 0.578 across the tertiles, Fig. 1). The results of Cox proportional hazard model analysis are shown in Table 2. Independent variables included in the models were age, BMI, body fat percentage, fasting blood glucose, HbA$_{1c}$, systolic blood pressure, triglyceride, HDL cholesterol, fibrinogen, PAI-1, smoking status, presence of metabolic syndrome, and serum osteocalcin

Table 1—*Baseline characteristics according to serum osteocalcin tertiles*

| | | Tertile | | | |
	Total	Bottom (*n* = 400)	Middle (*n* = 406)	Top (*n* = 423)	*P*
Osteocalcin (nmol/L, range)	1.32 ± 0.47 (0.34–3.69)	0.83 ± 0.20 (0.34–1.08)	1.28 ± 0.11 (1.09–1.45)	1.83 ± 0.32 (1.47–3.69)	<0.001
Age (years)	47.4 ± 5.8	47.5 ± 6.1	47.6 ± 5.5	47.1 ± 5.8	NS
BMI (kg/m^2)	23.8 ± 2.3	24.1 ± 2.1	23.7 ± 2.3	23.5 ± 2.5	<0.001
Body fat (%)	22.4 ± 4.5	23.0 ± 4.0	22.2 ± 4.7	22.1 ± 4.8	0.006
Blood pressure (mmHg)					
Systolic	120.7 ± 13.6	121.2 ± 13.3	119.7 ± 13.7	121.1 ± 13.9	NS
Diastolic	82.9 ± 10.0	83.6 ± 9.8	82.4 ± 10.0	82.8 ± 10.3	NS
Fasting plasma glucose					
(mmol/L)	5.52 ± 0.56	5.53 ± 0.57	5.56 ± 0.55	5.48 ± 0.55	NS
HbA$_{1c}$ (%)	5.3 ± 0.4	5.3 ± 0.4	5.3 ± 0.4	5.3 ± 0.4	NS
Log HOMA-IR	1.67 (1.20–2.32)	1.80 (1.25–2.44)	1.68 (1.19–2.30)	1.62 (1.15–2.20)	0.025
Log HOMA-B%	69.4 (50.4–95.4)	73.6 (52.9–100.8)	67.7 (48.4–91.1)	66.3 (50.0–94.0)	NS
Total cholesterol (mmol/L)	5.13 ± 0.82	5.17 ± 0.81	5.11 ± 0.82	5.12 ± 0.84	NS
Log triglycerides (mmol/L)	1.47 (1.07–2.05)	1.59 (1.14–2.21)	1.42 (1.07–2.01)	1.42 (1.02–1.94)	0.004
HDL cholesterol (mmol/L)	1.27 ± 0.30	1.24 ± 0.29	1.27 ± 0.30	1.30 ± 0.32	0.014
LDL cholesterol (mmol/L)	3.10 ± 0.75	3.12 ± 0.75	3.09 ± 0.74	3.09 ± 0.75	NS
Log γGT (units/L)	30.0 (22.0–44.5)	33.0 (24.0–52.0)	29.0 (22.0–45.0)	28.0 (21.0–39.0)	<0.001
Uric acid (μmol/L)	345.2 ± 63.0	348.4 ± 61.4	346.6 ± 64.8	340.9 ± 62.7	NS
Fibrinogen (μmol/L)	9.10 ± 1.73	9.14 ± 1.71	9.00 ± 1.65	9.15 ± 1.81	NS
Log lipoprotein (a) (μmol/L)	0.47 (0.25–0.90)	0.43 (0.21–0.87)	0.47 (0.26–0.90)	0.50 (0.28–0.94)	0.049
Log PAI-1 (μg/L)	26.0 (16.0–39.0)	28.0 (18.0–43.0)	26.0 (16.0–38.3)	23.0 (15.0–36.0)	0.004
Alcohol intake (%)	374 (30.4)	130 (32.5)	126 (31.0)	118 (27.9)	NS
Current smoking (%)	586 (47.7)	183 (45.8)	197 (48.5)	206 (48.7)	NS
Hypertension (%)	224 (18.2)	80 (20.0)	60 (14.8)	82 (19.4)	NS
Metabolic syndrome (%)	351 (28.6)	143 (35.8)	121 (29.8)	87 (20.6)	<0.001

Data are expressed as mean ± SD, median (interquartile range), or frequency.

level by tertiles. FPG (HR per 1-SD 10.79 [95% CI 6.47–17.99]) and HbA$_{1c}$ levels (2.51 [1.82–3.45]) independently predicted future development of type 2 diabetes, and HDL cholesterol was associated with a lower incidence of diabetes (0.61 [0.47–0.79]). However, serum total osteocalcin was not associated with incident type 2 diabetes across the tertiles. Likewise, serum total osteocalcin was not associated with incident type 2 diabetes in the IFG group (Supplementary Fig. 1 and Supplementary Table 2).

CONCLUSIONS—In the current study, we tried to determine whether serum osteocalcin level is independently associated with incident diabetes using a retrospective cohort recruited from the Health Promotion Center. Of the 2,435 apparently healthy subjects (1,761 men and 674 women), we limited the subjects to 1,229 nondiabetic men, aged 25–60 years, who were expected to have minimal bone turnover to minimize the effects of altered bone turnover on serum osteocalcin levels. Previous studies have demonstrated that serum osteocalcin levels showed dynamic changes with age and

sex (18). In men, serum osteocalcin levels were highest in the 20–29 year age group, declined and stabilized, then increased again in the seventh decade, and serum osteocalcin levels showed more dynamic changes, and increased abruptly around the time of menopause in women. Therefore, although we could not totally exclude the effects of altered bone turnover on osteocalcin level by measuring bone mineral density or other bone turnover markers, such as bone-specific alkaline phosphatase or other markers of bone resorption, we did attempt to minimize the effects of other confounders by uniformly enrolling middle-aged men, who were expected to have minimal bone turnover. In addition to middle-aged male subjects, we investigated the effect of osteocalcin on incident type 2 diabetes in another study population including total men, total women, women who were likely to be premenopausal (age ≤50 years), and total men and women. In Cox proportional hazard regression analyses, serum osteocalcin was not independently associated with incident type 2 diabetes in all of the aforementioned

study groups. Therefore, it appears that serum osteocalcin is not associated with incident type 2 diabetes, not only in middle-aged men but also in other groups (data not shown).

In agreement with previous reports (10–15), serum osteocalcin levels were inversely associated with the presence of obesity, atherogenic dyslipidemia (high triglyceride and low HDL cholesterol levels), inflammatory status (high PAI-1 level), and metabolic syndrome in the baseline cross-sectional analyses (Table 1 and Supplementary Table 1). However, although the levels of HOMA-IR, an indicator of IR, decreased progressively from lower to upper osteocalcin tertiles, no differences were observed in FPG and HbA$_{1c}$ levels across the osteocalcin tertiles. Instead, serum osteocalcin levels were significantly correlated with FPG ($r = -0.202$, $P = 0.041$) and HbA$_{1c}$ levels ($r = -0.211$, $P = 0.033$) in 103 patients with diabetes at baseline (data not shown). Therefore, it appears that serum osteocalcin is more closely associated with the parameters reflecting insulin resistance including obesity, atherogenic dyslipidemia, and
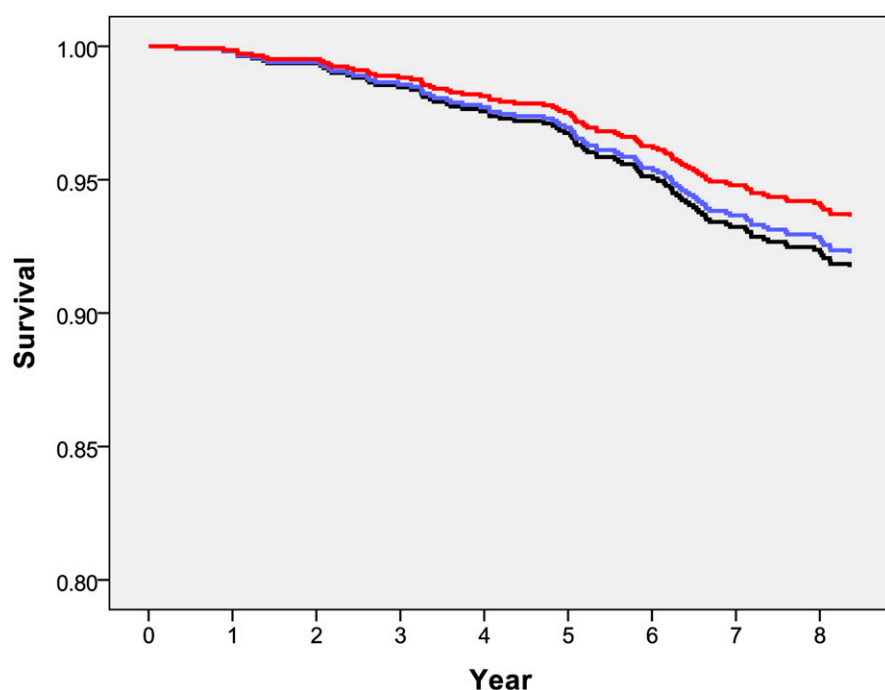
Figure 1—*Kaplan-Meier survival curve for incident type 2 diabetes according to serum total osteocalcin tertiles (red, top tertile; blue, middle tertile; black, bottom tertile) in total subjects (P = 0.578 across the tertiles).*

inflammation rather than glucose tolerance itself before the onset of diabetes. Presently, the association between serum osteocalcin and glucose tolerance became evident just after the onset of diabetes. In the current study, serum osteocalcin was not associated with the development of incident diabetes during the mean 8.4-year follow-up both in unadjusted and adjusted models. In addition, when we limited the study subjects to the IFG group, no differences in the progression rate to diabetes were observed (Supplementary Table 2 and Supplementary Fig. 1). Therefore, the role of circulating osteocalcin on glucose metabolism might be limited in human subjects.

To date, almost all human studies have agreed with the results of previous animal studies; that is, serum osteocalcin levels are inversely associated with blood glucose levels and positively associated with insulin secretion and insulin sensitivity. In addition, serum osteocalcin predicts all-cause and cardiovascular disease–related mortality in a prospective cohort of community-dwelling elderly men even though the relationship is U-shaped with men at both ends of the distribution at increased risk (19). However, a few studies have been conducted to determine the association between serum osteocalcin level and glucose tolerance with longitudinal follow-up. Pittas

et al. (12) reported that mean osteocalcin concentration during follow-up predicted change of FPG at year 3 with 198 study subjects, independently of baseline FPG, age, sex, physical activity, smoking, education, and change in BMI at year 3. Another study also showed that reduced serum total osteocalcin (odds ratio 0.90; 95% CI 0.81–0.99), but not undercarboxylated osteocalcin levels, was an independent risk factor for development of diabetes after adjustment for baseline FPG, age, BMI, and exercise in 126 subjects within a nested case-control cohort in Thailand during the 10-year follow-up (20). Collectively, contrary to our results, several cross-sectional and some longitudinal studies have consistently demonstrated that circulating osteocalcin plays a favorable role in glucose metabolism in humans. However, previous longitudinal follow-up studies were conducted with a relatively small sample size and short follow-up period. Thus, the association between serum osteocalcin level and glucose tolerance is not yet conclusive in human subjects.

The basis of the discrepancy between the present and previous studies is unclear. Several suggestions can be made. Contrary to animal models, it may be possible that osteocalcin has minimal effects on energy metabolism in humans, particularly for glucose homeostasis. There are data to support this suggestion. First, several studies have shown that antiresorptive agents such as bisphosphonates, raloxifene, strontium ranelate, and the RANK ligand antibody denosumab suppress bone turnover by inhibiting the osteoclasts, and thus decrease circulating total osteocalcin levels (21–25). In addition, it was reported that alendronate treatment significantly decreased serum undercarboxylated osteocalcin level, while serum carboxylated osteocalcin and the undercarboxylated-to-carboxylated osteocalcin ratio were not affected in postmenopausal women (26). These observations indicate that antiresorptive agents have the potential to increase the risk of diabetes in theory. However, no large clinical trials have reported an association between treatment with antiresorptive agents such as bisphosphonates and incident diabetes. Vestergaard (27) showed that the risk of developing type 2 diabetes was reduced with alendronate, etidronate, and raloxifene treatment in a nationwide Danish cohort. In particular, a dose-dependent risk reduction was observed for alendronate; therefore, antiresorptive drugs do not appear to

Table 2—*Cox proportional hazards regression analysis for incident type 2 diabetes in the total subjects*

	HR per 1-SD increase in variables	95% CI	*P*
Osteocalcin tertile			
Top (reference)	1	ND	ND
Middle	1.10	0.67–1.81	0.70
Bottom	1.13	0.67–1.91	0.66
FPG	10.79	6.47–17.99	<0.001
HbA$_{1c}$	2.51	1.82–3.45	<0.001
HDL cholesterol	0.61	0.47–0.79	<0.001

Values are adjusted for age, BMI, body fat percentage, fasting blood glucose, HbA$_{1c}$, systolic blood pressure, triglyceride, HDL cholesterol, fibrinogen, PAI-1, smoking status, and presence of metabolic syndrome. ND, not determined.

be associated with an increased risk of diabetes. Rather, they might be protective involving the suppression of bone turnover. Second, it was reported that chronic exposure to high glucose inhibited cell growth of a human osteoblast–like cell line in a dose-dependent manner and decreased osteocalcin mRNA levels in mouse osteoblast (28,29). In agreement with this observation, several human studies showed that osteocalcin and other bone turnover markers are lower in patients with established diabetes (30–33). Thus, it remains unclear whether osteocalcin precedes hyperglycemia or vice versa. Third, although almost all human studies have reported the positive association between serum osteocalcin levels and better glycemic control, Aoki et al. (34) showed that serum osteocalcin concentration was increased in Japanese subjects with early-stage type 2 diabetes who had never taken medication. Especially, serum osteocalcin level was positively correlated with postload 2-h glucose level on oral glucose tolerance test. Fourth, protein tyrosine phosphatase OST-PTP, the protein encoded by Esp that regulates osteocalcin carboxylation, is a mouse gene that is not expressed in humans (35). The collective observations highlight the controversy over whether osteocalcin plays a direct role in regulating glucose homeostasis in humans, as is the case from the animal studies. It is possible that previous human studies showing the association between circulating osteocalcin and favorable metabolic profiles were merely correlations.

In addition, it has been reported that about 25% of subjects with prediabetes progress to type 2 diabetes in 5 years (36). In addition, results of several recent prospective studies support the suggestion that the rate of progression to type 2 diabetes may be even higher, averaging 10–12% per year (37–39). However, only 13% (79 of 607) of our subjects with prediabetes progressed to diabetes during the mean 8-year mean follow-up. This may be partly because our study subjects presented for medical health check-ups. They may have been healthier than others in the general population. Consequently, although the rate of incident diabetes showed a tendency to decrease in subjects with upper serum osteocalcin tertile, it could not reach statistical significance.

Apart from its being a retrospective follow-up study in nature, this study had several limitations. First, according to the initial observations of Lee et al. (4), only the uncarboxylated form of osteocalcin has the ability to induce insulin secretion and the expression of genes encoding adiponectin and insulin. As a result, glucose metabolism is improved. In contrast, carboxylated osteocalcin was found to have none of these effects. However, we did not differentiate serum osteocalcin with respect to the γ-carboxylation status and only measured the total form of osteocalcin, instead of directly measuring carboxylated and uncarboxylated osteocalcin. Therefore, we do not know the differential mechanism of both types of osteocalcin to regulate insulin secretion and insulin sensitivity. Second, although we limited the subjects to middle-aged men to minimize the effects of age, sex, and altered bone turnover on serum osteocalcin levels, we could not entirely exclude the effects of age, sex, and other variables in the associations between plasma osteocalcin levels and glucose metabolism. Therefore, the additional measurement of bone resorption markers may further clarify the potential association between osteocalcin and glucose homeostasis. Third, data regarding concurrent medication, which may affect bone turnover and serum osteocalcin levels, including calcium supplements, vitamin D supplements, and osteoporosis medications, were not determined. Fourth, vitamin D deficiency increases bone remodeling and may increase serum osteocalcin levels. However, vitamin D status measured by serum 25-hydroxyvitamin D was not assessed in this study. Finally, we did not use the oral glucose tolerance test or HbA_{1c} for the diagnosis of diabetes. Thus, the incidence of diabetes might be underestimated. However, the development of diabetes was regularly followed not only at the Health Promotion Center but also on an out-patient basis and during hospitalization by medical record review. Thus, we could minimize the number of undiagnosed subjects with diabetes.

Despite these limitations, this is the first study to investigate whether serum osteocalcin is associated with the development of incident diabetes in a relatively large sample size and long-term follow-up period with valid statistical methods. In conclusion, serum osteocalcin level is not associated with the development of type 2 diabetes in middle-aged male Koreans. However, further prospective studies to examine the role of undercarboxylated osteocalcin on the development of diabetes are warranted.

References
1. Lee NK, Karsenty G. Reciprocal regulation of bone and energy metabolism. Trends Endocrinol Metab 2008;19:161–166
2. Ducy P. The role of osteocalcin in the endocrine cross-talk between bone remodelling and energy metabolism. Diabetologia 2011;54:1291–1297
3. Confavreux CB, Levine RL, Karsenty G. A paradigm of integrative physiology, the crosstalk between bone and energy metabolisms. Mol Cell Endocrinol 2009;310:21–29
4. Lee NK, Sowa H, Hinoi E, et al. Endocrine regulation of energy metabolism by the skeleton. Cell 2007;130:456–469
5. Ferron M, Hinoi E, Karsenty G, Ducy P. Osteocalcin differentially regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. Proc Natl Acad Sci USA 2008;105:5266–5270
6. Ferron M, McKee MD, Levine RL, Ducy P, Karsenty G. Intermittent injections of osteocalcin improve glucose metabolism and prevent type 2 diabetes in mice. Bone 2012;50:568–575
7. Fernández-Real JM, Izquierdo M, Ortega F, et al. 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
8. 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
9. 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
10. Kindblom JM, Ohlsson C, Ljunggren O, et al. Plasma osteocalcin is inversely

related to fat mass and plasma glucose in elderly Swedish men. J Bone Miner Res 2009;24:785–791

11. Kanazawa I, Yamaguchi T, Yamamoto M, et al. Serum osteocalcin level is associated with glucose metabolism and atherosclerosis parameters in type 2 diabetes mellitus. J Clin Endocrinol Metab 2009;94:45–49

12. Pittas AG, Harris SS, Eliades M, Stark P, Dawson-Hughes B. Association between serum osteocalcin and markers of metabolic phenotype. J Clin Endocrinol Metab 2009;94:827–832

13. Kanazawa I, Yamaguchi T, Yamauchi M, et al. Serum undercarboxylated osteocalcin was inversely associated with plasma glucose level and fat mass in type 2 diabetes mellitus. Osteoporos Int 2011;22:187–194

14. Bao Y, Zhou M, Lu Z, et al. Serum levels of osteocalcin are inversely associated with the metabolic syndrome and the severity of coronary artery disease in Chinese men. Clin Endocrinol (Oxf) 2011;75:196–201

15. Tan A, Gao Y, Yang X, et al. Low serum osteocalcin level is a potential marker for metabolic syndrome: results from a Chinese male population survey. Metabolism 2011;60:1186–1192

16. Hwang YC, Jee JH, Oh EY, et al. Metabolic syndrome as a predictor of cardiovascular diseases and type 2 diabetes in Koreans. Int J Cardiol 2009;134:313–321

17. Grundy SM, Cleeman JI, Daniels SR, et al.; American Heart Association; National Heart, Lung, and Blood Institute. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005;112:2735–2752

18. Gundberg CM, Looker AC, Nieman SD, Calvo MS. Patterns of osteocalcin and bone specific alkaline phosphatase by age, gender, and race or ethnicity. Bone 2002;31:703–708

19. Yeap BB, Chubb SA, Flicker L, et al. Associations of total osteocalcin with all-cause and cardiovascular mortality in older men. The Health In Men Study. Osteoporos Int 2012;23:599–606

20. 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 (Oxf). In press

21. Adami S, Passeri M, Ortolani S, et al. Effects of oral alendronate and intranasal salmon calcitonin on bone mass and biochemical markers of bone turnover in postmenopausal women with osteoporosis. Bone 1995;17:383–390

22. Meunier PJ, Confavreux E, Tupinon I, Hardouin C, Delmas PD, Balena R. Prevention of early postmenopausal bone loss with cyclical etidronate therapy (a double-blind, placebo-controlled study and 1-year follow-up). J Clin Endocrinol Metab 1997; 82:2784–2791

23. Bjarnason NH, Sarkar S, Duong T, Mitlak B, Delmas PD, Christiansen C. Six and twelve month changes in bone turnover are related to reduction in vertebral fracture risk during 3 years of raloxifene treatment in postmenopausal osteoporosis. Osteoporos Int 2001;12:922–930

24. Gulhan I, Bilgili S, Gunaydin R, Gulhan S, Posaci C. The effect of strontium ranelate on serum insulin like growth factor-1 and leptin levels in osteoporotic post-menopausal women: a prospective study. Arch Gynecol Obstet 2008;278:437–441

25. Kostenuik PJ, Smith SY, Jolette J, Schroeder J, Pyrah I, Ominsky MS. Decreased bone remodeling and porosity are associated with improved bone strength in ovariectomized cynomolgus monkeys treated with denosumab, a fully human RANKL antibody. Bone 2011;49:151–161

26. Hirao M, Hashimoto J, Ando W, Ono T, Yoshikawa H. Response of serum carboxylated and undercarboxylated osteocalcin to alendronate monotherapy and combined therapy with vitamin K2 in postmenopausal women. J Bone Miner Metab 2008;26:260–264

27. Vestergaard P. Risk of newly diagnosed type 2 diabetes is reduced in users of alendronate. Calcif Tissue Int 2011;89:265–270

28. Terada M, Inaba M, Yano Y, et al. Growth-inhibitory effect of a high glucose concentration on osteoblast-like cells. Bone 1998;22:17–23

29. Zayzafoon M, Stell C, Irwin R, McCabe LR. Extracellular glucose influences osteoblast

differentiation and c-Jun expression. J Cell Biochem 2000;79:301–310

30. Pietschmann P, Schernthaner G, Woloszczuk W. Serum osteocalcin levels in diabetes mellitus: analysis of the type of diabetes and microvascular complications. Diabetologia 1988;31:892–895

31. Bouillon R, Bex M, Van Herck E, et al. Influence of age, sex, and insulin on osteoblast function: osteoblast dysfunction in diabetes mellitus. J Clin Endocrinol Metab 1995;80:1194–1202

32. Dobnig H, Piswanger-Sölkner JC, Roth M, et al. Type 2 diabetes mellitus in nursing home patients: effects on bone turnover, bone mass, and fracture risk. J Clin Endocrinol Metab 2006;91:3355–3363

33. Akin O, Göl K, Aktürk M, Erkaya S. Evaluation of bone turnover in post-menopausal patients with type 2 diabetes mellitus using biochemical markers and bone mineral density measurements. Gynecol Endocrinol 2003;17:19–29

34. Aoki A, Muneyuki T, Yoshida M, et al. Circulating osteocalcin is increased in early-stage diabetes. Diabetes Res Clin Pract 2011;92:181–186

35. Kawai M, Devlin MJ, Rosen CJ. Fat targets for skeletal health. Nat Rev Rheumatol 2009;5:365–372

36. Larsson H, Lindgärde F, Berglund G, Ahrén B. Prediction of diabetes using ADA or WHO criteria in post-menopausal women: a 10-year follow-up study. Diabetologia 2000;43:1224–1228

37. Pan XR, Li GW, Hu YH, et al. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. Diabetes Care 1997;20:537–544

38. Tuomilehto J, Lindström J, Eriksson JG, et al.; Finnish Diabetes Prevention Study Group. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001;344:1343–1350

39. Knowler WC, Barrett-Connor E, Fowler SE, et al.; Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346:393–403