Low Serum Osteocalcin Level as a Risk Factor for Metabolic Syndrome in Korean Men: A Retrospective Longitudinal Study

Jin-Sook Moon (✉ s2moon@naver.com)  
Samsung Changwon Hospital

Mi Hyeon Jin  
Samsung Changwon Hospital

Research Article

Keywords: Osteocalcin, Metabolic Syndrome, Longitudinal study

Posted Date: October 20th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-965007/v1

License: ☒  This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

Background: Recent studies have suggested that low serum osteocalcin levels are associated with metabolic syndrome (MetS). However, the association between serum osteocalcin levels and the incidence of MetS remains unclear. This aim of this study was to investigate the relationship between serum osteocalcin levels and MetS development in Korean men.

Methods: This retrospective study included 2,837 Korean men who were not diagnosed with MetS and recruited from the Health Promotion Center from January 2003 through December 2018. They were followed up at the center until the participant was newly diagnosed with MetS or until the last follow-up visit if the participant was not diagnosed with MetS. Serum osteocalcin levels were measured using an electrochemiluminescence immunoassay. The participants were divided into quartiles (Q1–Q4) based on their serum osteocalcin levels. Cox proportional hazard models were used to test the independent association of serum osteocalcin levels with the development of MetS, and hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated.

Results: In the baseline analysis, blood pressure, body mass index, waist circumference, waist-hip ratio, body fat mass, fasting glucose, hemoglobin A1c ($P_{\text{for trend}} < 0.001$ for all), total cholesterol ($P_{\text{for trend}} = 0.026$), triglyceride ($P_{\text{for trend}} = 0.003$), and low-density lipoprotein cholesterol ($P_{\text{for trend}} = 0.031$) varied inversely with the osteocalcin quartile. In addition, the prevalence of abdominal obesity ($P_{\text{for trend}} = 0.001$), high blood pressure ($P_{\text{for trend}} = 0.008$), and hyperglycemia ($P_{\text{for trend}} < 0.001$) decreased as the osteocalcin quartile increased. After a mean follow-up time of 2.76 years, MetS occurred in 518 participants (18.26%), and baseline serum osteocalcin levels were found to be inversely associated with the incident risk of MetS ($P_{\text{for trend}} < 0.001$ across quartiles of osteocalcin levels). In a fully adjusted model, HR for MetS in the first quartile (the lowest osteocalcin concentration) was significantly higher than that for MetS in the fourth quartile (HR, 1.40; 95% CI, 1.06–1.84).

Conclusion: Low serum osteocalcin levels at baseline were associated with unfavorable metabolic profiles and a higher risk of incident MetS in Korean men.

Background

Metabolic syndrome (MetS) is characterized by a cluster of metabolic disturbances comprising central obesity, insulin resistance, atherogenic dyslipidemia, and high blood pressure, which appear to increase the risk of cardiovascular disease, diabetes mellitus and overall mortality [1]. The prevalence of MetS varies depending on the definition used, sex, race, and age, and an estimated one-quarter to one-third of adults with multiple ethnic backgrounds meet the MetS criteria [1]. A cross-sectional study based on the National Health and Nutrition Examination Survey in the United States and conducted from 2003 to 2012 showed that the overall prevalence of MetS was 33% [2]. According to the the Korean National Health Insurance Service in South Korea, the age-adjusted prevalence of MetS from 2009 to 2013 was 30.52% [3]. In most countries in the Asia-Pacific, $\geq 20\%$ of the adult population is affected by MetS, with an
observed increase in prevalence [4]. In fact, the prevalence of MetS has been increasing worldwide, and it has recently become a major public health issue with serious health and economic implications.

Osteocalcin is a well-known bone turnover marker that is clinically used to assess the response to anabolic and antiresorptive therapies for osteoporosis because it reflects bone formation with the N-terminal propeptide of type I procollagen [5]. In 2007, it was discovered that osteocalcin secreted from bone acts as a hormone that regulates energy metabolism; this provided evidence for a new function of bone [6]. The first evidence supporting regulation of energy metabolism by osteocalcin was provided through the investigation of osteocalcin-deficient mice [6]. Osteocalcin knockout (KO) mice exhibited a higher blood glucose level, lesser insulin secretion, higher insulin resistance, and greater fat pad mass and serum triglyceride levels than did with wild-type mice [6]. In agreement with these animal models, emerging human evidence has demonstrated that serum osteocalcin levels are associated with blood glucose, fat mass, and MetS [7–10]. Zhou et al. [7] showed that the serum total osteocalcin level is inversely associated with glucose levels and adipocytes in the Chinese population. Kindblom et al. [8] demonstrated that serum osteocalcin is negatively related to fat mass and plasma glucose in elderly men without diabetes. In addition, osteocalcin levels reportedly showed an inverse correlation with MetS in postmenopausal women [9] and men [10]. In a meta-analysis, low osteocalcin levels were consistently associated with the presence of type 2 diabetes and MetS [11].

Although serum osteocalcin is not one of the diagnostic criteria for MetS, it is considered to be correlated with MetS. However, almost all previous studies have involved cross-sectional analyses, and the relationship between osteocalcin levels and the risk of incident MetS remains unclear. Therefore, the aim of this study was to determine the association between serum osteocalcin levels and the development of MetS in Korean men.

Methods

1. Study population

This retrospective, longitudinal study included 44,502 Korean men whose total (D2 and D3) vitamin D (Vit D) levels were measured at the Health Promotion Center, Changwon from January 2003 through December 2018. A flow chart of the study population is shown in Figure 1. All the participants visited the center for a medical health check-up and were interviewed and examined by clinicians. A total of 41,665 men were excluded from the analysis because they were already diagnosed with MetS at baseline (6,829), lacked data at baseline and/or during follow-up (n=29,880), visited the hospital just once (n=12,671), or had an osteocalcin level of > 100 ng/ml (detection limit of the assay kit) (n=4). Participants with the following conditions were also excluded at baseline and/or during the follow-up period: history of malignancy (n=528); coronary artery disease (n=361); stroke (n=230); thyroid disease (n=519); chronic kidney disease (n=16); liver disease (n=1,295); use of medications for thyroid disease (n=1,352); use of medications for osteoporosis (n=1,099); use of steroids (n=8,510); use of sex hormones (n=724); use of anticoagulants (n=8,504); use of anticonvulsants (n=19); use of health supplements,
including vitamins (n=7,254); and abnormal results in liver function (n=1,056), thyroid function (n=546), or renal function (n=31) tests. Finally, 2,837 participants were enrolled. Each participant was monitored until he was newly diagnosed with MetS or until the last follow-up visit (if not diagnosed with MetS).

2. Baseline and follow-up measurements

1) Clinical and anthropometric evaluation

Information on habitual smoking and alcohol consumption and any history of medical and/or surgical disease was obtained via a self-reported questionnaire at baseline. Alcohol consumption was defined as the consumption of a cup or more of alcohol more than once a week in the past year, regardless of the type of alcohol [12, 13]. With regard to the smoking status, participants were classified as current smokers or non-current smokers. A current smoker was defined as an individual who had smoked at least 100 cigarettes in his lifetime and currently smoked cigarettes [14]. Height and weight were measured using an automatic stadiometer (BSM 370, Biospace Co., Ltd., Seoul, Korea). Body mass index (BMI) was calculated as weight divided by height in square meters (kg/m²). The percent body fat (PBF) and skeletal muscle mass (SMM) were measured using bioelectrical impedance analysis (InBody 3.0; Biospace, Seoul, Korea), while the waist circumference (WC) was measured using a standard protocol. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using an automatic sphygmomanometer (EASY X 800, Jawon medical Co., Ltd, Seoul, Korea) in a sitting position after 10 min of seated rest.

2) Biochemical analyses

Blood samples were collected after overnight fasting. Fasting plasma glucose (FPG) concentrations were measured using a glucose hexokinase in vitro diagnostic assay (Glucose HK gen.3, Roche Diagnostics, Mannheim, Germany) with a Roche-Hitachi Cobas 8000 c702 analyzer (Roche Diagnostics, Mannheim, Germany). Hemoglobin A1c (HbA1c) levels were analyzed by high-performance liquid chromatography using a Tosoh HLC-723 G8 (Tosoh Co., Tokyo, Japan). Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), serum high-sensitivity C-reactive protein (hs-CRP), and alkaline phosphatase (ALP) levels were measured using enzymatic procedures with Cobas reagents and analyzed with a Cobas 8000 c702 analyzer (Roche Diagnostics, Mannheim, Germany). Serum creatinine (Cr), uric acid (UA), calcium (Ca), phosphate (P), total Vit D, and insulin levels were also measured. Serum total osteocalcin levels were measured using an electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany), and the same detection method was maintained during the study period. Insulin resistance was estimated using the homeostatic model assessment of insulin resistance (HOMA-IR) index, calculated as follows: HOMA-IR = fasting serum insulin (mU/L) × FPG (mg/dL)/405 [15].

3. Definition of MetS
The diagnosis of MetS was based on three or more criteria as follows: (1) WC ≥ 90 cm for men (in accordance with the International Obesity Task Force criteria for the Asian-Pacific population), (2) SBP and/or DBP ≥ 130/85 mmHg or the use of antihypertensive medications, (3) FPG ≥ 100 mg/dL or the use of antidiabetic agents or insulin, (4) serum TG levels ≥ 150 mg/dL or the use of associated medication, and (5) HDL-C levels < 40 mg/dL for men or the use of associated medications, in agreement with the definition established by the modified National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) [16].

4. Statistical analyses

All statistical analyses were performed using Stata 14.0 (Stata Corporation, College Station, TX, USA). Statistical tests were two-tailed, and \( P < 0.05 \) was considered to indicate statistical significance. Baseline characteristics are expressed as the mean and standard deviation or median (interquartile range quartile 1 [Q1] to quartile 4 [Q4]). Categorical variables are reported as numbers \( (n) \) and percentages (%). One-way analysis of variance (ANOVA) was used to compare mean values among the osteocalcin quartiles. Trend analysis was performed to describe the linear component of the trend from the Q1 to Q4 groups of serum osteocalcin levels. Pearson's correlation test and partial correlation coefficients were used to evaluate the associations between osteocalcin and clinical and laboratory parameters. The incidence rate of MetS was estimated as the number of incidences divided by the total person-years. Kaplan–Meier curves were plotted to describe the incidence of MetS, and the log-rank test was used to examine the significance of differences among groups. Cox proportional hazards analysis was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for the incidence of MetS according to the osteocalcin quartile (the fourth quartile was used as the reference). In multivariable Cox proportional hazards analysis, independent variables included age, components of MetS (five elements included in the diagnostic criteria: BP, WC, FPG, TG, and HDL-C) and their closely related parameters (waist-hip ratio [WHR] and HbA1c), and a current smoking status and alcohol consumption (Model 1). In Model 1, confounding factors were selected to consider the effects of MetS components already presented at baseline. This was further adjusted for BMI, SMM, ALP, Vit D, Ca, P, Cr, hs-CRP, and UA in Model 2. These confounders were selected as parameters that were significantly correlated with osteocalcin levels in the partial correlation analysis after adjusting for age (SBP, DBP, BMI, WC, WHR, PBF, FPG, Hba1c, TC, TG, LDL-C, ALP, P, Cr, and hs-CRP). The additional factors (SMM [17], Vit D [18], and UA [19, 20]) that were not significantly correlated with osteocalcin levels in the current study; however, they had shown relevance in term of osteocalcin and/or MetS in previous studies. To avoid multicollinearity, the variance inflation factor (VIF) was calculated for the independent predictors. A VIF of <10 was considered optimal to ensure stability, and WC, WHR, and PBF were excluded from the analysis because of their VIF of ≥ 10.

Results

1. Baseline characteristics and risk of incident MetS
A total of 2,837 participants with a mean age of 44.6 ± 5.8 years were included. The participants were categorized into quartiles based on their serum osteocalcin levels at baseline. The baseline characteristics of the study participants within each osteocalcin quartile are shown in Table 1 (see at the end of the manuscript). Higher serum osteocalcin levels were associated with favorable metabolic profiles. Age, SBP, DBP, BMI, WC, WHR, PBF, FPG, HbA1c ($P$ for trend < 0.001 for all), TC ($P$ for trend = 0.026), TG ($P$ for trend = 0.003), LDL-C ($P$ for trend = 0.031), and hs-CRP ($P$ for trend = 0.001) varied inversely with the osteocalcin quartiles. However, no difference was observed in SMM, HOMA-IR, HDL-C, Vit D, and UA across the different quartiles. In line with the results in Table 1, the correlation analysis showed similar results (Table 2). As expected, serum osteocalcin levels significantly correlated with ALP, which is a bone formation marker expressed in osteoblasts [21].
Table 1
Baseline characteristics according to the quartiles of the serum osteocalcin level

| Variables         | Q1 (n=710) | Q2 (n=709) | Q3 (n=712) | Q4 (n=706) | P for trend |
|-------------------|------------|------------|------------|------------|------------|
| Age (years)       | 45.24±5.80 | 44.89±5.90 | 44.32±5.64 | 44.03±5.74 | <0.001     |

**Anthropometric parameters**

| Variables     | Q1          | Q2          | Q3          | Q4          | P for trend |
|---------------|-------------|-------------|-------------|-------------|-------------|
| SBP (mmHg)    | 124.51±15.00| 123.59±15.56| 121.95±14.64| 121.18±15.93| <0.001      |
| DBP (mmHg)    | 74.22±10.77 | 73.35±11.20 | 71.99±10.67 | 71.41±11.55 | <0.001      |
| BMI (kg/m²)   | 24.24±2.69  | 24.05±2.65  | 23.68±2.44  | 23.32±2.89  | <0.001      |
| WC (cm)       | 84.05±6.91  | 86.64±6.67  | 82.95±6.39  | 81.82±6.87  | <0.001      |
| WHR           | 0.88±0.03   | 0.88±0.03   | 0.88±0.03   | 0.87±0.04   | <0.001      |
| PBF (%)       | 22.56±5.12  | 21.88±5.00  | 21.15±4.90  | 20.56±5.16  | <0.001      |
| SMM (kg)      | 30.83±3.56  | 31.03±3.46  | 31.07±3.49  | 30.70±3.72  | 0.569       |

**Laboratory parameters**

| Variables      | Q1            | Q2            | Q3            | Q4            | P for trend |
|----------------|---------------|---------------|---------------|---------------|-------------|
| Osteocalcin (ng/mL) | 10.77±1.57 | 14.09±0.78 | 16.96±0.95 | 22.43±3.60 | <0.001      |
| FPG (mg/dL)    | 92.32±18.88  | 90.63±12.53  | 88.96±9.41  | 88.23±9.13  | <0.001      |
| HbA1c (%)      | 5.58±0.74    | 5.49±050     | 5.44±0.38    | 5.43±0.38    | <0.001      |
| HOMA-IR        | 0.98±0.75    | 1.05±0.63    | 1.02±0.65    | 1.05±0.75    | 0.267       |
| TC (mg/dL)     | 202.38±34.61 | 200.41±33.57 | 199.43±34.47 | 198.54±30.77 | 0.026       |
| TG (mg/dL)     | 122.61±70.82 | 123.69±72.94 | 118.94±67.80 | 112.66±63.24 | 0.003       |
| HDL-C (mg/dL)  | 56.79±13.37  | 55.85±13.52  | 55.72±12.79  | 57.39±13.83  | 0.461       |
| LDL-C (mg/dL)  | 133.88±32.85 | 132.74±32.22 | 131.91±31.77 | 130.34±29.07 | 0.031       |
| ALP (IU/L)     | 66.64±15.07  | 69.48±15.77  | 73.73±16.30  | 78.79±17.80  | <0.001      |
| Vit D (ng/mL)  | 19.89±7.82   | 19.34±7.62   | 19.21±7.60   | 19.39±7.86   | 0.226       |
| Ca (mg/dL)     | 9.60±0.44    | 9.63±0.36    | 9.64±0.34    | 9.64±0.39    | 0.019       |
| P (m/dL)       | 3.31±0.44    | 3.34±0.44    | 3.35±0.43    | 3.42±0.40    | <0.001      |
| Cr (mg/dL)     | 0.96±0.13    | 0.97±0.13    | 0.99±0.12    | 1.00±0.13    | <0.001      |
| hs-CRP (mg/L)  | 1.46±4.77    | 1.09±2.95    | 1.02±1.86    | 0.92±2.07    | 0.001       |
| UA (mg/dL)     | 5.89±1.27    | 5.94±1.18    | 5.91±1.19    | 5.99±1.30    | 0.192       |

**Health habits**
| Variables                          | Q1 (n=710) | Q2 (n=709) | Q3 (n=712) | Q4 (n=706) | P for trend |
|-----------------------------------|------------|------------|------------|------------|-------------|
| Current smoking [n (%)]           | 300 (47.62)| 282 (45.05)| 300 (45.39)| 279 (43.06)| 0.440       |
| Alcohol drinking [n (%)]          | 643 (90.56)| 632 (89.14)| 662 (92.98)| 655 (92.78)| 0.027       |
| **Component of MetS criteria [n (%)]** |           |            |            |            |             |
| Abdominal obesity                 | 114 (16.06)| 106 (14.95)| 84 (11.80) | 69 (9.77)  | 0.001       |
| Hyper TG                          | 206 (29.01)| 227 (32.02)| 205 (28.79)| 183 (25.92)| 0.094       |
| Low HDL-C                         | 31 (4.37)  | 54 (7.62)  | 38 (5.34)  | 41 (5.81)  | 0.064       |
| High BP                           | 267 (37.61)| 241 (33.99)| 218 (30.62)| 212 (30.03)| 0.008       |
| Hyperglycemia                     | 101 (14.23)| 82 (11.57) | 58 (8.15)  | 56 (7.93)  | <0.001      |
| **Number of MetS components [n (%)]** |           |            |            |            |             |
| 0                                 | 214 (30.14)| 220 (31.03)| 270 (37.92)| 289 (40.93)| 0.004       |
| 1                                 | 273 (38.45)| 268 (37.80)| 281 (39.47)| 273 (38.67)| 0.820       |
| 2                                 | 223 (31.41)| 221 (31.17)| 161 (22.61)| 144 (20.40)| 0.027       |

Values are expressed as mean ± standard deviation or number (%). Linear regression was used for trend analysis between the quartiles.

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WC, waist circumference; WHR, waist-hip ratio; PBM, percent body fat; SMM, skeletal muscle mass; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostatic model assessment of insulin resistance; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALP, alkaline phosphatase; Vit D, total vitamin D; Ca, calcium; P, phosphate; Cr, creatinine; hs-CRP, high sensitivity C-reactive protein; UA, uric acid; MetS, metabolic syndrome; Current smoking—participants who had smoked at least 100 cigarettes in their lifetime and currently smoked cigarettes; Alcohol drinking—participants who had consumed a cup or more of alcohol, more than once a week in the past year, regardless of the type of alcohol; MetS components—elements included diagnostic criteria of MetS; Abdominal obesity—WC ≥ 90 cm; Hyper TG—serum TG levels ≥ 150 mg/dL or use of associated medication; Low HDL-C—HDL-C levels < 40 mg/dL or use of associated medications; High BP—SBP and/or DBP ≥ 130/85 mmHg or use of antihypertensive medications; Hyperglycemia—FPG ≥ 100 mg/dL or use of antidiabetic agents or insulin.
Table 2  
Correlations of serum osteocalcin with anthropometric and laboratory variables

| Variables     | Model 1 |       |       | Model 2 |       |       |
|---------------|---------|-------|-------|---------|-------|-------|
|               | r       | P-value | r     | P-value |
| Age (years)   | -0.081  | <0.001 | -0.093| <0.001  |
| SBP (mmHg)    | -0.090  | <0.001 | -0.096| <0.001  |
| DBP (mmHg)    | -0.096  | <0.001 | -0.096| <0.001  |
| BMI (kg/m²)   | -0.128  | <0.001 | -0.137| <0.001  |
| WC (cm)       | -0.123  | <0.001 | -0.139| <0.001  |
| WHR           | -0.132  | <0.001 | -0.142| <0.001  |
| PBF (%)       | -0.142  | <0.001 | -0.145| <0.001  |
| SMM (kg)      | -0.016  | <0.001 | -0.037| 0.052   |
| FPG (mg/dL)   | -0.130  | <0.001 | -0.124| <0.001  |
| HbA1c (%)     | -0.112  | <0.001 | -0.102| <0.001  |
| HOMA-IR       | 0.024   | 0.207  | 0.021 | 0.261   |
| TC (mg/dL)    | -0.051  | 0.007  | -0.053| 0.004   |
| TG (mg/dL)    | -0.049  | 0.009  | -0.050| 0.008   |
| HDL-C (mg/dL) | 0.008   | 0.679  | 0.011 | 0.572   |
| LDL-C (mg/dL) | -0.050  | 0.008  | -0.053| 0.005   |
| ALP (IU/L)    | 0.294   | <0.001 | 0.292 | <0.001  |
| Vit D (ng/mL) | -0.039  | 0.036  | -0.030| 0.107   |
| Ca (mg/dL)    | 0.034   | 0.074  | 0.022 | 0.245   |
| P (m/dL)      | 0.095   | <0.001 | 0.090 | <0.001  |
| Cr (mg/dL)    | 0.107   | <0.001 | 0.102 | <0.001  |
| hs-CRP (mg/L) | -0.077  | <0.001 | -0.081| <0.001  |
| UA (mg/dL)    | 0.025   | 0.185  | 0.015 | 0.436   |

Correlations were determined using Pearson's correlation coefficients and partial correlation coefficients.

Model 1: crude, Model 2: adjusted for age
Among MetS components, abdominal obesity as measured by WC ($P$ for trend = 0.001), high blood pressure ($P$ for trend = 0.008), and hyperglycemia ($P$ for trend < 0.001) showed significant difference prevalences between quartiles. Across the quartiles, the number and percentage of participants without any MetS component increased ($P$ for trend = 0.004), while the number and percentage of participants with two MetS components decreased ($P$ for trend = 0.027).

### 2. Relationship between baseline serum osteocalcin and development of MetS

Kaplan–Meier curves showing the probability of MetS-free survival during the follow-up period according to the osteocalcin quartiles are shown in Figure 2. When compared with the highest quartile (Q4, reference group), the Q2 and Q3 quartiles showed no difference with regard to the development of MetS, whereas the lowest quartile (Q1) showed a significantly increased incidence of MetS (log-rank test, $P$ < 0.001).

In total, 518 of the 2,837 men (18.26%) developed MetS during a mean follow-up period of 2.76 years. Table 3 shows HRs for MetS developed according to the osteocalcin quartile. Osteocalcin levels were inversely associated with the risk of MetS ($P$ for trend < 0.001). The lowest quartile (Q1) at baseline was associated with an increased risk of incident MetS. When the quartiles were compared, the risk of MetS development was higher in the first quartile (Q1) than in the fourth quartile (Q4) in the age-adjusted model (Model 1) (HR, 2.06; 95% CI, 1.62–2.63). HRs remained significant after further adjustments for a current smoking status and alcohol consumption (Model 2) (HR, 1.95; 95% CI, 1.52–2.51).
Table 3
Adjusted hazard ratios for the incidence of metabolic syndrome according to serum osteocalcin quartiles

| Osteocalcin | Person-years | Number of incidence | Incident rate/1000 person-years | Model 1 HR (95% CI) | P for trend | Model 2 HR (95% CI) | P for trend |
|-------------|--------------|---------------------|---------------------------------|---------------------|------------|---------------------|------------|
| Q1 1822.97  | 181          | 99.29 (85.83–114.86)| 2.06 (1.62–2.63)              | <0.001              | 1.95 (1.52–2.51)| <0.001              |
| Q2 1953.03  | 123          | 62.98 (52.78–75.15)| 1.25 (0.96–1.62)              |                     | 1.17 (0.89–1.54)|                     |
| Q3 1990.29  | 108          | 54.26 (44.94–65.53)| 1.06 (0.81–1.39)              |                     | 1.04 (0.79–1.37)|                     |
| Q4 2061.21  | 106          | 51.43 (42.51–62.21)| Reference                        |                     | Reference                        |           |

Model 1: adjusted for age
Model 2: adjusted as in Model 1, plus smoking status and alcohol consumption

HR, hazard ratio; CI, confidence interval; Q, quartile (Q1 was the lowest quartile)

Table 4 shows the results of multivariable Cox proportional hazards regression analysis after adjusting for age, MetS components (BP, WC, FPG, TG, and HDL-C), WHR, HbA1c, current smoking, and alcohol consumption (Model 1). Age (HR, 1.02; 95% CI, 1.01–1.04; P = 0.014), DBP (HR, 1.02; 95% CI, 1.01–1.04; P = 0.006), WC (HR, 1.13; 95% CI, 1.01–1.04; P < 0.001), FPG (HR, 1.02; 95% CI, 1.01–1.03; P < 0.001), TG (HR, 1.00; 95% CI, 1.00–1.00; P < 0.001), current smoking (HR, 1.44; 95% CI, 1.19–1.73; P < 0.001), and the lowest serum osteocalcin level (Q1) (HR, 1.39; 95% CI, 1.08–1.80; P = 0.011) independently predicted future development of MetS, while HDL-C was associated with a lower risk of MetS development (HR, 0.97; 95% CI, 0.96–0.98; P < 0.001). Model 2 shows the results after further adjustments for BMI, SMM, ALP, Vit D, Ca, P, Cr, hs-CRP, and UA. WC, WHR, and PBF were excluded to avoid multicollinearity (VIF ≥ 10). Current smoking (HR, 1.42; 95% CI, 1.17–1.71; P < 0.001) and the lowest osteocalcin level (Q1) (HR, 1.40; 95% CI, 1.06–1.84; P = 0.017) remained significant risk factors for the incidence of MetS. Age (HR, 1.03; 95% CI, 1.01–1.05; P = 0.008), DBP (HR, 1.03; 95% CI, 1.01–1.04; P = 0.002), BMI (HR, 1.22; 95% CI, 1.17–1.27; P < 0.001), SMM (HR, 1.06; 95% CI, 1.03–1.10; P < 0.001), FPG (HR, 1.02; 95% CI, 1.01–1.03; P < 0.001), TG (HR, 1.00; 95% CI, 1.00–1.00; P < 0.001), Ca (HR, 1.42; 95% CI, 1.07–1.89; P = 0.016), and UA (HR, 1.13; 95% CI, 1.05–1.22; P = 0.002) were also shown as independent risk factors for the development of MetS, and HDL-C (HR, 0.97; 95% CI, 0.96–0.98; P < 0.001) also remained a preventive factor in Model 2.
We found that low serum osteocalcin levels at baseline were significantly associated with an increased risk of incident MetS in the fully adjusted model.

Table 4
Multivariable Cox proportional hazard model for metabolic syndrome incidence, with further adjustment for confounders

| Variables               | Model 1          |          | Model 2          |          |
|-------------------------|------------------|----------|------------------|----------|
|                         | HR (95% CI)      | P-value  | HR (95% CI)      | P-value  |
| Age                     | 1.02 (1.01–1.04) | 0.014    | 1.03 (1.01–1.05) | 0.008    |
| SBP                     | 1.00 (0.99–1.01) | 0.571    | 1.00 (0.99–1.01) | 0.752    |
| DBP                     | 1.02 (1.01–1.04) | 0.006    | 1.03 (1.01–1.04) | 0.002    |
| BMI                     |                 |          | 1.22 (1.17–1.27) | <0.001   |
| WC                      | 1.13 (1.01–1.04) | <0.001   |                 |          |
| SMM                     |                 |          | 1.06 (1.03–1.10) | <0.001   |
| FPG                     | 1.02 (1.01–1.03) | <0.001   | 1.02 (1.01–1.03) | <0.001   |
| TG                      | 1.00 (1.00–1.00) | <0.001   | 1.00 (1.00–1.00) | <0.001   |
| HDL-C                   | 0.97 (0.96–0.98) | <0.001   | 0.97 (0.96–0.98) | <0.001   |
| Ca                      |                 |          | 1.42 (1.07–1.89) | 0.016    |
| UA                      | 1.13 (1.05–1.22) | 0.002    |                 |          |
| Current smoking         | 1.44 (1.19–1.73) | <0.001   | 1.42 (1.17–1.71) | <0.001   |
| Osteocalcin             |                 |          |                 |          |
| Q1                      | 1.39 (1.08–1.80) | 0.011    | 1.40 (1.06–1.84) | 0.017    |
| Q2                      | 0.97 (0.74–1.28) | 0.829    | 0.96 (0.72–1.27) | 0.773    |
| Q3                      | 1.04 (0.79–1.37) | 0.799    | 1.02 (0.77–1.35) | 0.885    |
| Q4                      | Reference        |          | Reference        |          |

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WC, waist circumference; SMM, skeletal muscle mass; FPG, fasting plasma glucose; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; Ca, calcium; UA, uric acid; Current smoking—participants who had smoked at least 100 cigarettes in their lifetime and currently smoked cigarettes; HR, hazard ratio; CI, confidence interval; Q, quartile (Q1 was the lowest quartile)

Model 1: adjusted for age, smoking status, alcohol consumption, BP, WC, FPG, TG, HDL-C, WHR and HbA1c

Model 2: adjusted as in model 1, plus BMI, SMM, ALP, Vit D, Ca, P, Cr, hs-CRP, and UA
At the time of MetS diagnosis, the most common combination of components was increased BP, TG, and FPG (26.64%), followed by increased WC, BP, and TG (18.53%) (Table 5).

| Component combination          | N (%) |
|-------------------------------|-------|
| WC + BP + TG                  | 96 (18.53) |
| WC + BP + HDL-C               | 5 (0.97) |
| WC + BP + FPG                 | 51 (9.85) |
| WC + TG + HDL-C               | 27 (5.21) |
| WC + TG + FPG                 | 38 (7.34) |
| WC + HDL-C + FPG              | 2 (0.39) |
| BP + TG + HDL-C               | 49 (9.46) |
| BP + TG + FPG                 | 138 (26.64) |
| BP + HDL-C + FPG              | 3 (0.58) |
| TG + HDL-C + FPG              | 25 (4.83) |
| WC + BP + TG + HDL-C          | 18 (3.47) |
| WC + BP + TG + FPG            | 44 (8.49) |
| WC + BP + HDL-C + FPG         | 2 (0.39) |
| WC + TG + HDL-C + FPG         | 2 (0.39) |
| BP + TG + HDL-C + FPG         | 10 (1.93) |
| WC + BP + TG + HDL-C + FPG    | 8 (1.54) |

Table 5
Component combinations for the newly developed MetS

Discussion

The results of this retrospective, longitudinal study showed that low serum osteocalcin levels at baseline were significantly related to worse metabolic profiles and independently related to an increased risk of MetS incidence in Korean men. After adjusting for conventional risk factors for MetS, such as PBF, BMI, and smoking status, as well as factors considered to be criteria for MetS, the serum osteocalcin level remained an independent protective factor against MetS. Therefore, a low serum osteocalcin level may be an indicator of increased risk of MetS in Korean men.
Experimental animal studies have showed that osteocalcin regulates glucose and fat metabolism. Lee et al. [6] showed that osteocalcin promotes the proliferation of pancreatic β cells, while increasing insulin and adiponectin secretion in adipocytes via osteocalcin-targeted KO mice. The KO mice exhibited higher blood glucose levels, greater fat pad mass, and higher serum TG levels than did the wild-type mice [6]. Consistent with the findings in mouse models, our study showed higher TG levels, PBF, and serum glucose levels in participants with low serum osteocalcin levels. FPG and HbA1c levels decreased progressively from the lower to upper quartiles of serum osteocalcin levels, consistent with previous findings in humans [11]. However, no differences were observed in HOMA-IR, which approximates insulin resistance, across the osteocalcin quartiles; this finding was similar to that in some previous studies [22, 23] and different from that in others studies [9, 24, 25]. Insulin resistance is a fundamental cause of diabetes and MetS. However, the relationship between osteocalcin levels and the incidence of diabetes is controversial. Studies showed that a low serum osteocalcin level was independently related to an increased risk of incident type 2 diabetes mellitus in Japanese postmenopausal women [26] and the Chinese population [27]. In a nested case-control study, low serum osteocalcin levels were associated with an increased risk of incident diabetes during a mean 5-year follow-up period [28]. An inverse association between the osteocalcin level and the change in FPG was found in a prospective analysis by Pittas et al. [29]. On the other hand, Hwang et al. [30] found that the serum osteocalcin level was not associated with the development of type 2 diabetes after a mean of 8.4 years in a retrospective cohort study, despite the presence of a positive association between serum osteocalcin and favorable metabolic parameters at baseline. Another prospective research showed that lower serum osteocalcin levels did not predict future development of diabetes [31]. Taken together, these results suggest that osteocalcin may not play a major role in the development of diabetes in humans.

The G protein-coupled receptor class C, group 6, subtype A (GPRC6A) is known as a receptor for osteocalcin and transduces the signal through the phospholipase C-inositol 1,4,5-trisphosphate-calcium$^{2+}$ pathway and adenylyl cyclase-cyclic adenosine monophosphate-protein kinase A pathway to promote the secretion of insulin and adiponectin and modulate insulin sensitivity [32]. However, recently, Jørgensen et al. found that full locus GPRC6A KO mice presented normal glucose metabolism that did not differ from that exhibited by wild-type mice; this was not consistent with the finding in previous KO mice models known as GPRC6A exon II KO and exon IV KO [33]. Furthermore, it was found that insulin signaling in murine osteoblasts enhanced the conversion of osteocalcin into the active, undercarboxylated form and regulated glucose homeostasis by signaling through GPRC6A in mice [34–36]. However, in another study, no significant changes were observed between mice with postnatal deletion of the insulin receptor in osteoprogenitor cells and control mice, although undercarboxylated osteocalcin levels were significantly decreased in the mutant mice [37]. Therefore, more studies are needed to clarify the molecular mechanism of action and functions of osteocalcin.

Considerable controversy has arisen over several findings that mouse models may not satisfactorily represent the human osteocalcin physiology [38]. The reasons for the variable metabolic phenotypes in terms of glucose and energy metabolism in different mouse models remain unclear [37]. However, the
involvement of serum osteocalcin in MetS has been largely studied through cross-sectional research in humans [10, 11, 24, 25, 39–41], which showed that serum osteocalcin levels were negatively associated with MetS. Although serum osteocalcin levels are affected by age, sex, menopausal status, and ethnicity [40], Saleem et al. [41] found an inverse association between serum osteocalcin and the presence of MetS in black and non-Hispanic white individuals. Yang et al. [39] investigated postmenopausal women in China and found similar results, which were also consistent with those in some studies involving Korean men and postmenopausal women [24] and older Caucasian men [10, 25]. Furthermore, a meta-analysis demonstrated significantly lower circulating levels of serum osteocalcin in participants with MetS than in those without MetS in 12 cross-sectional studies [11].

The benefits of exercise in terms of muscle energy metabolism and insulin sensitivity have been well documented [42]. Increases in undercarboxylated osteocalcin levels following aerobic exercise have been observed in animal models [43] and humans [44]. Recently, Rahimi et al. [45] found that exercise-induced elevation of undercarboxylated osteocalcin was a potential cause of increased adiponectin, which has insulin-sensitizing, anti-atherogenic and anti-inflammatory properties. Meanwhile, osteocalcin is synthesized in the presence of vitamin K and undergoes post-translational carboxylation in a vitamin K-dependent manner [46]. Although clinical trials of vitamin K supplementation have yielded mixed results [47], several studies have shown that vitamin K supplementation increased carboxylated osteocalcin [48] and decreased undercarboxylated osteocalcin, thus improving insulin sensitivity [49]. Although there is controversy regarding the form of osteocalcin that is functionally active in humans [27, 28, 49], exercise and vitamin K may act as regulators of glucose metabolism by influencing serum osteocalcin.

This retrospective follow-up study had some limitations. First, the participants were volunteers for a health check-up. The prevalence of MetS in our study was lower than that in the general population [3] because our participants might be particularly concerned about their health. Another limitation is women were excluded from in this study because female serum osteocalcin levels are affected by menopausal status (i.e., premenopause, perimenopause, and postmenopause) [21, 50]. In addition, our questionnaire did not permit obtaining detailed information about menstrual pattern changed that could have otherwise determined menopause status [51]. Furthermore, because this study only included men recruited from a single-center, the representative capacity of the results may be limited. Second, we did not consider dietary or exercise habits, which are well-known effective factors for the development or resolution of MetS. Third, whether or not undercarboxylated osteocalcin is the active form in humans remains unclear [27, 28, 52, 53]. Undercarboxylated osteocalcin alone has a biological effect in mice [6]. However, we did not differentiate serum osteocalcin with respect to the ë-carboxylation status and measured the total osteocalcin levels alone. However, to minimize the possibility of this effect, we excluded men who took vitamin K antagonists or multivitamin supplements including vitamin K, as carboxylation is a vitamin K-dependent process [6, 46]. Fourth, although we excluded participants who used medication for osteoporosis at baseline and/or during the follow-up period, we could not consider other bone conditions such as Paget’s disease and fracture. If these diseases are considered, the results may be different. Fifth, because the follow-up period was relatively short, we could not confirm the long-term effects of serum osteocalcin levels. Nevertheless, to our knowledge, this is one of the few studies investigating the
association between serum osteocalcin and the development of incident MetS in a relatively large sample.

**Conclusion**

MetS is not a disease by definition; rather, it is a predictor of cardiovascular disease risk. Therefore, a diagnosis of MetS could be expected to predict the risks and prevent diseases, particularly cardiovascular disease and diabetes [1]. In the current study, we found that low serum osteocalcin levels could predict the future development of MetS in Korean men. Therefore, men with a low serum osteocalcin level should pay more attention to lifestyle-related factors such as exercise, intake of green leafy vegetables that supply vitamin K, and smoking cessation (Table 4). Further prospective studies are needed to verify the optimal cutoff value of serum osteocalcin for predicting incident MetS in general populations.

**Abbreviations**

ALP, alkaline phosphatase; ALT, alanine aminotransferase; BMI, body mass index; Ca, calcium; CAD, coronary artery disease; CKD, chronic kidney disease; Cr, creatinine; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high sensitivity C-reactive protein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; MetS, metabolic syndrome; P, phosphate; PBM, percent body fat; SBP, systolic blood pressure; SMM, skeletal muscle mass; TC, total cholesterol; TFT, thyroid function test; TG, triglyceride; UA, uric acid; UNL, upper normal limit; Vit D, total vitamin D; WC, waist circumference; WHR, waist-hip ratio

**Declarations**

**Acknowledgements**

The authors would like to thank Dr. Yun-Jin Kim from Department of Family Medicine, Pusan National University Graduate School of Medicine for his advice and Editage (www.editage.co.kr) for its English language editing service.

**Authors’ contributions**

MJS contributed to study conceptualization and design, data collection and interpretation, and manuscript writing and editing. JMH participated in data collection and interpretation and performed statistical analysis. Both authors read and approved the final manuscript.

**Funding**

Not applicable.

**Availability of data and materials**

...
The datasets analyzed during the current study are available from the corresponding author on reasonable request.

**Ethic approval and informed consent**

This study was approved by the Institutional Review Board of Samsung Changwon Hospital, Changwon, Sungkyunkwan University (No: 2019-08-006-001). The requirement for informed consent from the participants was waived owing to the retrospective nature of the study.

**Consent for publication**

Not applicable.

**Competing interests**

The authors have no competing interests.

**References**

1. Samson SL, Garber AJ. Metabolic syndrome. Endocrinol Metab Clin North Am. 2014;43(1):1-23.
2. Aguilar M, Bhuket T, Torres S, Liu B, Wong RJ. Prevalence of the Metabolic Syndrome in the United States, 2003-2012. JAMA. 2015;313(19):1973-4.
3. Lee SE, Han K, Kang YM, Kim SO, Cho YK, Ko KS, et al. Trends in the prevalence of metabolic syndrome and its components in South Korea: Findings from the Korean National Health Insurance Service Database (2009-2013). PloS one. 2018;13(3):e0194490.
4. Park SY, Park YK, Cho KH, Choi HJ, Han JH, Han KD, et al. Normal range albuminuria and metabolic syndrome in South Korea: the 2011-2012 Korean National Health and Nutrition Examination Survey. PloS one. 2015;10(5):e0125615.
5. Eastell R, Szulc P. Use of bone turnover markers in postmenopausal osteoporosis. Lancet Diabetes Endocrinol. 2017;5(11):908-23.
6. Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, et al. Endocrine regulation of energy metabolism by the skeleton. Cell. 2007;130(3):456-69.
7. Zhou M, Ma X, Li H, Pan X, Tang J, Gao Y, et al. Serum osteocalcin concentrations in relation to glucose and lipid metabolism in Chinese individuals. Eur J Endocrinol. 2009;161(5):723-9.
8. Kindblom JM, Ohlsson C, Ljunggren Ö, Karlsson MK, Tivesten Å, Smith U, et al. Plasma Osteocalcin Is Inversely Related to Fat Mass and Plasma Glucose in Elderly Swedish Men. J Bone Miner Res. 2009;24(5):785-91.
9. Lee SW, Jo HH, Kim MR, Kim JH, You YO. Association between osteocalcin and metabolic syndrome in postmenopausal women. Arch Gynecol Obstet. 2015;292(3):673-81.
10. Confavreux CB, Szulc P, Casey R, Varennes A, Goudable J, Chapurlat RD. Lower serum osteocalcin is associated with more severe metabolic syndrome in elderly men from the MINOS cohort Eur J
11. Kunutsor SK, Apekey TA, Laukkanen JA. Association of serum total osteocalcin with type 2 diabetes and intermediate metabolic phenotypes: systematic review and meta-analysis of observational evidence. Eur J Epidemiol. 2015;30(8):599-614.

12. Lee SW, Jo HH, Kim MR, You YO, Kim JH. Association between obesity, metabolic risks and serum osteocalcin level in postmenopausal women. Gynecol Endocrinol. 2012;28(6):472-7.

13. Topiwala A, Allan CL, Valkanova V, Zsoldos E, Filippini N, Sexton C, et al. Moderate alcohol consumption as risk factor for adverse brain outcomes and cognitive decline: longitudinal cohort study. BMJ. 2017;367:j2353.

14. Cornelius ME, Wang TW, Jamal A, Loretan CG, Neff LJ. Tobacco Product Use Among Adults — United States, 2019. MMWR Morb Mortal Wkly Rep. 2020;69(46):1736-42.

15. Matthews DR, Hosker JP, Rudensi AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.

16. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120(16):1640-5.

17. Nomura K, Eto M, Ogawa S, Kojima T, Iijima K, Nakamura T, et al. Association between low muscle mass and metabolic syndrome in elderly Japanese women. PloS one. 2020;15(12):e0243242.

18. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of Calcium and Vitamin D Supplementation on Bone Density in Men and Women 65 Years of Age or Older. N Engl J Med. 1997;337(10):670-6.

19. De Pergola G, Giagulli VA, Bartolomeo N, Gaeta F, Petruzzella A, Guastamacchia E, et al. Independent Relationship between Serum Osteocalcin and Uric Acid in a Cohort of Apparently Healthy Obese Subjects. Endocr Metab Immune Disord Drug Targets. 2017;17(3):207-12.

20. Jeong J, Suh YJ. Association between Serum Uric Acid and Metabolic Syndrome in Koreans J Korean Med Sci. 2019;34(48):e307.

21. Atalay S, Elci A, Kayadibi H, Onder CB, Aka N. Diagnostic Utility of Osteocalcin, Undercarboxylated Osteocalcin, and Alkaline Phosphatase for Osteoporosis in Premenopausal and Postmenopausal Women. Ann Lab Med. 2012;32(1):23-30.

22. Giudici KV, Fisberg RM, Marchioni DML, Peters BSE, Martini LA. Crosstalk Between Bone and Fat Tissue: Associations Between Vitamin D, Osteocalcin, Adipokines, and Markers of Glucose Metabolism Among Adolescents. J Am Coll Nutr. 2017;36(4):273-80.

23. Ma XY, Chen FQ, Hong H, Lv XJ, Dong M, Wang QY. The Relationship between Serum Osteocalcin Concentration and Glucose and Lipid Metabolism in Patients with Type 2 Diabetes Mellitus - The Role of Osteocalcin in Energy Metabolism. Ann Nutr Metab. 2015;66(2-3):110-6.
24. Bae SJ, Choe JW, Chung YE, Kim BJ, Lee SH, Kim HY, et al. The association between serum osteocalcin levels and metabolic syndrome in Koreans. Osteoporos Int. 2011;22(11):2837-46.

25. Yeap BB, Chubb SAP, Flicker L, McCaul KA, Ebeling PR, Beilby JP, et al. Reduced serum total osteocalcin is associated with metabolic syndrome in older men via waist circumference, hyperglycemia, and triglyceride levels. Eur J Endocrinol. 2010;163(2):265-72.

26. Urano T, Shiraki M, Kuroda T, Tanaka S, Urano F, Uenishi K, et al. Low serum osteocalcin concentration is associated with incident type 2 diabetes mellitus in Japanese women. J Bone Miner Metab. 2018;36(4):470-7.

27. Shu H, Pei Y, Chen K, Lu J. Significant inverse association between serum osteocalcin and incident type 2 diabetes in a middle-aged cohort. Diabetes Metab Res Rev. 2016;32(8):867-74.

28. Díaz-López A, Bulló M, Juanola-Falgarona M, Martínez-González MA, Estruch R, Covas MI, et al. Reduced serum concentrations of carboxylated and undercarboxylated osteocalcin are associated with risk of developing type 2 diabetes mellitus in a high cardiovascular risk population: a nested case-control study. J Clin Endocrinol Metab. 2013;98(11):4524-31.

29. 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(3):827-32.

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(9):1919-24.

31. Liatis S, Sfikakis PP, Tsiakou A, Stathi C, Terpos E, Katsilambros N, et al. Baseline osteocalcin levels and incident diabetes in a 3-year prospective study of high-risk individuals. Diabetes Metab. 2014;40(3):198-203.

32. Diaz-Franco MC, Franco-Diaz de Leon R, Villafan-Bernal JR. Osteocalcin-GPRC6A: An update of its clinical and biological multi-organic interactions (Review). Mol Med Rep. 2019;19(1):15-22.

33. Jørgensen CV, Gasparini SJ, Tu J, Zhou H, Seibel MJ, Bräuner-Osborne H. Metabolic and skeletal homeostasis are maintained in full locus GPRC6A knockout mice. Sci Rep 2019;9(1):5995.

34. Pi M, Wu Y, Quarles LD. GPRC6A Mediates Responses to Osteocalcin in β-Cells In Vitro and Pancreas In Vivo. J Bone Miner Res. 2011;26(7):1680–3.

35. Fulzele K, Riddle RC, DiGirolamo DJ, Cao X, Wan C, Chen D, et al. Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition. Cell. 2010;142(2):309-19.

36. Ferron M, Wei J, Yoshizawa T, Del Fattore A, DePinho RA, Teti A, et al. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. Cell. 2010;142(2):296-308.

37. Fowlkes JL, Clay Bunn R, Kalaitzoglou E, Ray P, Popescu I, Thraillkill KM. Postnatal loss of the insulin receptor in osteoprogenitor cells does not impart a metabolic phenotype. Sci Rep. 2020;10(1):8842.

38. Manolagas SC, Kronenberg HM. Reproducibility of results in preclinical studies: a perspective from the bone field. J Bone Miner Res. 2014;29(10):2131-40.
39. Yang R, Ma X, Pan X, Wang F, Luo Y, Gu C, et al. Serum osteocalcin levels in relation to metabolic syndrome in Chinese postmenopausal women. Menopause. 2013;20(5):548-53.
40. 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(6):703-8.
41. 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, thrombosis, and vascular biology. 2010;30(7):1474-8.
42. Devlin JT, Horton ES. Effects of prior high-intensity exercise on glucose metabolism in normal and insulin-resistant men. Diabetes 1985;34(10):973-9.
43. Mera P, Laue K, Ferron M, Confavreux C, Wei J, Galán-Díez M, et al. Osteocalcin Signaling in Myofibers Is Necessary and Sufficient for Optimum Adaptation to Exercise. Cell Metab. 2016;23(6):1078-92.
44. Levinger I, Jerums G, Stepto NK, Parker L, Serpiello FR, McConell GK, et al. The effect of acute exercise on undercarboxylated osteocalcin and insulin sensitivity in obese men. J Bone Miner Res. 2014;29(12):2571-6.
45. Mohammad Rahimi GR, Niyazi A, Alaee S. The effect of exercise training on osteocalcin, adipokines, and insulin resistance: a systematic review and meta-analysis of randomized controlled trials. Osteoporos Int. 2021;32(2):213-24.
46. Hauschka PV, Lian JB, Cole DE, Gundberg CM. Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. Physiol Rev. 1989;69(3):990-1047.
47. Lin X, Brennan-Speranza TC, Levinger I, Yeap BB. Undercarboxylated Osteocalcin: Experimental and Human Evidence for a Role in Glucose Homeostasis and Muscle Regulation of Insulin Sensitivity. Nutrients. 2018;10(7):847.
48. Aguayo-Ruiz JI, García-Cobián TA, Pascoe-González S, Sánchez-Enríquez S, Llamas-Covarrubias IM, García-Iglesias T, et al. Effect of supplementation with vitamins D3 and K2 on undercarboxylated osteocalcin and insulin serum levels in patients with type 2 diabetes mellitus: a randomized, double-blind, clinical trial. Diabetol Metab Syndr. 2020;12:73.
49. Choi HJ, Yu J, Choi H, An JH, Kim SW, Park KS, et al. Vitamin K2 supplementation improves insulin sensitivity via osteocalcin metabolism: a placebo-controlled trial. Diabetes Care. 2011;34(9):e147.
50. Park SG, Jeong SU, Lee JH, Ryu SH, Jeong HJ, Sim YJ, et al. The Changes of CTX, DPD, Osteocalcin, and Bone Mineral Density During the Postmenopausal Period. Ann Rehabil Med 2018;42(3):441-8.
51. Soules MR, Sherman S, Parrott E, Rebar R, Santoro N, Utian W, et al. Executive summary: Stages of Reproductive Aging Workshop (STRAW). Climacteric. 2001;4(4):267-72.
52. Funakoshi S, Yoshimura K, Hirano S, Ohmi S, Amano E, Fukuda Y, et al. Undercarboxylated osteocalcin correlates with insulin secretion in Japanese individuals with diabetes. Diabetol Metab Syndr 2020;12:72.
53. Mori K, Emoto M, Motoyama K, Lee E, Yamada S, Morioka T, et al. Undercarboxylated osteocalcin does not correlate with insulin resistance as assessed by euglycemic hyperinsulinemic clamp
Figures

Men with serum total vitamin D levels measured from 2003 through 2018 (n = 44,502)

Excluded (n = 41,665)
- Missing data (n = 29,880)
- Presence of MetS at baseline (n = 6,829)
- Serum osteocalcin level > 100 (n = 4)

Past History
- Excluded at baseline and/or during follow-up
  - Any cancer (n = 528)
  - CAD (n = 361)
  - Stroke (n = 230)
  - Thyroid disease (n = 519)
  - CKD (n = 16)
  - Liver disease (n = 1,295)

Medication History
- Excluded at baseline and/or during follow-up
  - Thyroid disease (n = 1,352)
  - Osteoporosis (n = 1,099)
  - Steroid (n = 8,510)
  - Sex hormone (n = 724)
  - Anticoagulants (n = 8,504)
  - Anticonvulsants (n = 19)
  - Health supplements, including vitamins (n = 7,254)

Abnormal laboratory test results
- Excluded at baseline and/or during follow-up
  - ALT > ×3 UNL (n = 1,056)
  - TFT (n = 546)
  - Creatinine ≥ 2 (n = 31)

Participants without MetS at baseline (n = 2,837)

Incident MetS (n = 518)

No MetS (n = 2,319)

Figure 1
Figure 2

Kaplan–Meier curve showing the incidence of metabolic syndrome during the follow-up period according to serum osteocalcin quartiles. The lowest quartile (Q1) shows a significantly increased incidence of MetS. The log-rank test was used to examine the significance of differences among groups. Q, quartile