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Osteoprotegerin is a Better Serum Biomarker of Coronary Artery Calcification than Osteocalcin in Type 2 Diabetes

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Abstract

Objective—Coronary artery calcification (CAC) is a prominent feature of atherosclerosis and is associated with cardiovascular events. In vitro studies have suggested that osteoprotegerin (OPG) and osteocalcin (OC) exert anticalcification potential in the vessel wall. The objective of this study was to investigate the association of CAC and serum bone biomarkers in persons with type 2 diabetes.

Methods—We examined 50 individuals with type 2 diabetes. CAC imaging was performed by multidetector computed tomography. CAC scores ≥ 10 , expressed in Agatston units, were considered abnormal. OC, undercarboxylated OC (ucOC), and OPG levels were determined by enzyme-linked immunosorbent assay.

Results—Abnormal CAC scores were found for 64% of the study cohort. OPG levels were significantly elevated (5.5 ± 2.0 pmol/L vs. 4.2 ± 1.7 pmol/L; $P = .026$) for those with abnormal CAC scores. No univariate differences were found for OC or ucOC. Logistic regression analyses revealed that an increase in serum OPG level was significantly associated with an increase in CAC score (odds ratio, 3.324; 95% confidence interval, 1.321 to 8.359; $P = .011$). Longer duration of diabetes was a significant covariate ($P = .026$), whereas nonsignificant covariates in the final model were age, gender, systolic blood pressure, body mass index, insulin resistance determined by the homeostasis model assessment for insulin resistance, leptin, adiponectin, and glycemic control. The Nagelkerke R^2 for the model was 0.66. Neither OC nor ucOC were significantly associated with elevated CAC scores.

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DISCLOSURE

The authors have no multiplicity of interest to disclose.

Conclusion—Our results suggest that OPG is a more useful serum biomarker than OC or ucOC for identifying those at increased risk of arterial calcification in type 2 diabetes.

INTRODUCTION

Calcification of the coronary arteries is highly correlated with atherosclerosis. Coronary artery calcification (CAC) is characterized by the deposition of calcium in coronary arteries and CAC scores are predictive of cardiac events in type 2 diabetes mellitus (1, 2).

Osteoprotegerin (OPG), a secreted glycoprotein, belongs to the tumor necrosis factor–receptor superfamily (3). OPG is known to inhibit osteoclastogenesis by acting as a decoy receptor for the receptor activator of nuclear factor- κ B ligand (RANKL). The coordinated synthesis of RANKL and OPG is an essential feature of bone remodeling. The OPG/RANKL signaling pathway also plays a role in regulating bone matrix proteins in vascular smooth muscle cells (4), with an imbalance in the OPG/RANKL signaling pathway potentially central to the process of vascular calcification (5). In vascular smooth muscle cells, RANKL appears to promote calcification, whereas the action of OPG may be protective (6). OPG circulates in the blood but at lower levels than in the tissues (5). Previous studies have shown that elevated serum OPG levels are associated with the presence and severity of coronary artery disease (CAD) determined by coronary angiography (7) and with CAC scores in asymptomatic individuals with type 2 diabetes (8).

Osteocalcin (OC) is a hydroxyapatite-binding protein synthesized by osteoblasts and contains 3 residues of gamma-carboxyglutamic acid that are responsible for the protein's calcium-binding properties (9). A fraction of newly synthesized OC is released into the circulation, providing a noninvasive marker of osteoblastic activity (10). OC functions as a continuous inhibitor of calcification in vessel walls and has been demonstrated in nondiseased aortas as well as in all stages of atherosclerosis (11). Recently, an increased risk of coronary heart disease was shown to be associated with serum OC levels in Chinese patients (31% diagnosed with type 2 diabetes) who underwent coronary angiography, where the number of stenotic coronary arteries was associated with decreasing serum OC levels (12).

Posttranslational carboxylation of OC by a vitamin K–dependent carboxylase yields both carboxylated and undercarboxylated (uc) OC molecules in serum (13). When OC is undercarboxylated, its avidity for hydroxyapatite is lower, providing a means for ucOC to more easily enter the circulation (14). OC appears to be not only involved in bone metabolism but may also be involved in glucose homeostasis and adipocyte secretion of adipokines (e.g., adiponectin) (15), with in vitro studies having suggested that ucOC may be the isoform that mediates metabolic functions (16). Some human studies (17, 18) (but not all [19]) have shown an inverse association between OC and insulin resistance measured by the homeostasis model assessment for insulin resistance (HOMA-IR). An inverse relationship between ucOC and fasting plasma glucose and glycated hemoglobin (HbA_{1c}) levels was reported for Japanese men with type 2 diabetes (20). OC and ucOC levels were also recently shown to be inversely associated with abdominal aortic calcification in men with type 2 diabetes (21). It should be noted that this association was independent of HbA_{1c} and

HOMA-IR (21). In patients with essential hypertension, ucOC was associated with carotid calcification (10). There is, however, a paucity of studies that have examined the relationship between OPG, OC, ucOC, and CAC in type 2 diabetes. There is a need for serum biomarkers to help identify patients at risk for future cardiovascular events. Thus, the objective in this study was to investigate the association between CAC and serum bone biomarkers in persons with type 2 diabetes without a history of CAD or ischemic cardiac events.

METHODS

Subjects

A total of 51 participants volunteered to participate in this study and were evaluated at the Diabetes and Metabolic Research Center, Christiana Care Health System, Newark, Delaware. Individuals with type 2 diabetes mellitus were eligible for the study if they were 18 years old. Exclusion criteria included: (1) history of a myocardial infarction, percutaneous coronary interventions, coronary artery bypass graft surgery, acute coronary syndromes, recent/ ongoing atrial fibrillation, or acute myocardial ischemia; (2) dose changes for antihypertensive and antidiabetes medications 2 months prior to enrollment; and (3) chronic kidney disease stage 3b. It should be noted that 1 individual that was enrolled in the study had stage 3b chronic kidney disease and thus after exclusion of this individual the results for 50 individuals were utilized. This study had approval of the Institutional Review Board of Christiana Care Corporation, and each participant gave written informed consent before participating in the study.

Clinical Measurements and CAC Scoring

Weight and height were measured using a stadiometer. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2). Blood pressure (BP) was monitored electronically in the supine posture using an oscillometric automatic recorder. CAC scores were determined via a 64-slice multidetector computed tomography scanner (Somatom Definition, Siemens Medical Solutions, Forchheim, Germany). Threshold calcium was set using a density of 130 Hounsfield units, and approximately 80 scans were obtained in 3-mm contiguous sections of the heart. CAC scores were calculated on U.S. Food and Drug Administration–approved postprocessing workstations according to the method of Agatston et al (22). CAC scores <10 were considered normal, whereas scores ≥ 10 were considered abnormal (23).

Blood Analytes

OPG and OC (intact) levels were measured using enzyme-linked immunosorbent assay (ELISA) kits (ALPCO, Salem, NH). An ELISA kit was also used to determine ucOC concentrations (Takara Bio, Inc, Otsu, Shiga, Japan). An electrochemiluminescence immunoassay was used to measure insulin, C-peptide, parathyroid hormone, and 25-hydroxyvitamin D levels. Specifically, insulin and C-peptide concentrations were determined on a Roche Cobas e601 chemistry analyzer; parathyroid hormone was measured using a Roche Elecsys 2010 analyzer; and 25-hydroxyvitamin D levels were assayed via a Roche Cobas e411 analyzer (Roche Diagnostics, Indianapolis, IN). Glucose measurements

were performed using an enzymatic method, and serum creatinine levels were determined using an enzymatic colorimetric assay. Insulin resistance was calculated using the HOMAIR online calculator downloaded from <http://www.dtu.ox.ac.uk> (24). HbA_{1c} was measured by high-performance liquid chromatography (HPLC) using a Tosoh G7 automated HPLC analyzer (Tosoh Bioscience, Inc, South San Francisco, CA). Calcium levels were determined using the Vitros Ca slide method on the Vitros 5,1 FS chemistry system (Ortho-Clinical Diagnostics, Rochester, NY). Leptin and total adiponectin levels were measured by radioimmunoassay (Millipore Corporation, Billerica, MA).

Statistical Analyses

Comparisons of demographic and metabolic parameters between those with normal versus abnormal CAC scores were made with unpaired *t* tests for continuous data and contingency table (chi-square) analysis for categorical variables. Pearson and Spearman rank correlation coefficients, where appropriate, were used to evaluate associations between demographic and metabolic parameters and continuous CAC scores and serum bone biomarkers. Logistic regression was used to assess the association between normal versus abnormal CAC scores and serum bone biomarkers (i.e., OPG, OC, ucOC) and other potential independent variables (i.e., age, duration of diabetes, HOMA-IR, BMI, gender, systolic BP, HbA_{1c}, leptin, and adiponectin). Normality was tested and if violated, a natural logarithmic transformation (e.g., ucOC) was used where possible.

RESULTS

Table 1 provides participants' physical demographic and metabolic parameters. Individuals with abnormal CAC scores (i.e., ≥ 10) had increased OPG and lower total adiponectin concentrations. No participants had known CAD or a history of ischemic cardiac events.

Table 2 shows correlation coefficients for demographic and metabolic parameters with continuous CAC scores and serum bone biomarkers. CAC scores were only positively associated with one serum bone biomarker (i.e., OPG [$r = 0.38$; $P < .01$]). It should be noted that neither HOMA-IR nor HbA_{1c} was associated with any of the serum bone biomarkers.

Table 3 shows a comparison of serum bone biomarkers for those with good glycemic control (i.e., HbA_{1c} $< 7\%$) versus those with poorer control. Although all biomarkers were higher for those with good glycemic control, there were no statistically significant differences between the groups. Likewise, we also compared serum bone biomarkers based on amount of insulin resistance (Table 4). HOMA-IR > 1.8 has been suggested as a cut-off value to indicate increased insulin resistance (25). There were no statistically significant differences between the groups with respect to OPG, OC, or ucOC.

Table 5 provides results of logistic regression analyses, with normal versus abnormal CAC scores as the dependent variable. A series of regression models were used to evaluate the relationship between CAC scores and the 3 serum bone biomarkers, individually and jointly. These models included adjusting for the covariates mentioned above. Individually, OPG was significantly associated with an abnormal CAC score. Neither OC nor ucOC were significantly associated with CAC scores. In a model including all 3 of the serum bone

biomarkers, OPG remained the only significant biomarker. The fact that OPG was significant regardless of the model suggests that increased OPG levels are robustly associated with elevated CAC scores. Based on these results, the final model presented here included OPG as the serum bone biomarker. Longer duration of diabetes was the only significant covariate associated with elevated CAC scores. Nonsignificant covariates in the model were age, gender, systolic BP, BMI, HOMA-IR, leptin, adiponectin, and HbA_{1c}. The model Nagelkerke R^2 was 0.66, indicating that approximately 66% of the variation in CAC scores could be explained by this model.

We were concerned with the number of covariates, given our sample size; thus, an automated logistic regression procedure was employed. A backward, stepwise selection with removal testing based on the likelihood-ratio statistic from the maximum partial likelihood estimates was used. The initial model contained all 3 serum bone biomarkers and the 9 covariates. After backwards elimination, the final model included 4 effects (i.e., OPG, duration, systolic BP, and gender) with increased OPG levels, longer duration of diabetes, higher systolic BP, and male gender being associated with elevated CAC scores (Table 6). It should be noted that although HOMA-IR remained in the final model, it was not statistically significant ($P = .054$). The Nagelkerke R^2 was 0.59 and accounted for almost as much variance as the model above.

DISCUSSION

In this study, we investigated whether serum bone biomarkers (i.e., OPG, OC, and ucOC) are associated with arterial calcification in the coronary arteries in persons with type 2 diabetes who did not have known CAD or a history of ischemic cardiac events. The results of this study demonstrate that serum OPG levels, not OC or ucOC levels, are associated with the presence of abnormal CAC scores. Our results support the results of an earlier study (8) in which increased serum OPG levels were associated with elevated CAC scores in asymptomatic individuals with type 2 diabetes. Our results are in agreement with several other studies that have shown an increase in serum OPG in association with vascular injury assessed by various methods in patients with and without type 2 diabetes (7, 26–28). The mechanisms for the increased serum OPG levels are, however, not clear.

OPG is widely expressed in human tissues, including bone and the vasculature (7). OPG is a regulator of bone resorption and prevents osteoclast differentiation. Growing evidence suggests that OPG/RANKL proteins may be important in vascular calcification (6). Insulin resistance is a key feature of type 2 diabetes. It is possible that elevated serum OPG may reflect this underlying condition. Indeed, one study involving a group of newly diagnosed individuals with diabetes and normal controls found that OPG and HOMA-IR are positively correlated (29). Our results, however, in a group of persons with type 2 diabetes with a longer duration of diabetes were in contrast to these findings but in agreement with another study of individuals with abnormal glucose tolerance in which no association between HOMA-IR and OPG was found (30). It is also possible that poor glycemic control may affect OPG levels. This was suggested in a study of 166 individuals with type 2 diabetes showing that those with higher HbA_{1c} levels have increased OPG concentrations (31). Our results are in contrast, not revealing an association between OPG and glycemic control. Our

results suggest that the elevation in serum OPG in our study cohort was due to factors other than insulin resistance and glycemic control. Guzel et al (5) suggested that increased OPG levels may function as a compensatory mechanism to limit vascular damage and to keep the activation of inflammatory pathways in check. Thus, it is possible that a rise in serum OPG levels may reflect silent vascular calcification, with OPG's compensatory process occurring in a self-defensive response to the progression of disease. Clearly, additional investigations are needed to determine the mechanism responsible for increased serum OPG levels, but it appears that serum OPG is a promising biomarker to identify patients at risk.

In vitro studies have shown that OC is a continuous inhibitor of calcification in the vessel wall (11). Studies of patients with and without CAD have revealed conflicting results. Two studies (12, 32) showed that OC is lower in those with CAD, with decreasing serum OC associated with an increasing number of diseased vessels. In contrast, Pongvarin et al (33) found no difference in OC between controls and those with CAD. Our results are in more agreement with the latter study (33), as we failed to show an association between OC and abnormal CAC scores. Potential mechanisms that have been hypothesized to explain an underlying association between OC and CAD involve beneficial effects of OC on glucose metabolism via enhancement of insulin sensitivity (32). Recent clinical studies in humans have demonstrated some conflicting associations between OC and glucose metabolism and insulin resistance. For example, using multiple regression analysis adjusted for age, BMI, and creatinine, Kanazawa et al (34) showed that OC levels are negatively associated with fasting glucose, HbA^{1c}, and HOMA-IR in persons with type 2 diabetes. Likewise, Zhang et al (12) found an inverse relationship between OC and fasting glucose and HbA^{1c} after adjusting for CAD status. These results would seem to indicate that individuals with poorer glycemic control are more likely to have lower OC levels. Diaz-Lopez et al (18), studying a group of individuals newly diagnosed with diabetes, showed that OC concentrations are inversely associated with HOMA-IR after adjustment for gender, age, BMI, and other confounding factors. Wang et al (19), however, showed conflicting results in 66 cases with type 2 diabetes. Although OC was inversely correlated with HbA^{1c} in that study, the authors found no relationship between OC and HOMA-IR. Likewise, Gower et al (35) did not show an association between OC and HOMA-IR in 63 overweight/obese adults. These investigators did, however, observe a positive association between OC and insulin sensitivity as assessed with an intravenous glucose tolerance test. These observations suggest that the potential effect of OC on insulin sensitivity may be associated with skeletal muscle and not the liver. It is tempting to speculate that OC's function in glucose metabolism may be a more localized (rather than systemic) effect for mechanical support of new bone formation via OC's role in bone growth. Nonetheless, the results of the current study failed to show any associations between OC and measures of glucose metabolism or CAC.

Studies that have examined the association between ucOC levels and arterial disease are limited. Okura et al (10) showed that in patients with essential hypertension, circulating levels of ucOC are higher for patients with carotid calcification compared to those without calcification. In addition, logistic regression analysis showed that ucOC is an independent determinant, suggesting that ucOC is a circulating biomarker of carotid artery calcification (10). To the best of our knowledge, our study is the first to address ucOC and CAC. We did not, however, find any significant difference in ucOC levels between those with and without

abnormal CAC scores. Results from animal studies initially suggested that ucOC is the isoform that mediates metabolic functions (16). Studies in humans have produced conflicting results. For example, Iki et al (36) showed that ucOC is inversely associated with HOMA-IR, fasting glucose, and HbA^{1c} in elderly Japanese men with and without type 2 diabetes. In contrast, Mori et al (37) found no association between ucOC and insulin resistance in patients with type 2 diabetes, where insulin resistance was assessed using the euglycemic hyperinsulinemic clamp technique. Likewise, in this study, we did not find any association with ucOC in terms of insulin resistance or glycemic control. Clearly, additional research is needed to determine the isoform of OC that is associated with indices of glucose metabolism in humans.

We also investigated two other potential surrogate markers (leptin and adiponectin) of coronary calcification, as these biomarkers of adipose function may add value to predicting vascular disease and due to their relationship with bone biomarkers. Adiponectin appears to exert cardioprotective effects with low levels of adiponectin shown to be associated with elevated CAC scores (38). We also demonstrated that individuals with elevated CAC scores had lower adiponectin levels. However, in logistic analysis, adiponectin was not an independent determinant of CAC scores. The association between leptin and CAC has produced conflicting reports (39, 40). Although our results do not support an independent association between leptin or adiponectin and vascular calcification, it is possible that the relationship between these adipocytes and CAC is driven by covariation with other related risk factors.

Our study has several strengths, including simultaneous assessment of OPG, OC, and ucOC in the same cohort along with the measurement of adipokines. This study also has some potential limitations that deserve mention. This was a cross-sectional study and thus cause-effect relationships cannot be determined. It is known that with increasing age, the risk of having calcium deposits in the coronary arteries increases. This was confirmed in this study via a positive correlation between age and CAC scores as a continuous variable. When CAC scores were dichotomized to form two groups, age was not significantly different between the groups, and the final backward stepwise logistic regression model selected longer duration of diabetes and higher systolic BP as significant effects. It should be noted, however, that both effects coincide with increasing age. In addition, other investigators (7) showed that age is not significantly associated with CAD in multivariate logistic regression analyses in which OPG levels were evaluated. We used HOMA-IR as a surrogate measure of insulin resistance. Although HOMA-IR is not the gold standard, it is a clinically useful index used in many studies. We measured only intact molecules of OC, levels of which would be lower than total OC levels. Our study did not include a control group (i.e., nondiabetic individuals of a similar age range), nor did we have normal reference ranges from our local population for the serum bone biomarkers. It should be noted, however, that our results for these biomarkers appear comparable to other studies that have included small control groups (5, 18). Some participants may have been using medications that could have influenced coronary calcification and/or serum bone biomarkers. It should be noted, however, that there were no dose changes for antihypertensive and antidiabetes medications 2 months prior to enrollment. No invasive measures to determine the presence/absence or

extent of atherosclerotic disease were used in this study, but the determination of CAC scores does provide a valid marker of atherosclerotic burden.

CONCLUSION

In summary, the present study measured 3 serum biomarkers of bone metabolism in patients with type 2 diabetes. We confirm that in patients with abnormal CAC scores, elevated serum OPG levels are present. Because cardiovascular disease morbidity and mortality are high in persons with type 2 diabetes, it is important to identify biochemical markers of increased risk. The identification of such biomarkers could be useful in the clinical setting for determination of subclinical cardiovascular disease. This would permit the development of strategies designed to help in the reduction of cardiovascular events. Further studies will be required, however, to determine exactly what information elevated serum OPG levels are indicating with regard to underlying changes that are occurring in the vascular wall.

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Abbreviations

BMI	body mass index
BP	blood pressure
CAC	coronary artery calcification
CAD	coronary artery disease
HbA^{1c}	glycated hemoglobin
HOMA-IR	homeostasis model assessment for insulin resistance
OC	osteocalcin
OPG	osteoprotegerin
RANKL	receptor activator of nuclear factor- κ B ligand
ucOC	undercarboxylated osteocalcin

Table 1Participant Demographics and Metabolic Parameters^a

	CAC scores <10 (n = 18)	CAC scores ≥ 10 (n = 32)	P value
Age (years)	60 ± 13	65 ± 7	.134
Duration (years)	11 ± 6	14 ± 9	.284
Male/female (n)	5/13	16/16	.149
Systolic blood pressure (mm Hg)	121 ± 12	127 ± 14	.082
Diastolic blood pressure (mm Hg)	73 ± 6	74 ± 8	.877
HbA _{1c} (%)	7.4 ± 1.6	7.2 ± 0.9	--
Ln HbA _{1c}	1.99 ± 0.19	1.97 ± 0.12	.944
Body mass index (kg/m ²)	32.8 ± 6.4	32.6 ± 4.7	.912
HOMA-IR	1.8 ± 0.8	2.3 ± 1.3	--
Ln HOMA-IR	0.48 ± 0.47	0.65 ± 0.62	.319
Leptin (ng/mL)	28.6 ± 20.3	19.6 ± 14.5	--
Ln leptin	3.0 ± 0.88	2.7 ± 0.77	.167
Total adiponectin (mg/L)	8.6 ± 6.8	6.0 ± 4.3	--
Ln total adiponectin	1.97 ± 0.56	1.62 ± 0.59	.042
25-hydroxyvitamin D (ng/mL)	29.9 ± 11.5	30.0 ± 10.6	.979
Parathyroid hormone (pg/mL)	40.4 ± 17.6	33.6 ± 13.9	.137
Calcium (mg/dL)	9.5 ± 0.5	9.5 ± 0.4	.808
Serum creatinine (mg/dL)	0.81 ± 0.21	0.89 ± 0.27	.290
Osteoprotegerin (pmol/L)	4.2 ± 1.7	5.5 ± 2.0	.026
Osteocalcin (ng/mL)	7.2 ± 3.0	7.2 ± 2.2	.929
Undercarboxylated osteocalcin (ng/mL)	3.5 ± 2.2	6.4 ± 5.9	--
Ln undercarboxylated osteocalcin	0.99 ± 0.77	1.4 ± 1.0	.128

Abbreviations: CAC = coronary artery calcification; HbA_{1c} = glycated hemoglobin; HOMA-IR = homeostasis model assessment for insulin resistance; Ln = natural logarithmic transformation.

^aData are presented as mean ± SD.

Table 2

Correlation Analyses

Variable	CAC scores r_s^a	OPG r^b	Osteocalcin r	Ln ucOC r
Age (years)	0.29 ^c	0.22	0.12	-0.15
Duration (years)	0.10	0.06	-0.05	-0.13
Gender	-0.28 ^c	0.40 ^d	0.19	-0.07
Systolic blood pressure (mm Hg)	0.10	0.25	0.22	0.07
Diastolic blood pressure (mm Hg)	0.01	-0.02	0.15	0.37 ^d
HbA _{1c} (%)	0.02	-0.07	-0.14	-0.21
Body mass index (kg/m ²)	-0.01	0.02	-0.13	0.09
HOMA-IR	0.24	-0.10	0.06	-0.09
Leptin (ng/mL)	-0.11	0.30 ^c	0.05	0.04
Total adiponectin (mg/L)	-0.29 ^c	0.13	0.09	0.04
25-hydroxyvitamin D (ng/mL)	-0.02	0.09	-0.25	-0.05
Parathyroid hormone (pg/mL)	-0.02	0.17	-0.02	-0.11
Calcium (mg/dL)	0.01	0.33 ^c	-0.06	0.03
Serum creatinine (mg/dL)	0.15	-0.24	-0.08	0.10
Osteocalcin (ng/mL)	-0.01	0.13	--	--
ucOC (ng/mL)	0.15	0.00	0.13	--
OPG (pmol/L)	0.38 ^d	--	--	--

Abbreviations: CAC = coronary artery calcification; HbA_{1c} = glycated hemoglobin; HOMA-IR = homeostasis model assessment for insulin resistance; OPG = osteoprotegerin; r = Pearson correlation coefficient; r_s = Spearman rank correlation coefficient; ucOC = undercarboxylated osteocalcin.

^a Presented for continuous CAC scores that were not normally distributed.

^b Presented for OPG, osteocalcin, and the natural logarithmic transformation of undercarboxylated osteocalcin.

^c $P < .05$.

^d $P < .01$.

Table 3Serum Bone Biomarkers by Glycemic Control Level^a

Measure	HbA _{1c} <7 % (n = 24)	HbA _{1c} 7% (n = 26)	P value
Osteoprotegerin (pmol/L)	5.3 ± 2.3	4.7 ± 1.6	.284
Osteocalcin (ng/mL)	7.7 ± 3.0	6.8 ± 1.9	.208
Undercarboxylated osteocalcin (ng/mL)	6.9 ± 6.2	3.8 ± 3.2	--
Ln undercarboxylated osteocalcin	1.5 ± 1.0	1.0 ± 0.8	.072

Abbreviations: HbA_{1c} = glycated hemoglobin; Ln = natural logarithmic transformation.^aData are presented as mean ± SD.

Table 4Serum Bone Biomarkers by Insulin Resistance^a

Measure	HOMA-IR 1.8 (n = 27)	HOMA-IR >1.8 (n = 23)	P value
Osteoprotegerin (pmol/L)	5.1 ± 1.9	4.8 ± 2.1	.608
Osteocalcin (ng/mL)	7.3 ± 2.6	7.1 ± 2.4	.698
Undercarboxylated osteocalcin (ng/mL)	5.6 ± 5.8	5.0 ± 4.1	--
Ln undercarboxylated osteocalcin	1.3 ± 1.0	1.2 ± 0.9	.870

Abbreviations: HOMA-IR = homeostasis model assessment for insulin resistance; Ln = natural logarithmic transformation.

^aData are presented as mean ± SD.

Table 5

Full Logistic Regression Model with CAC Scores as the Dependent Variable

Parameter	Odds Ratio	95% Confidence Interval	P value
Osteoprotegerin (pmol/L)	3.324	1.321–8.359	.011
Duration of diabetes (years)	1.175	1.020–1.355	.026
Age (years)	1.083	0.951–1.234	.230
Systolic blood pressure (mm Hg)	1.109	0.974–1.262	.120
Gender	0.082	0.003–2.614	.157
Body mass index (kg/m ²)	1.159	0.858–1.565	.337
HOMA-IR	3.056	0.681–13.726	.145
Leptin (ng/mL)	0.919	0.831–1.016	.097
Adiponectin (mg/L)	0.990	0.781–1.254	.931
HbA _{1c} (%)	0.741	0.283–1.943	.542

Abbreviations: CAC = coronary artery calcification; HbA_{1c} = glycated hemoglobin; HOMA-IR = homeostasis model assessment for insulin resistance.

Table 6

Final Backward Selected Logistic Regression Model With CAC Score as the Dependent Variable

Parameter	Odds Ratio	95% Confidence Interval	P value
Osteoprotegerin (pmol/L)	2.823	1.356–5.878	.006
Duration of diabetes (years)	1.157	1.021–1.311	.022
Systolic blood pressure (mm Hg)	1.125	1.016–1.245	.024
Gender	0.021	0.001–0.297	.004
HOMA-IR	2.903	0.982–8.582	.054

Abbreviations: CAC = coronary artery calcification; HOMA-IR = homeostasis model assessment for insulin resistance.