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ORIGINAL ARTICLE

Reduced serum osteocalcin concentrations are associated with type 2 diabetes mellitus and the metabolic syndrome components in postmenopausal women: the crosstalk between bone and energy metabolism

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Abstract Although it has been shown that osteocalcin functions as a hormone in the regulation of glucose metabolism and fat mass, no population-based study to date has addressed serum osteocalcin levels in relation to energy metabolism concurrent with bone metabolism in postmenopausal women. In a population-based study, cardiovascular risk factors, high-sensitivity C-reactive protein (hs-CRP), osteoprotegerin, receptor activator of nuclear factor-κB ligand, osteocalcin, CrossLaps, alkaline phosphatase, and bone mineral density (BMD) at the lumbar spine (L2–L4) and the proximal femur were measured in 382 Iranian postmenopausal women. In multiple logistic regression analysis, lower osteocalcin and CrossLaps levels were associated with a higher odds ratio (OR) of having type 2 diabetes mellitus when adjustments were made for age, hs-CRP, cardiovascular risk factors, BMD, and markers of bone metabolism [OR 5.17, CI (2.66–10.04), $p < 0.0001$ and OR 2.51, CI (1.37–4.61), $p = 0.003$, respectively]. However, lower alkaline phosphatase levels were associated with a lower OR of having type 2 diabetes mellitus [OR 0.28, CI (0.15–0.52), $p < 0.0001$] in regression analysis. No significant difference was found between serum osteocalcin levels of those with and without metabolic syndrome. Among the metabolic syndrome components, low osteocalcin levels had significant associations with elevated blood glucose [OR 1.89, CI (1.16–3.07), $p = 0.010$] and elevated waist circumference [OR 2.53, CI (1.13–5.67), $p = 0.024$] in multivariate analyses. In conclusion, serum osteocalcin was independently associated with glucose intolerance and abdominal obesity as the components of metabolic syndrome and type 2 diabetes mellitus in postmenopausal women. Since CrossLaps and alkaline phosphatase levels were independently associated with the presence of type 2 diabetes mellitus, the unique contribution of osteocalcin in glucose metabolism could not be concluded.

Keywords Osteocalcin · Diabetes mellitus · Metabolic syndrome · Postmenopausal women

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Introduction

In recent years, a growing body of scientific and clinical evidence has indicated that we have encountered a paradigm of integrative physiology in bone and energy homeostasis [1, 2]. One of the osteoblast-specific proteins, osteocalcin, is encoded by the bone γ-carboxyglutamate (Gla) protein BGLAP and is secreted in the general circulatory system during osteogenesis [3]. The revolutionary

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finding that osteocalcin positively regulates energy metabolism through increased fat metabolism, energy expenditure, the insulin secretion capacity of the pancreas, and release of adiponectin from adipocytes, in addition to inducing mitochondrial proliferation and function in genetically modified mice [1], confirmed that a complex crosstalk between bones and adipocytes in energy metabolism may be present [2].

Although these animal-based studies are certainly of interest, the clinical relevance of osteocalcin has seldom been explored so far. Observational and cross-sectional human studies showed that in type 2 diabetes, osteocalcin was associated with plasma glucose level and fat mass [4], insulin sensitivity and secretion [5], glucose metabolism and atherosclerosis parameters [6], and glycemic variability [7].

Consistent with animal studies, it was reported that a circulating osteocalcin level was associated with improved glucose tolerance, insulin secretion, and sensitivity [8]. In addition, serum osteocalcin was closely associated with lipid metabolism [9] and measures of adiposity [10].

Recently, human studies reported that low osteocalcin levels were associated with metabolic syndrome [11–15], independent from well-known metabolic syndrome risk factors [12] and glucose metabolism [11], suggesting that osteocalcin may be involved in the crosstalk between bone and adipose tissue.

The aforementioned studies all describe significant inverse associations of osteocalcin with type 2 diabetes, insulin resistance, metabolic syndrome, measures of adiposity and lipid metabolism after adjustment for glucose and lipid metabolism variables in multivariate analyses. However, although many previous studies focused on metabolic syndrome in relation to bone metabolism [15, 16], they did not clarify that the associations of circulating osteocalcin with insulin resistance and energy metabolism exist independently of bone mineral metabolism. The components of metabolic syndrome were described as being associated with decreased bone mineral density (BMD) [17]. The rate of decline in BMD has been reported to be faster at the hip and slower at the lumbar spine in women with diabetes mellitus across menopause [18], and in comparison to controls, postmenopausal women with type 2 diabetes mellitus had lower levels of bone formation markers [19].

A few studies focused on postmenopausal women regarding osteocalcin in relation to type 2 diabetes mellitus [6, 20], glucose metabolism [9], and metabolic syndrome [11].

In the present population-based study, we investigated whether serum osteocalcin was associated with the components of metabolic syndrome and type 2 diabetes mellitus in postmenopausal women. In this integrative study, bone metabolism was also considered concurrently with markers of energy and lipid metabolism.

Materials and methods

Community sampling

The study design was described in a previous study [21]. In brief, 382 postmenopausal women who participated in the extension part of the Iranian Multicentral Osteoporosis Study were examined from 4 April to 22 September 2006. The mean age (mean ± SD) of the women was 58.78 ± 7.8 years (range 50–83 years). They were randomly selected from 13 clusters in Bushehr Port (the center of Bushehr province, which has the longest border with the Persian Gulf). All were community dwelling and ambulatory. The following exclusion criteria were used: (1) the known presence of generalized bone diseases including hyperparathyroidism, hypoparathyroidism, thyroid disorders, rheumatoid arthritis, Cushing disease, and steroid-induced osteoporosis; renal osteodystrophy; or other metabolic diseases; (2) a history of malignant diseases and liver diseases; (3) drug addiction; and (4) restriction to bed rest within the last 2 weeks after an illness or complete bed rest for 3 months.

Physical examinations

A standard mercury sphygmomanometer was used to measure blood pressure twice at the right arm after a 15-min rest in the sitting position. A stadiometer was used to measure height and weight. Heavy outer garments and shoes were removed before the participants' height and weight were measured. Body mass index (BMI) was calculated. Waist circumference was defined at the midway level between the costal margins and the iliac crests. Hip circumference was measured at the level of the greater trochanters.

BMD was determined at the lumbar spine (L2–L4) and proximal femur (neck) using dual-energy X-ray absorptiometry on an Osteocore II bone densitometer (Osteocore II Osteodensitometer; Medilink, France). To eliminate operator discrepancies, the same operator tested all the women during the study. Duplicate measurements were obtained from 30 women who agreed to undergo a repeat assessment on the same day, and the precision errors were calculated using the root mean square method. The coefficients of variation (CVs; precision) of measurements of the lumbar spine and femoral neck were 0.8 and 1.6 %, respectively.

Laboratory measurements

A fasting blood sample was taken. All samples were promptly centrifuged and separated, and the analyses were carried out at the Persian Gulf Health Research Center on the day of blood collection using a Selectra 2 autoanalyzer

(Vital Scientific, Spankeren, The Netherlands). Glucose was assayed by the enzymatic (glucose oxidase) colorimetric method using a commercial kit (Pars Azmun, Tehran, Iran). Serum TC and HDL-C were measured using a cholesterol oxidase phenol aminoantipyrine method, and TG was measured using a glycerol-3 phosphate oxidase phenol aminoantipyrine enzymatic method. Serum LDL-C was calculated using the Friedewald formula. LDL-C was not calculated when the TG concentration was greater than 400 mg/dl.

The measurement of C-reactive protein (CRP) by a high-sensitivity (hs) CRP assay and CRP HS enzyme-linked immunosorbent assay (ELISA) (DRG International) was conducted. The minimum detectable concentration of the CRP HS ELISA assay was estimated to be 0.1 mg/l. In addition, the functional sensitivity was determined to be 0.1 mg/l (as determined with interassay coefficient of variation <20 %).

Serum osteoprotegerin (OPG) levels were measured using an ELISA commercial kit (Biomedica Gruppe, Vienna, Austria). The detection limit of the assay was 0.14 pmol/l. The mean intra- and interassay CVs of the OPG assay were 4–10 and 7–8 %, respectively.

The receptor activator of nuclear factor-κB ligand (RANKL) levels were measured using an ELISA with an additional enhancement system (ampli-sRANKL; Biomedica Gruppe). The detection limit of the assay was 0.4 pg/ml. The mean intra- and interassay CVs of the RANKL assay were 8–9 and 6–3 %, respectively.

The N-MID Osteocalcin ELISA (Nordic Bioscience Diagnostics A/S) was used for the quantitative measurement of osteocalcin in sera. The intra-assay CVs for the low (7.0 ng/ml), medium (21.8 ng/ml), and high (43.2 ng/ml) values were 3.4, 2.0, and 2.4 %, respectively.

The serum CrossLaps ELISA (Nordic Bioscience Diagnostics A/S, Herlev, Denmark) was used for the quantification of degradation products of C-terminal telopeptides of type I collagen in sera. The intra-assay CVs for low (0.242 ng/ml), medium (0.375 ng/ml), and high (0.476 ng/ml) values were 5.4, 5.0, and 5.1 %, respectively.

Serum alkaline phosphatase was determined by spectrophotometry using *p*-nitrophenylphosphate as substrate (Pars Azmun, Tehran, Iran). Intra- and interassay CVs were 1.5 and 2.6 %, respectively.

Definitions

In accordance with the American Diabetes Association criteria, diabetes was defined as either a fasting plasma glucose of at least 126 mg/dl or use of antidiabetic measures [22].

The cutoff points of serum total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), and serum triglycerides (TG) distributions used to assign subjects to different levels of risk were those derived from the National Cholesterol Education Program (NCEP) guidelines in the USA [Adult Treatment Panel (ATP) III, September 2002] [23]. A subject was considered hypertensive if blood pressure was at least 140/90 mmHg or centrally obese if the waist-to-hip ratio was at least 0.9.

Metabolic syndrome was diagnosed using the criteria indicated by the NCEP-ATP III [23]. According to these criteria, subjects with metabolic syndrome have any combination of three or more of the following risk determinants: fasting plasma glucose of at least 110 mg/dl, blood pressure of at least 130/85 mmHg or antihypertensive treatment, plasma TG of at least 150 mg/dl, HDL-C less than 50 mg/dl, and waist circumference of at least 88 cm in women.

Statistical analysis

The distribution of variables was studied using probability plots and the Shapiro–Wilk test. We found that log transformation of osteocalcin, hs-CRP, OPG, RANKL, and CrossLaps gave a better fit to a Gaussian distribution. The geometric mean for those biochemical variables was defined as the arithmetic mean of the log-transformed data $\pm$ SD, raised to the power of 10.

A two-tailed *t* test was used to compare the mean values across groups. Pearson correlation analysis was employed to study the relationships between bone turnover markers and the anthropometric, BMD, and biochemical variables. For variables without normal distribution, log-transformed data were used.

Binary logistic regression analysis was used to ascertain the associations between low serum bone turnover markers levels (below the median) and type 2 diabetes mellitus or metabolic syndrome. In the full model, a low serum osteocalcin level versus a high level (below or equal to the median) was the independent variable of interest. Age, hs-CRP, OPG, cardiovascular risk factors (according to NCEP-ATP III criteria), CrossLaps, alkaline phosphatase, BMD at femoral neck, and lumbar spine BMD were considered as covariates.

A *p* value less than 0.05 was accepted as the value of significance. All statistical analyses were performed using the PASW Statistics GradPack 18 (SPSS Inc., Chicago, IL, USA).

Results

Subjects were stratified into low (below or equal to the median) and high (above the median) serum osteocalcin

groups. Serum osteocalcin had a median level of 9.82 ng/ml for the total population ($n = 382$). The characteristics of the study participants according to their serum osteocalcin levels (osteocalcin below or osteocalcin above median) are shown in Table 1.

There were no differences between the two groups with regard to systolic and diastolic blood pressures, TC, LDL-cholesterol, HDL-cholesterol, and RANKL levels. However, women with low osteocalcin levels (below median) were younger, had higher serum hs-CRP, fasting glucose, and TG levels, in addition to higher BMI, waist circumference, measures of truncal obesity, and BMD at the femoral neck and lumbar spine. They had lower CrossLaps and alkaline phosphatase levels (Table 1).

Serum osteocalcin showed correlations with age ($r = 0.25$, $p < 0.0001$), waist-to-hip ratio ($r = -0.10$, $p = 0.036$), BMI ($r = -0.20$, $p < 0.0001$), BMD at the femoral neck ($r = -0.19$, $p < 0.0001$) and lumbar spine ($r = -0.26, p < 0.0001$), hs-CRP ($r = -0.20, p < 0.0001$), fasting glucose ($r = -0.25$, $p < 0.0001$), TC ($r = -0.12$, $p = 0.017$), TG ($r = -0.15$, $p = 0.002$), CrossLaps ($r = 0.49, p < 0.0001$), and alkaline phosphatase ($r = 0.29$, $p < 0.0001$) levels in bivariate analyses (Table 2). However, serum osteocalcin levels had no significant correlations with systolic and diastolic blood pressures, TC, LDL-cholesterol, HDL-cholesterol, and OPG ($p > 0.05$).

Serum CrossLaps showed correlations with age, waist circumference, BMI, fasting glucose, TC, hs-CRP, RANKL, osteocalcin, alkaline phosphatase, and BMD at the femoral neck and lumbar spine ($p < 0.05$, Table 2).

Serum alkaline phosphatase showed correlations with fasting glucose, TC, LDL-cholesterol, osteocalcin, CrossLaps, and BMD at the lumbar spine ($p < 0.05$, Table 2).

Of the studied population, 102 subjects (26.7 %) had type 2 diabetes mellitus, and 261 women (68.32 %) met the metabolic syndrome criteria (NCEP-ATP III criteria).

Table 3 shows a comparison of cardiovascular risk factors, age, OPG, RANKL, hs-CRP, BMD and bone turnover markers in patients with type 2 diabetes mellitus and normal postmenopausal women. The serum osteocalcin level was lower in those with type 2 diabetes mellitus [median 8.54 ng/ml, interquartile range (7.69–9.94 ng/ml)] than those without diabetes [10.50 (8.55–13.67) ng/ml] ($p < 0.0001$). The patients with type 2 diabetes mellitus had lower serum CrossLaps levels ($p < 0.0001$). However, they had higher levels of serum alkaline phosphatase ($p = 0.020$, Table 3).

Age-adjusted lower osteocalcin levels (lower than median) were associated with a higher odds ratio (OR) of having type 2 diabetes mellitus (OR 4.29, CI [2.55–7.22], $p < 0.0001$) (Table 4). This association remained unchanged after adjusting for further variables, including

Table 1 Characteristics of 382 postmenopausal women, stratified by serum osteocalcin below/equal or above median

	All	Serum osteocalcin ≤ median	Serum osteocalcin > median	*p*
Age (years)	58.71 ± 7.50	57.41 ± 6.53	60.02 ± 8.59	0.001
Waist circumference (cm)	99.12 ± 10.62	101.55 ± 9.95	97.19 ± 10.67	<0.0001
Body mass index (kg/m^2)	28.34 ± 4.73	29.07 ± 4.99	27.55 ± 4.67	0.003
Waist-to-hip ratio	0.92 ± 0.06	0.93 ± 0.06	0.91 ± 0.06	0.019
Systolic blood pressure (mmHg)	126.19 ± 19.64	125.10 ± 19.87	126.95 ± 19.64	0.365
Diastolic blood pressure (mmHg)	78.80 ± 10.67	78.55 ± 10.30	78.97 ± 11.08	0.707
Fasting glucose (mg/dl)	115.71 ± 52.63	129.59 ± 65.68	102.75 ± 30.92	<0.0001
Total cholesterol (mg/dl)	235.02 ± 47.89	238.47 ± 47.89	232.24 ± 46.71	0.203
LDL-cholesterol (mg/dl)	157.35 ± 43.20	158.22 ± 43.29	156.91 ± 42.68	0.768
HDL-cholesterol (mg/dl)	40.98 ± 10.57	41.04 ± 11.25	41.01 ± 9.92	0.977
Triglyceride (mg/dl)	183.52 ± 96.26	196.09 ± 107.13	171.106 ± 82.53	0.014
Log(hs-CRP) (mg/l)[a]	1.89 ± 2.84	2.33 ± 2.93	1.55 ± 2.68	<0.0001
Osteoprotegerin (pg/ml)[a]	3.62 ± 1.55	3.58 ± 1.55	3.62 ± 1.56	0.792
RANKL (pg/ml)[a]	1.59 ± 3.00	1.43 ± 2.94	1.74 ± 2.97	0.085
Osteocalcin (ng/ml)[a]	11.45 ± 1.85	8.18 ± 1.1.10	14.09 ± 1.41	<0.0001
CrossLaps (ng/ml)[a]	0.58 ± 1.68	0.45 ± 1.57	0.73 ± 1.42	<0.0001
Alkaline phosphatase (U/l)	245.36 ± 90.21	223.08 ± 66.64	259.75 ± 91.12	<0.0001
Femoral neck BMD (g/cm^2)	0.844 ± 0.184	0.881 ± 0.208	0.816 ± 0.162	0.001
Lumbar BMD (g/cm^2)	0.946 ± 0.186	1.001 ± 0.189	0.902 ± 0.164	<0.0001

Data are given as mean ± SD unless otherwise indicated

BMD bone mineral density, *hs-CRP* high-sensitivity C-reactive protein, *RANKL* receptor activator of nuclear factor-κB ligand

[a] Geometric mean ± SD

Table 2 Bivariate correlation analysis between bone turnover markers and cardiovascular risk factors, age, osteoprotegerin, RANKL, hs-CRP, and BMD in postmenopausal women

	Log(osteocalcin)		Log(CrossLaps)		Alkaline phosphatase	
	r	*p* value	*r*	*p* value	*r*	*p* value
Age	0.25	<0.0001	−0.14	0.004	0.09	0.056
Waist circumference	−0.22	<0.0001	−0.19	<0.0001	−0.05	0.247
Body mass index	−0.20	<0.0001	−0.16	0.001	−0.05	0.308
Waist-to-hip ratio	−0.10	0.036	−0.10	0.053	−0.02	0.694
Systolic blood pressure	0.09	0.063	−0.01	0.730	0.05	0.315
Diastolic blood pressure	0.01	0.719	−0.05	0.305	0.01	0.830
Fasting glucose	−0.25	<0.0001	−0.27	<0.0001	0.19	<0.0001
Total cholesterol	−0.12	0.017	−0.11	0.025	0.11	0.028
LDL-cholesterol	−0.05	0.291	−0.06	0.195	0.12	0.013
HDL-cholesterol	−0.04	0.410	−0.08	0.109	−0.02	0.600
Triglyceride	−0.15	0.002	−0.09	0.083	0.01	0.882
Log(hs-CRP)	−0.20	<0.0001	−0.23	<0.0001	0.09	0.079
Log(osteoprotegerin)	0.04	0.402	0.01	0.721	0.05	0.258
Log(RANKL)	0.07	0.151	0.17	0.001	0.08	0.094
Log(osteocalcin)	–	–	0.49	<0.0001	0.29	<0.0001
Log(CrossLaps)	0.49	<0.0001	–	–	0.11	0.024
Alkaline phosphatase	0.29	<0.0001	0.11	0.024	–	–
Femoral neck BMD	−0.19	<0.0001	−0.11	0.024	−0.09	0.062
Lumbar BMD	−0.26	<0.0001	−0.22	<0.0001	−0.11	0.031

Correlation coefficients and *p* values were calculated using Pearson correlation analysis

BMD bone mineral density, *hs-CRP* high-sensitivity C-reactive protein, *RANKL* receptor activator of nuclear factor-κB ligand

cardiovascular risk factors, hs-CRP, OPG, RANKL, CrossLaps, alkaline phosphatase, and BMD at the femoral neck and lumbar spine [OR 5.17, CI (2.66–10.04), $p < 0.0001$] (Table 4).

In multiple logistic regression analysis, lower CrossLaps levels were associated with a higher OR of having type 2 diabetes mellitus when adjustments were made for age, hs-CRP, cardiovascular risk factors, BMD, and markers of bone metabolism [OR 2.51, CI (1.37–4.61), $p = 0.003$]. However, lower alkaline phosphatase levels were associated with a lower OR of having type 2 diabetes mellitus [OR 0.28, CI (0.15–0.52), $p < 0.0001$] in regression analysis (Table 4).

No significant difference was found between serum osteocalcin, CrossLaps, alkaline phosphatase levels of those with metabolic syndrome and those without metabolic syndrome in different models using multiple logistic regression analysis. Of the metabolic syndrome components, low osteocalcin levels had significant associations with elevated blood glucose [OR 1.89, CI (1.16–3.07), $p = 0.010$] and elevated waist circumference [OR 2.53, CI (1.13–5.67), $p = 0.024$] after adjustments for age, hs-CRP, cardiovascular risk factors, BMD, and markers of bone metabolism (Fig. 1).

Serum osteocalcin levels did not differ significantly between subjects with and without hypertension and dyslipidemia, according to the NCEP-ATP III criteria.

Discussion

We found that serum osteocalcin levels were independently associated with glucose intolerance and abdominal obesity, which are components of metabolic syndrome, and type 2 diabetes mellitus in postmenopausal women.

Several human studies were conducted in patients with type 2 diabetes mellitus to assess the reciprocal relationship between circulating osteocalcin and glucose metabolism [4–6, 20, 24, 25].

In agreement with the results of our study, a significant reduction in osteocalcin levels among type 2 diabetes mellitus patients compared with the normal glucose and impaired fasting glucose groups was reported for the first time in postmenopausal Korean women [20]. However, in contrast to the Korean study for which the participants were recruited from a periodic health checkup in a health promotion center of a hospital [20], the current study was conducted with a relatively large number of well-characterized

Table 3 Comparison of cardiovascular risk factors, age, osteoprotegerin, RANKL, hs-CRP, BMD, and bone turnover markers in patients with type 2 diabetes mellitus and normal postmenopausal women

	Normal	Type 2 diabetes mellitus	*p* value
Age (years)	58.47 ± 8.07	59.67 ± 7.01	0.185
Waist circumference (cm)	98.63 ± 10.52	100.48 ± 10.86	0.134
Body mass index (kg/m^2)	28.01 ± 4.46	28.36 ± 5.11	0.532
Waist-to-hip ratio	0.92 ± 0.07	0.94 ± 0.05	0.010
Systolic blood pressure (mmHg)	125.61 ± 18.25	127.76 ± 22.99	0.345
Diastolic blood pressure (mmHg)	78.73 ± 10.57	78.97 ± 10.97	0.849
Fasting glucose (mg/dl)	93.50 ± 13.56	176.45 ± 69.49	<0.0001
Total cholesterol (mg/dl)	235.93 ± 47.84	232.54 ± 48.17	0.542
LDL-cholesterol (mg/dl)	159.09 ± 43.06	152.59 ± 45.44	0.194
HDL-cholesterol (mg/dl)	42.04 ± 10.71	38.08 ± 9.70	0.001
Triglyceride (mg/dl)	174.03 ± 93.75	209.48 ± 98.70	0.001
Log(hs-CRP) (mg/l)[a]	1.77 ± 2.75	2.18 ± 2.95	0.066
Osteoprotegerin (pg/ml)[a]	69.18 ± 1.54	79.43 ± 1.54	0.009
RANKL (pg/ml)[a]	1.58 ± 2.88	1.58 ± 3.09	0.964
Osteocalcin (ng/ml)[a]	11.22 ± 1.44	9.12 ± 1.31	<0.0001
CrossLaps (ng/ml)[a]	0.64 ± 1.62	0.47 ± 1.63	<0.0001
Alkaline phosphatase (U/l)	238.88 ± 84.93	263.10 ± 101.62	0.020
Femoral neck BMD (g/cm^2)	0.851 ± 0.183	0.838 ± 0.209	0.568
Lumbar BMD (g/cm^2)	0.945 ± 0.189	0.961 ± 0.173	0.452

Data are given as mean ± SD unless otherwise indicated

BMD bone mineral density, *hs-CRP* high-sensitivity C-reactive protein, *RANKL* receptor activator of nuclear factor-κB ligand

[a] Geometric mean ± SD

Table 4 Odds ratio and 95 % CI relating diabetes mellitus or metabolic syndrome as dependent variable, and bone turnover markers including osteocalcin, cardiovascular risk factors, age, osteoprotegerin, RANKL, hs-CRP, and BMD as independent parameters in postmenopausal women

	Type 2 diabetes mellitus			Metabolic syndrome		
	OR	CI	*p*	OR	CI	*p*
Osteocalcin						
Unadjusted	3.71	2.26–6.11	<0.0001	1.37	0.87–2.14	0.164
Age-adjusted	4.29	2.55–7.22	<0.0001	1.48	0.94–2.33	0.089
Full model[a]	5.17	2.66–10.04	<0.0001	1.41	0.74–2.69	0.295
CrossLaps						
Unadjusted	3.46	2.11–5.66	<0.0001	1.13	0.72–1.75	0.581
Age-adjusted	3.78	2.28–6.28	<0.0001	1.02	0.99–1.05	0.099
Full model[a]	2.51	1.37–4.61	0.003	1.24	0.67–2.28	0.479
Alkaline phosphatase						
Unadjusted	0.60	0.38–0.95	0.030	0.63	0.41–0.99	0.047
Age-adjusted	0.61	0.38–0.96	0.036	1.02	0.99–1.05	0.123
Full model[a]	0.28	0.15–0.52	<0.0001	0.62	0.34–1.11	0.109

[a] Full model included bone turnover markers (lower than median), cardiovascular risk factors [low HDL-cholesterol (<40 mg/dl), high LDL-cholesterol (≥160 mg/dl), hypertension (≥140/90 mmHg), waist-to-hip ratio (≥0.9), smoking], log(hs-CRP), log(osteoprotegerin), log(RANKL), and BMD at the femoral neck and lumbar area

postmenopausal women who were selected in a population-based setting.

Another main strength of our study is that bone metabolism was included concurrently with energy and lipid metabolism in the regression analyses. In all previous studies [4–6, 20, 24–26], the researchers considered only energy and/or lipid metabolism markers in their analyses to show inverse association of osteocalcin and glucose metabolism.

Because our data are cross-sectional, only limited inferences can be made regarding temporality and causation. Thus, we cannot indicate that a reduced serum osteocalcin level is not caused by hyperglycemia or other unidentified mechanisms that operate in diabetes mellitus. Indeed, in previous human studies, it has been shown that levels of bone turnover markers are lower in patients with diabetes [27–29]. Our findings are consistent with these previous studies that bone turnover markers (osteocalcin

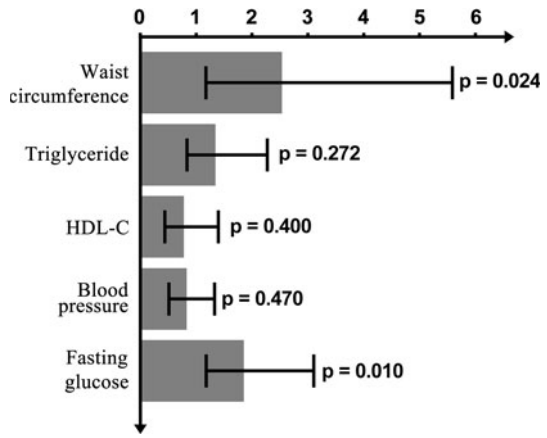

Fig. 1 Odds ratio and 95 % CI relating the metabolic syndrome components (as dependent variable), and osteocalcin (lower than median) after adjustments for age, hs-CRP, cardiovascular risk factors, bone mineral density, and markers of bone metabolism in postmenopausal women

and CrossLaps) were suppressed by hyperglycemia in patients with diabetes.

The status of bone resorption in hyperglycemia and diabetes has not been well characterized and the results of previous studies are conflicting [4, 20, 30, 31]. In contrast to previous studies [4, 20, 30, 31], we found lower levels of CrossLaps and higher alkaline phosphatase levels in patients with diabetes. This discrepancy in levels of osteocalcin, a marker for mature osteoblasts, and bone alkaline phosphatase, a marker for immature osteoblasts, in diabetic patients could be explained by the results of in vitro studies that showed chronic hyperglycemia increases the activity and expression of alkaline phosphatase, but decreases osteocalcin expression [6, 32]. In fact, osteocalcin was elevated and bone alkaline phosphatase was reduced after treatment of diabetes [33, 34].

Recently, lower bone turnover was suggested as a skeletal parameter that is present in type 2 diabetes mellitus [19]. The results of our regression analyses revealed that osteocalcin, CrossLaps, and alkaline phosphatase levels were independently associated with type 2 diabetes mellitus. Thus, the unique contribution of osteocalcin as a bone turnover marker in glucose metabolism could not be concluded in the current study and the observed association between osteocalcin and type 2 diabetes may simply reflect the effects of diabetes and hyperglycemia on bone metabolism.

To our knowledge, five clinical studies examined the association between osteocalcin and metabolic syndrome [11–15]. Of these studies, three examined the relationship of circulating osteocalcin and metabolic syndrome in men. Yeap et al. [15] reported a lower osteocalcin level in older men with metabolic syndrome. An inverse relationship between osteocalcin levels and the presence of metabolic syndrome was reported among Chinese men [12, 14]. Osteocalcin levels in the highest quartile were associated with a lower OR of having metabolic syndrome in blacks and non-Hispanic whites [13]. The participants in this cohort were old and predominantly hypertensive [13].

In contrast to the results of a Korean study that found the ORs for metabolic syndrome were significantly higher in the lowest quartile than in the highest quartile among postmenopausal women [11], we did not find a significant difference between osteocalcin levels of postmenopausal women with metabolic syndrome and those without metabolic syndrome. The discrepancies between our obtained results in postmenopausal women with the Korean study cannot be explained until the results of more studies are available. However, differences in the selection and the characteristics of the studied populations, including race/ethnicity, may be potential contributing factors.

Of the metabolic syndrome components, elevated blood glucose and elevated waist circumference had a significant association with lower osteocalcin levels when adjustments were made for age, hs-CRP, cardiovascular risk factors, BMD, and markers of bone metabolism in regression models.

Our finding that abdominal obesity was inversely associated with serum osteocalcin is in line with other animal [35] and human studies [12, 14, 15]. Lee et al. [35] reported that osteocalcin-deficient mice were obese. Osteocalcin was a strong negative predictor of fat mass and trunk fat in elderly Swedish men [25]. Im et al. [20] showed that serum osteocalcin levels had a significant negative correlation with BMI among postmenopausal women. In another study, lower serum osteocalcin concentrations were reported in obese postmenopausal women in comparison with non-obese women [36]. Although obesity is associated with low bone turnover [26, 37], the relationship between serum osteocalcin and adiposity needs a full explanation.

We acknowledge that our study has several limitations. In the present study, we used the NCEP-ATP III definition of metabolic syndrome. Although this definition is the most often used, other definitions of metabolic syndrome do exist. Another limitation of our study includes the lack of measurement of insulin resistance from fasting glucose and insulin concentrations using the HOMA method. Although we considered the participants' lifestyle risk factors for osteoporosis and cardiovascular diseases, a limitation is that subjects who underwent insulin or sulfonylurea treatments were not excluded.

In the current study, total serum osteocalcin was measured. Thus, the differential effects of carboxylated and uncarboxylated forms of osteocalcin on glucose metabolism and metabolic syndrome could not be examined.

Although animal and in vitro studies showed that the uncarboxylated form appeared to mediate the effects of osteocalcin in the regulation of glucose and energy metabolism [35, 38], other in vitro and clinical studies reported the importance of both types of osteocalcin in glucose metabolism [4, 39]. Kanazawa et al. [4] reported that both carboxylated and uncarboxylated forms of osteocalcin were associated with blood glucose level in type 2 diabetes. Vitamin K is a cofactor for gamma-carboxylation of osteocalcin (OC). This vitamin reduces circulating osteocalcin while increasing calcification of the bone [2]. In fact, phylloquinone (K1) and menaquinone 7 (MK-7) correlated inversely with uncarboxylated OC [40] and a negative correlation between undercarboxylated OC and MK-7 was reported in elderly women with type II diabetes mellitus [41]. Furthermore, vitamin K supplementation for 36 months reduced progression of insulin resistance in older men [42]. These findings demonstrate that vitamin K status should be included in crosstalk between bone and energy metabolism.

Since we assessed the investigated osteocalcin with single measurements, the changes in osteocalcin levels over time could not be reflected in the current study. Another limitation of our study includes the lack of measurement of other bone formation markers such as amino-terminal procollagen type I propeptide (PINP).

In conclusion, serum osteocalcin was independently associated with glucose intolerance and abdominal obesity, which are components of metabolic syndrome, and type 2 diabetes mellitus in postmenopausal women. These findings may suggest the independent role of osteocalcin in the crosstalk between bone and energy metabolism in humans. However, we also observed that low CrossLaps and high alkaline phosphatase levels were independently associated with type 2 diabetes mellitus. Therefore, the unique contribution of osteocalcin as a bone turnover marker in type 2 diabetes mellitus could not be concluded in the current study.

In a recent study, daily injections of osteocalcin significantly affected glucose handling in wild-type mice, improved insulin sensitivity, prevented obesity, and protected against liver steatosis [43]. Therefore, on the basis of the results of this animal study, further research is required to evaluate whether interventions that raise osteocalcin levels might contribute to the development of treatments for components of metabolic syndrome such as obesity and diabetes mellitus in humans.

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Conflict of interest All authors have no conflicts of interest.

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