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The Association between Serum under carboxylated Osteocalcin Level and Type-2 Diabetes Mellitus: Results from the Kyushu and Okinawa Population Study

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Abstract

Objectives: Diabetes can affect bone via obesity, hyperglycemia, hyperinsulinemia, and hypoinsulinemia. Osteocalcin (OC) is a non-collagenous protein of the bone that contributes to bone formation. A population-based study was done to examine the relationship between the serum level of under carboxylated osteocalcin (ucOC) and diabetes.

Methods: A cross-sectional study was done in 2,062 women aged 40 to 69 years living in a suburban Japanese town. Serum ucOC level by electro chemiluminescent immunoassay, Fasting Plasma Glucose (FPG), and glycohemoglobin A1c (HbA1c) were measured for each participant. Participants who were previously diagnosed with diabetes or who were receiving its treatment were defined as having pre-existing diabetes. Participants without pre-existing diabetes who had a FPG level >7.0 mmol/L or HbA1c level of ≥6.5 % were defined as having newly diagnosed diabetes.

Results: The median serum ucOC level of 1,433 postmenopausal women (5.2 ng/mL) was significantly higher than that of 629 premenopausal women (3.1 ng/mL) (P<0.0001). The distribution of postmenopausal women with pre-existing diabetes, newly diagnosed diabetes, and non-diabetes was 38 (2.7%), 88 (6.1%), and 1,307 (91.2%), respectively. The median serum ucOC levels of the 3-groups of postmenopausal women significantly decreased with worsened glucose metabolism (pre-existing diabetes 3.38, newly diagnosed diabetes 4.51, and non-diabetes 5.51 ng/mL) (P<0.0001). Multivariate regression analysis showed the FPG and HbA1c values to be negative factors independently associated with the serum ucOC level of postmenopausal women.

Conclusions: These findings indicate that the serum ucOC levels decrease in postmenopausal diabetic women, suggesting a relationship between diabetes and osteoporosis.

Abbreviations: OC: Osteocalcin; UCOC: Under Carboxylated Osteocalcin; FPG: Fasting Plasma Glucose; Hba1c: Glycohemoglobin A1c; BMD: Bone Mineral Density; BMI: Body Mass Index; BP: Blood Pressure; NGSP: US National Glycohemoglobin Standardization Program; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; Gla: Γ-Carboxyglutamic; Gla-OC: Carboxylated-Type Osteocalcin

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Keywords: Diabetes; Osteocalcin; Postmenopausal Women

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Introduction

Recent evidence suggests an endocrine cross-talk between bone remodelling and energy metabolism [1]. The relationship between diabetes and osteoporosis is under especially intense investigation due to the increasing prevalence of both diseases and evidence that type 2 diabetes mellitus is associated with an increased risk of fracture [2]. Osteoporosis occurs when the rate of bone resorption is greater than the rate of formation, leading to changes in bone structure, malformation, pain, and fractures. The World Health Organization fracture risk assessment tool, used clinically to generate estimated probabilities for major osteoporotic and hip fractures over 10 years, demonstrated good concordance with observed fractures for non-diabetes patients, but underestimated observed major osteoporotic and hip fracture risk in diabetes patients, suggesting that diabetes confers an increased risk of fracture that is independent of Bone Mineral Density (BMD) [3].

Osteocalcin (OC) is a non-collagenous protein of the bone that contributes to hard bone formation and calcium ion homeostasis and that also serves as a marker for bone formation. OC is both released from the bone matrix and activated by bone resorption activity of the osteoblasts following multiple post-translational modifications of energy metabolism [1]. OC-deficient mice are hyperglycemic, hypoinsulinemic, have low pancreatic β-cell mass, decreased insulin sensitivity, increased fat mass and decreased energy expenditure [4]. Conversely, subcutaneous infusion of recombinant OC into wild-type mice causes an increase in blood insulin levels, enhances glucose tolerance and improves insulin sensitivity [5]. OC is in a fully carboxylated form, implying that OC needs to be decarboxylated to become active [1]. The active form of OC is undercarboxylated OC (ucOC). Bone resorption, and therefore OC decarboxylation and activity (ucOC is released), is positively regulated by insulin signaling in osteoblast. In contrast, leptin, an adipokine secreted by adipocytes, acts via its central serotonin-mediated stimulation of sympathetic output, and indirectly inhibits OC activation, leading to inhibition of insulin secretion and causing glucose tolerance [6]. Bone tissue can regulate glucose metabolism through an endocrine cross-talk involving osteoblasts, adipocytes, and organs such as the pancreas.

The serum ucOC level increases in postmenopausal women [7], and the frequency of osteoporosis increases concurrently with postmenopausal decreases in the estrogen level [8]. There is a positive correlation between serum ucOC and serum OC levels [9], which affect glucose metabolism, as described above. To date, few large-scale, epidemiological studies that focused on serum ucOC levels have been conducted. Therefore, we conducted a large-scale, epidemiological study to determine the relationship between the serum ucOC level and type 2 diabetes of over 2,000 pre- and post- menopausal female participants undergoing routine medical examinations.

Materials and Methods

Study population

The Kyushu and Okinawa Population Study has been in progress since 2007 as a survey of the incidence of vascular events associated with lifestyle-related diseases among the general population of Japan [10, 11]. In the present sub-study, we recruited residents of Kasuya Town, a suburban area with about 35,500 residents adjacent to Fukuoka City, Kyushu Island. The participants were residents notified by local newspaper and public announcements of a free annual health examination given by our department. Eligible participants were residents aged 40-69 years at the time of this investigation. Between June 2007 and November 2009, 2,611 female residents took the examination, 2,398 of whom agreed to enroll to our

study (participation rate 91.8%). Because serum ucOC levels are affected by renal dysfunction [12], we excluded 241 participants from the study whose estimated glomerular filtration rate values, inferred from serum creatinine level, were less than 60 mL/min/1.73 m^2 as calculated by the Modification of Diet in Renal Disease Study equation [13] or who showed positive results for macro-albuminuria by dipstick test for spot urine. Moreover, 95 were excluded for taking anti-osteoporosis drugs (bisphosphonate, selective estrogen receptor modulator, calcium, vitamin D, vitamin K, and calcitonin), estrogen analogs, parathyroid hormones, multi-vitamin drugs, systematic antiviral, antineoplastic, immune suppressive drugs, or immune-modulating drugs within the 12 months before enrolment. We included 2,062 participants for the final analysis. The study was approved by the Kyushu University Hospital Ethics Committee. Written informed consent was obtained from all participants prior to the examination. This study was carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000.

Physical examination, interview, and blood examination

All participants completed a self-administered questionnaire as described below and at the same time underwent anthropometric measurements and venous blood drawing.

Anthropometric measurements were performed with each participant wearing indoor clothing and without shoes. Body Mass Index (BMI) was calculated as weight (kg) divided by height (m) squared. Waist circumference was measured at a level midway between the lowest rib and the iliac crest, in a standing position. Systolic Blood Pressure (BP) and diastolic BP were measured on the right arm in the sitting position with an automated sphygmomanometer after a 5 min rest.

The questionnaire inquired about the following: smoking, alcohol consumption, diseases under current or previous treatment, use of over-the-counter drugs and vitamin supplements, and family history of selected diseases, and menopausal status. The postmenopausal status of each participant was determined through the interview and was defined as the absence of menstrual bleeding during the preceding 12 months.

Blood collection for each participant occurred at least 10 h after fasting, early in the morning. Aliquots of whole blood, and serum and plasma samples from each participant were immediately sent at 4°C to a clinical laboratory testing company (SRL, Fukuoka, Japan) for the measurement of serum ucOC, Fasting Plasma Glucose (FPG), fasting serum insulin, glycohemoglobin A1c (HbA1c), serum lipid profiles and serum creatinine concentrations. The HbA1c level was initially calculated using the method of the Japanese Diabetes Society and Japanese Society for Clinical Chemistry, and then converted to US National Glycohemoglobin Standardization Program (NGSP) format. The HbA1c levels are expressed as NGSP levels (%) [14]. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: FPG (mmol/L) × fasting serum insulin (pmol/L) /156. Serum total cholesterol, HDL-cholesterol, LDL-cholesterol, and triacylglycerol levels were measured determined by a direct method.

The serum ucOC level of each participant was determined by a commercially available kit (Picolumi ucOC, Eidia Co., Ltd., Tokyo). It is a one-step sandwich electrochemiluminescence immunoassay that utilizes two mouse monoclonal undercarboxylated OC antibodies (Glu-OC4-5 and OC G3) (one of which is coated on the plate and the other ruthenium-labeled) for detection of ucOC. When used with an autoanalyzer, the kit achieved highly sensitive and rapid measurement over a range of 0.39 to 50 ng/mL. Assessment of reproducibility testing showed good results, with a coefficient of variation of ≤ 10%, a recovery rate for spiked samples of 93-106%, and no influence of interfering substances at normal levels [15].

Diagnosis of glucose metabolism

For this study, we defined pre-existing diabetes as participants who had been diagnosed with type 2 diabetes or those who were receiving treatment for the disease. For participants with no history of diabetes or current treatment for diabetes, a new diagnosis of diabetes mellitus was made if FPG was ≥7.0 mmol/L, and/or HbA1c was ≥6.5%. All others were considered

to have normal glucose metabolism. The study analysis was performed by dividing the participants into the above three categories of glucose metabolism.

Statistical analysis

Statistical analyses were conducted using SPSS Statistics 19.0 (IBM SPSS Inc., Chicago, IL, USA). Continuous data are expressed as median with first - third quartiles, and categorical variables are reported as frequencies and percentages. Univariate analyses were done using the Chi-square, Student's t test, Fisher's Exact test, Mann–Whitney U test, Kruskal-Wallis test, or Spearman's rank correlation coefficient, as appropriate. Variables with a P value<0.05 in univariate analysis were evaluated using stepwise multiple regression analysis with forward selection method to identify variables significantly associated with serum ucOC level as a continuous variable. All statistical tests were two-sided and the significance level was chosen as P value<0.05.

Results

Comparison of premenopausal and postmenopausal women

629 of the 2,062 participants (30.5%) were premenopausal and 1,433 (69.5%) were postmenopausal. As shown in **Table 1**, compared to premenopausal women, the postmenopausal women had a significantly more frequent history of bone fractures and significantly higher BMI, waist circumference, and systolic and diastolic blood pressures (all P<0.0001). The frequencies of past and current smoking of the postmenopausal women (6.5% and 4.4%) were significantly lower than those of the premenopausal women (16.1% and 8.4%) (P=0.013). Of the 629 premenopausal women, 5 (0.8%) had pre-existing diabetes, 15 (2.4%) did newly diagnosed diabetes, and 609 (96.8%) did normal glucose metabolism. The numbers of postmenopausal women with pre-existing diabetes, newly diagnosed diabetes, and normal glucose metabolism were 38 (2.7%), 88 (6.1%), and 1,307 (91.2%), respectively. The frequencies of pre-existing diabetes and newly diagnosed diabetes of postmenopausal women were significantly higher than those of premenopausal women (P<0.0001).

Table 1: Comparison between premenopausal and postmenopausal women of 2,062 female participants of Fukuoka Prefecture, Japan

Factors	Premenopausal n = 629	Postmenopausal n = 1,433	P values
Age (years)	45.0 [42.0 - 47.0]	59.0 [55.0 - 63.0]	<0.0001
Period of postmenopausal status (years)	-	10.0 [6.0 - 15.0]	-
History of bone fractures, n (%)	1 (0.1)	11 (0.8)	<0.0001
Body mass index (kg/m2)	21.4 [19.6 - 23.9]	22.8 [20.8 - 25.2]	<0.0001
Waist circumference (m)	0.77 [0.72 - 0.83]	0.82 [0.76 - 0.89]	<0.0001
Systolic blood pressure (mmHg)	110.0 [101.5 - 126.0]	126.0 [114.0 - 140.0]	<0.0001
Diastolic blood pressure (mmHg)	70.0 [61.0 - 80.0]	75.0 [68.0 - 80.0]	<0.0001
Current smoking, n (%)	101 (16.1)	92 (6.4)	0.013
Past smoking, n (%)	53 (8.4)	63 (4.4)	
Never smoking, n (%)	475 (75.5)	1,278 (89.2)	
Everyday drinking, n (%)	15 (2.4)	23 (1.6)	0.762
Sometimes drinking, n (%)	216 (34.3)	309 (21.6)	
No drinking, n (%)	398 (63.3)	1,101 (76.8)	

Continuous data are reported as median [first - third quartiles]. Categorical data are reported as number (%). P values are calculated for the comparison between premenopausal and postmenopausal women.

Differences in blood chemistry were found between premenopausal and postmenopausal women, as shown in **Table 2**. All measured values, including FPG, fasting serum insulin, HbA1c, serum total cholesterol, LDL-cholesterol, and triacylglycerol were significantly higher for postmenopausal than for premenopausal women, except for HDL-cholesterol, which was significantly lower (all P<0.0001). The median serum ucOC level of the postmenopausal women (5.2 ng/mL) was significantly higher than that of the premenopausal women (3.1 ng/mL) (P<0.0001).

Table 2: Differences in blood chemistry between premenopausal and postmenopausal women of 2,062 female participants of Fukuoka Prefecture, Japan

Tests	Premenopausal women *n* = 629	Postmenopausal women *n* = 1,433	P values
Fasting plasma glucose (mmol/L)	4.9 [4.7 - 5.2]	5.2 [4.8- 5.6]	<0.0001
Fasting serum insulin (pmol/L)	31.9 [21.5 - 55.0]	36.1 [22.3 - 57.0]	0.161
Homeostasis model assessment of insulin resistance	1.0 [0.6 - 1.8]	1.2 [0.7 - 2.0]	0.134
Glycohemoglobin A1c (%)	5.3 [5.1 - 5.5]	5.5 [5.3 - 5.7]	<0.0001
Serum total cholesterol (mmol/L)	5.2 [4.7 - 5.8]	5.7 [5.1 - 6.2]	<0.0001
Serum low-density lipoprotein-cholesterol (mmol/L)	2.8 [2.4 - 3.3]	3.3 [2.7 - 3.8]	<0.0001
Serum high-density lipoprotein-cholesterol (mmol/L)	1.8 [1.6 - 2.1]	1.6 [1.4 - 1.9]	<0.0001
Serum triacylglycerol (mmol/L)	1.9 [1.5 - 2.6]	2.5 [1.9 - 3.6]	<0.0001
Serum undercarboxylated osteocalcin level (ng/mL)	3.1 [2.1 - 4.7]	5.2 [3.6 - 7.5]	<0.0001

Continuous data are reported as median [first - third quartiles]. Categorical data are reported as number (%).

Homeostasis model assessment of insulin resistance was calculated using the following formula: fasting plasma glucose (mmol/L)×fasting serum insulin (pmol/L) / 156.

P values are calculated for the comparison between premenopausal and postmenopausal women.

Relationship of serum ucOC level to related factors, classified by menopause status

A univariate analysis was performed (**Table 3**) to determine the relationship between serum ucOC and background factors. There was a significant negative correlation between serum ucOC level and HbA1c level of both premenopausal and postmenopausal women (P=0.015 and P<0.0001, respectively). Only in premenopausal women, a positive correlation was observed between serum ucOC level and serum HDL cholesterol level (P=0.015).

Table 3: Relationships between serum undercarboxylated osteocalcin level as a continuous variable, glucose metabolism, and serum lipid profiles using *Spearman's* rank correlation coefficient (*r*) for 2,062 female participants, classified by menopausal status

	Premenopausal women *n* = 629			Postmenopausal women *n* = 1,433	
Variables	*r*	P value		*r*	P value
Fasting plasma glucose (mmol/L)	-0.045	0.069		-0.065	0.055
Fasting serum insulin (pmol/L)	-0.051	0.828		-0.024	0.764
Homeostasis model assessment of insulin resistance	-0.063	0.681		-0.039	0.844
Glycohemoglobin A1c (%)	-0.011	0.015		-0.149	<0.0001
Serum total cholesterol (mmol/L)	0.050	0.297		-0.054	0.268
Serum low-density lipoprotein -cholesterol (mmol/L)	0.051	0.057		0.030	0.262
Serum high-density lipoprotein -cholesterol (mmol/L)	0.096	0.015		0.037	0.160
Serum triacylglycerol (mmol/L)	-0.060	0.369		-0.061	0.337

Continuous data are reported as median [first - third quartiles]. Categorical data are reported as number (%).

Homeostasis model assessment of insulin resistance was calculated using the following formula: fasting plasma glucose (mmol/L)×fasting serum insulin (pmol/L) / 156.

P values are calculated for the comparison between premenopausal and postmenopausal women.

Relationship between HbA1c and serum ucOC level, classified by menopause status

The relationship between the HbA1c and ucOC levels of premenopausal and postmenopausal women is shown in **Table 4**. In premenopausal women, a higher HbA1c level was associated with a significantly more decrease in the serum ucOC level (P<0.0001), while this phenomenon was not observed in postmenopausal women. Regardless of the HbA1c level, the ucOC level was significantly higher in postmenopausal than in premenopausal women (all P<0.0001).

Table 4: Relationship between the serum undercarboxylated osteocalcin and glycohemoglobin A1c levels 2,062 female participants, classified by a glycohemoglobin A1c level or menopausal status

	Serum undercarboxylated osteocalcin level Median [first - third quartiles]		
Glycohemoglobin A1c (%)	Premenopausal women *n* = 629	Postmenopausal women *n* = 1,433	P values *
<6.5	3.18 [2.13 - 4.78]	5.49 [3.81 - 7.77]	<0.0001
6.5 - 6.9	2.63 [2.20 - 4.56]	4.02 [2.68 - 5.88]	<0.0001
≥7.0	1.38 [1.01 - 2.94]	4.21 [2.69 - 5.51]	<0.0001
P values **	<0.0001	0.723	

* P values are calculated for the comparison between premenopausal and postmenopausal women. ** P values are calculated for a trend of serum undercarboxylated osteocalcin level.

Relationship between serum ucOC level and glucose metabolism, classified by menopause status

Figure 1 shows the relationship between the serum ucOC level and the pre-existing or newly diagnosed diabetes of premenopausal and postmenopausal women. No significant difference was observed in the median ucOC levels of the pre-existing diabetes 3.20 ng/mL, newly diagnosed diabetes 2.63 ng/mL and normal glucose metabolism 3.18 ng/mL of premenopausal women. However, The median serum ucOC levels of the 3-groups of postmenopausal women significantly decreased with worsened glucose metabolism (pre-existing diabetes 3.38 ng/mL, newly diagnosed diabetes 4.51 ng/mL, and non-diabetes 5.51 ng/mL) (P<0.0001), suggesting that the serum ucOC level is an indicator of abnormal glucose metabolism.

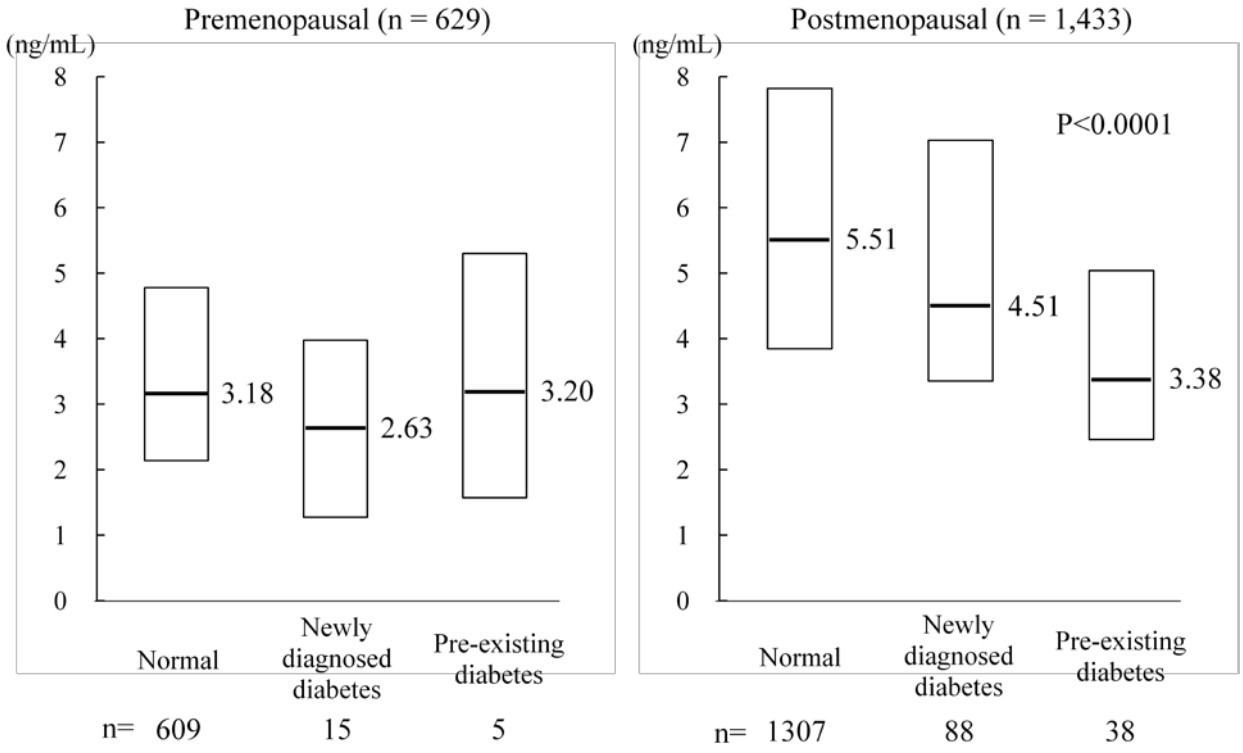

Figure 1: Relationship between the serum undercarboxylated osteocalcin (ucOC) level and the pre-existing or newly diagnosed diabetes of premenopausal and postmenopausal women

The premenopausal and postmenopausal participants were classified into pre-existing diabetes, newly diagnosed diabetes, and normal glucose metabolism) subgroups. Data are shown as median [first-quartile (Q1), third-quartile (Q3)] due to the skewed distribution. The bottom and top of the box are the Q1 and Q3, and the band near the middle of the box is the median.

Factors that contribute to serum ucOC level, classified by menopause status

We performed a multivariate analysis to determine the factors that contribute to changes in serum ucOC level as a continuous variable (**Table 5**). For both premenopausal and postmenopausal women, current smoking was an independent, negative factor for serum ucOC level (P=0.003 and P=0.014, respectively). Age and serum HDL-cholesterol level were positive factors for the serum ucOC level for premenopausal women only (P=0.002 and P=0.034, respectively). However, the FPG, HOMA-IR, HbA1c, and serum triacylglycerol values were independent, negative factors for postmenopausal women only (all P<0.05).

Table 5: Factors that contribute to serum undercarboxylated osteocalcin level as a continuous variable using multiple regression analysis for 2,062 female participants, classified by menopausal status

	Premenopausal women *n* = 629				Postmenopausal women *n* = 1,433		
Factors	β	Standard error (β)	P value		β	Standard error (β)	P value
Age (year)	0.068	0.023	0.002		-	-	-
Fasting plasma glucose (mmol/L)	-	-	-		-0.008	0.002	0.003
Homeostasis model assessment of insulin resistance	-	-	-		-0.037	0.019	0.004
Glycohemoglobin A1c (%)	-	-	-		-0.594	0.108	<0.0001
Serum high-density lipoprotein -cholesterol (mmol/L)	0.012	0.005	0.034				
Serum triacylglycerol (mmol/L)					-0.280	0.128	0.008
Current smoking (vs. non- or past smoking)	-0.456	0.144	0.003		-0.397	0.163	0.014

Discussion

The most interesting finding of our study was that the decreased serum ucOC level of the diabetic women, especially postmenopausal women, suggesting a relationship between diabetes and osteoporosis. A similar epidemiological study involving 2,174 Japanese men over 65 year of age showed negative correlations between serum ucOC and blood glucose, HbA1c, and HOMA-IR values [16]. The other study from Australia showed the reduced serum total OC was associated with metabolic syndrome in 70 year-old or over men via waist circumference, hyperglycemia, and serum triacylglycerol levels [17]. Our study that included a large number of postmenopausal women confirmed these results.

Intracellular pro-OC undergoes posttranslational modification by the vitamin K- and CO_2-dependent synthesis of three γ-carboxyglutamic (Gla) residues, allowing for α-helical conformation of the protein, a prerequisite for osteocalcin to adsorb calcium ions on hydroxyapatite crystals in bone [18]. Hence, carboxylated-type osteocalcin (Gla-OC) accumulates in bone [19]. In contrast, ucOC, with less than three carboxylated Gla residues, does not undergo α-helical conformation and has lesser affinity for Ca^{2+} and hydroxyapatite. While both forms of OC are found in bone, concentrations of total OC in the systemic circulation are relatively smaller, with a greater proportion of serum OC being the undercarboxylated form [20]. Therefore, the serum ucOC level reflects the 3-dimensional architecture of the bone matrix and can be used to assess bone weakening.

The serum ucOC levels of patients with renal dysfunction can be artificially inflated [12]. Therefore, participants with suspected renal dysfunction were removed from the analysis. Moreover, it has been suggested that circulating OC fragments are generated by proteolysis in the circulation or during the processing and storage of samples [21]. Unfortunately, we have no information on the possible overestimation of OC fragments by the electrochemiluminescence immunoassay kit used in the present study. This assay kit is based on the same monoclonal undercarboxylated OC antibodies (OC 4-5 and OC G3) as those of another commercially available kit for measuring ucOC level (Takara Bio Inc. Otsu, Japan). Such immunoassays, including the Takara kit, may not detect fragments because they are specific for either carboxylated or undercarboxylated OC, but these values can be normalized to reflect the total intact OC in the sample [22].

Our study revealed a negative correlation between the serum ucOC level and three well-known markers of glucose metabolism: the values of FPG, fasting serum insulin, and HbA1c. Diabetes confers an increased risk of fracture that is independent of BMD [4]. Patients with diabetes display an increased fracture risk despite a higher BMD, which is mainly attributable to the increased risk of falling [23]. Diabetes can

itself affect bone through several mechanisms: (a) Obesity, widespread in type 2 diabetes, is strongly associated with higher BMD, probably through mechanical loading and hormonal factors, including insulin, estrogen, and leptin [24]. (b) Hyperinsulinemia promotes bone formation, while hypoinsulinemia and the progression of type 2 diabetes causes a reduction of BMD [25]. (c) Hyperglycemia interacts with several proteins to generate a higher concentration of Advanced Glycation End-Products (AGE) in collagen that may reduce bone strength [26]. Accumulated AGEs in the body stimulate apoptosis of osteoblast, thereby contributing to deficient bone formation [27]. (d) Low levels of vitamin D are associated with the incidence of diabetes, and altered vitamin D metabolism leads to diabetic osteopenia [28].

The incidence of osteoporosis increases in postmenopausal women because of decreased estrogen. It is well recognized that estrogen aggravates insulin sensitivity. Premenopausal women are more insulin sensitive with associated improved glucose tolerance, are more resistant to develop insulin resistance compared with men, and display increased expression of glucose transporter 4, while postmenopausal women lose such favorable conditions for glucose metabolism [29]. We have already reported that the serum ucOC level increases with age in women: There is a notable increase after 50 years of age or post menopause [30]. In the present study, we showed that postmenopausal women had a significantly greater incidence of abnormal glucose tolerance than premenopausal women. This finding is a combined effect of other risk factors, including age and BMI, which also influences abnormal glucose metabolism. However, multivariate analysis, classified by menopausal status, revealed significantly reverse correlations of serum ucOC level with FPG, HOMA-IR, and HbA1c concentrations for postmenopausal women only. These results clearly indicate a correlation between abnormal glucose metabolism and the risk of osteoporosis, especially for postmenopausal women.

We also showed that a decrease in the serum HDL-cholesterol level is an indicator of decreased serum ucOC in premenopausal women. Similarly, a low HDL-cholesterol level indirectly indicates a high LDL-cholesterol level, which is linked to arteriosclerosis [31]. Furthermore, abnormalities in the serum lipid profile can accelerate the production of reactive oxygen species, leading to oxidative stress that influences arteriosclerosis [32]. Aggravation of oxidative stress inhibits osteoblast-bone formation and induces bone-cell apoptosis, leading to weakening of the bones [33]. In contrast to postmenopausal women, because there is no decrease in the estrogen level of premenopausal women, it is possible that the changes in lipid homeostasis described above can affect bone integrity. This new insight suggests that a link exists between dyslipidemia and bone weakening. However, further research will be necessary to determine the full extent of the link.

The present study had several limitations. First, it was cross-sectional and we did not prospectively examine the incidence of bone fractures or diabetes. Second, the study was limited to the Japanese population. Further research with other markers and populations will be necessary to confirm our findings. Third, we have no data on other bone turnover markers such as serum procollagen type 1 amino- terminal propeptide, urinary/serum collagen type 1 cross-linked C-telopeptide, or vitamin D level. However, we recruited participants from a large population for analysis in the present study, which means that it is representative of the general community and thus selection bias should make little contribution to the results. We believe this epidemiological study is of high significance, because no large-scale, population study to date has been centered on a comparison of premenopausal and postmenopausal women.

In summary, the serum ucOC level of postmenopausal women was decreased. An important bidirectional relationship was shown between the serum ucOC level and glucose metabolism.

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