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ORIGINAL ARTICLE

Serum undercarboxylated osteocalcin levels are inversely associated with glycemic status and insulin resistance in an elderly Japanese male population: Fujiwara-kyo Osteoporosis Risk in Men (FORMEN) Study

M. Iki · J. Tamaki · Y. Fujita · K. Kouda · A. Yura · E. Kadowaki · Y. Sato · J. S. Moon · K. Tomioka · N. Okamoto · N. Kurumatani

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Abstract
Summary Recent animal studies have demonstrated that undercarboxylated osteocalcin upregulates insulin secretion via osteoblast-insulin signaling. However, it remains unclear whether such a pathway exists in humans. This study showed that serum undercarboxylated osteocalcin levels were inversely associated with fasting plasma glucose, hemoglobin A_{1c}, and homeostasis model assessment of insulin resistance (HOMA-IR) levels in community-dwelling elderly Japanese men.
Introduction Undercarboxylated osteocalcin (ucOC) was reported to increase insulin secretion and improve glucose tolerance via osteoblast-insulin signaling in animal-based studies. Whether this pathway also exists in humans is unknown. We aimed to clarify whether serum ucOC levels are associated with glycemic status and insulin resistance in the general Japanese population.
Methods We included 2,174 Japanese men (≥65 years) who were able to walk without aid from others and lived at home in four cities of Nara Prefecture. We excluded participants with a history of diseases or medications that affect bone metabolism, other than type 2 diabetes mellitus (T2DM). Fasting plasma glucose, glycated hemoglobin A_{1c}, and HOMA-IR levels were determined as outcome measures.
Results Of the 1,597 participants included in the analysis, both intact OC (iOC) and ucOC levels showed significant inverse correlations with all outcome measures, even after adjusting for potential confounders. Mean values of outcome measures showed a significant decreasing trend with higher quintiles of iOC or ucOC after adjusting for confounders. This trend remained significant for ucOC quintiles after further adjustment for iOC levels, but was not significant for iOC quintiles after adjusting for ucOC levels. These results were attenuated, but still apparent, after excluding participants receiving drug therapy for T2DM.
Conclusions Levels of ucOC, but not iOC, were inversely associated with glycemic index and insulin resistance in a population of Japanese men. These findings will need to be confirmed with longitudinal studies.

Keywords Community-dwelling Japanese elderly men · Fasting plasma glucose · Hemoglobin A_{1c} · HOMA-IR · Undercarboxylated osteocalcin

M. Iki (✉) · J. Tamaki · Y. Fujita · K. Kouda · A. Yura · E. Kadowaki
Department of Public Health,
Kinki University Faculty of Medicine,
377-2 Oono-higashi,
Osaka-Sayama, Osaka 589-8511, Japan
e-mail: masa@med.kindai.ac.jp

Y. Sato
Department of Human Life, Jin-ai University,
3-1-1 Ohdecho,
Echizen, Fukui 915-8586, Japan

J. S. Moon
Faculty of Human Sciences, Taisei Gakuin University,
1060-1 Hirao, Mihara-ku,
Sakai, Osaka 587-8555, Japan

K. Tomioka · N. Okamoto · N. Kurumatani
Department of Community Health and Epidemiology,
Nara Medical University School of Medicine,
840 Shijocho,
Kashihara, Nara 634-8521, Japan

Introduction

Osteocalcin (OC) is a peptide synthesized by mature osteoblasts that is used as a biochemical marker for bone formation [1]. Like other bone turnover markers, OC is expected to predict changes in bone mineral density [2–4] and risk of subsequent fractures in postmenopausal women [5, 6]. However, OC shows somewhat different features from other bone turnover markers in patients with type 2 diabetes mellitus (T2DM). It has been consistently documented that patients with T2DM have lower circulating levels of OC and other bone turnover markers compared to people without T2DM [7–11]. Further, Okazaki et al. [12] reported that serum OC levels in Japanese patients with poorly controlled T2DM significantly increased after glycemic control was achieved. Interestingly, other biochemical markers of bone formation or resorption decreased significantly after glycemic control in the same patients. Similar findings were observed in another group of Japanese patients [13] and in other ethnic group patients [14, 15]. These findings suggest that OC may play a role in glucose metabolism.

Recently, Lee et al. [16] reported the results of a series of animal and cell-based experiments. They demonstrated that OC deficiency in mice reduced islet β-cell proliferation, lowered serum insulin concentrations, decreased responsiveness to insulin, and increased glucose intolerance. Administration of recombinant OC improved these conditions in OC-deficient mice, and enhanced insulin secretion and glucose tolerance in wild-type mice [17]. Notably, these effects were associated with OC that was not γ-carboxylated. Posttranslational γ-carboxylation activates OC in extracellular matrix mineralization processes but may inactivate OC in glucose metabolism.

As expected from animal studies, inverse associations between circulating OC levels and fasting plasma glucose (FPG) [10, 11, 18–21], homeostasis model assessment of insulin resistance (HOMA-IR) [19–21], and glycated hemoglobin A_{1c} (HbA1c) levels [9, 11, 13, 19] have been observed in patients with T2DM [9, 10, 13] and in population-based cohorts [19–21]. However, these studies did not differentiate between the effects of carboxylated OC and those of undercarboxylated OC. Recently, Shea et al. [22] reported that only carboxylated OC was inversely associated with HOMA-IR in non-diabetic primarily white elderly men and women. This finding was supported by a longitudinal analysis of a subgroup from the same subjects. However, in a study of middle-aged Chinese men, of whom 73% were diabetic, serum concentrations of both undercarboxylated (ucOC) and carboxylated OC were inversely associated with FPG and HbA1c levels [23]. More recently, Kanazawa et al. reported that serum ucOC levels were inversely correlated with FPG and HbA1c levels in Japanese men and women with T2DM [24]. In that study, however, a similar correlation was also observed for total OC levels, but not for the ucOC/total OC ratio. Thus, the form of OC involved in human glucose metabolism remains unclear.

In the present study, we tested the hypothesis that ucOC levels are associated with FPG, HbA1c, and HOMA-IR levels independently of intact OC levels in elderly Japanese men in a population-based large-scale study.

Materials and methods

Study settings

The Fujiwara-kyo Osteoporosis Risk in Men (FORMEN) study is an ancillary study of a larger prospective cohort study, the Fujiwara-kyo Study (Primary Investigator: Norio Kurumatani, M.D., Ph.D., Professor and Chairman, Department of Community Health and Epidemiology, Nara Medical University School of Medicine), which aims to provide a scientific basis for a comprehensive strategy to prevent frailty, increase the number of healthy life years, and enhance functioning and quality of life of elderly men and women in Japan. The FORMEN Study evaluates bone health in male participants of the Fujiwara-kyo Study. Details of the Fujiwara-kyo Study and the FORMEN Study have been described elsewhere [25].

Study participants

Participants for the Fujiwara-kyo Study were enrolled in four cities of Nara Prefecture, Japan on a volunteer basis. Inclusion criteria were 65 years or older at enrollment, living at home, ability to walk without the assistance from another person, and ability to provide self-reported information and written informed consent. Of the 4,427 participants of the Fujiwara-kyo Study, 2,174 men were included in the FORMEN Study. Exclusion criteria were set as incomplete tests in the FORMEN Study, past or current illness, or medications known to affect bone metabolism (e.g., uncontrolled hyperthyroid disease, parathyroid disease, type 1 diabetes mellitus (T1DM), connective tissue disease, gastrectomy due to cancer or ulcer, prostate cancer with anti-androgen therapy, oral glucocorticoid therapy ≥5 mg/day for more than 3 months, bisphosphonate therapy for more than 6 months, vitamin D use for more than 2 years, and current warfarin use or vitamin K supplementation).

The study protocol of the Fujiwara-kyo Study was approved by the Medical Ethics Committee of Nara Medical University, and the protocol of the FORMEN Study was approved by the Ethics Committee of Kinki University Faculty of Medicine.

Medical history and lifestyle factors

Participants completed a questionnaire consisting of 250 items that covered medical history of malignant diseases, hypertension, diabetes mellitus, coronary heart disease, hyperlipidemia, asthma, kidney disease, prostate disease, medications due to these diseases, items related to the exclusion criteria of the present study, smoking and drinking, diet including regular consumption of milk, and the fermented soy bean product *natto*—a traditional Japanese food that is a major source of vitamin K_2 in Japan [26]. In addition, participants answered questions from the International Physical Activity Questionnaire [27] to calculate energy expenditure due to physical activity. Participants were asked to bring current prescriptions of medications to the baseline visit, and interviewers recorded the names and doses of the medications including insulin, thiazolidinediones, and other anti-diabetic drugs.

Definition of type 2 diabetes mellitus

T2DM was defined as T2DM or non-insulin-dependent diabetes mellitus that was diagnosed by a physician, or middle-age or elderly onset of physician-diagnosed diabetes mellitus without specification of type 1 or type 2, or by one of the following biochemical test results obtained in the present study according to guidelines of the American Diabetes Association [28] and the Japan Diabetes Society [29]: FPG level ≥126 mg/dl or HbA1c levels ≥6.5%. Participants reporting a history of diabetes mellitus were considered to be non-diabetic if they were not on drug therapy and their FPG and HbA1c levels were both normal.

Laboratory measurements

We drew blood from each participant after an overnight fast and obtained plasma and serum for conventional biochemical tests: FPG, HbA1c, and serum insulin (FSI). We stored the remaining serum at −80°C until we performed measurements of bone turnover markers: intact OC (iOC), ucOC, and tartrate-resistant acid phosphatase isoenzyme 5b (TRACP5b).

FPG levels (mg/dl) were determined by the hexokinase-glucose-6-phosphate dehydrogenase method (L-type Glu 2, Wako Pure Chemical Industries, Ltd., Osaka, Japan); HbA1c levels (%) by the latex aggregation immunoassay (Determiner L HbA1C, Kyowa Medex Co., Tokyo, Japan); and FSI levels (μU/ml) by the chemiluminescent enzyme immunoassay (Lumipulse Presto II/Insulin, Fujirebio Inc., Tokyo, Japan). HbA1c values were converted to National Glycohemoglobin Standardization Program values according to guidelines established by the Japan Diabetes Society [30]. To estimate insulin resistance, HOMA-IR was calculated using FPG and FSI values [31].

We measured iOC (ng/ml) with a two-site immunoradiometric assay (BGP IRMA kit Mitsubishi, Mitsubishi Kagaku Iatron Inc., Tokyo, Japan) that had a sensitivity of 1 ng/ml [32] with a 4.9% intra-assay coefficient of variation (CV), 3.7% inter-assay CV, and 6.1% overall CV. We used an electrochemiluminescence immunoassay to measure ucOC (ng/ml; Picolumi ucOC, Sanko Junyaku Co. Ltd., Tokyo, Japan) with a sensitivity of 0.39 ng/ml [33], 4.1% intraassay CV, 3.5% inter-assay CV, and 5.4% overall CV. The monoclonal antibodies used in the assay recognized OC molecules with two uncarboxylated glutamic acid residues; therefore, the ucOC measured in this study may have contained one carboxylated residue per OC molecule. TRACP5b was measured by a fragment-absorbed immunocapture enzyme assay (Osteolinks-TRAP-5b, Nitto Boseki, Koriyama, Japan) with a sensitivity of 19.2 mU/dl [34], 4.9% intraassay CV, 7.3% inter-assay CV, and 8.8% overall CV.

Body size measurements

We measured the height (cm) and weight (kg) of participants with an automatic scale, and calculated BMI (kg/m²).

Statistical analysis

All statistical calculations were performed with SAS software (Version 9.13, SAS Institute, Cary, NC, USA) on a personal computer. Levels of biochemical markers for bone turnover and glucose metabolism all distributed log-normally; therefore, statistical analyses were performed after logarithmic conversion of values. These data are expressed as geometric means and SDs. Since ucOC levels were affected by bone formation, the ucOC/iOC ratio was calculated to adjust for this effect. Mean levels of glucose metabolism indices among quintile groups of iOC or ucOC and mean levels of iOC or ucOC in participants with or without T2DM were calculated after adjusting for potential confounding factors including age, height, weight, smoking pack-years, weekly ethanol consumption, physical activity in metabolic equivalent minutes per day, and milk intake by the general linear model with the Tukey–Kramer multiple comparison procedure when appropriate. Multiple logistic regression was used to determine odds ratios (ORs) of prevalent T2DM among quintile groups of iOC or ucOC after adjusting for potential confounding variables.

Results

Basic characteristics of participants

Among 2,174 male participants of the Fujiwara-kyo Study, 2,012 completed the study procedures of the FORMEN

Study. We excluded 313 men who met the exclusion criteria, and an additional 102 men did not provide complete information regarding smoking and drinking habits, physical activity, or milk and natto intake.

Table 1 shows the basic characteristics of the remaining 1,597 participants classified by quintiles of iOC or ucOC levels. The correlation coefficient between iOC and ucOC was 0.655 ($p<0.0001$), indicating that basic characteristics of participants according to quintiles of iOC and ucOC were very similar. In the higher iOC or ucOC quintiles, participants were older, weighed less, and consumed less ethanol. The prevalence of T2DM in the lowest iOC quintile was twice that in the highest quintile, and the T2DM prevalence in the lowest quintile of ucOC was three times that in the highest quintile.

Table 2 shows laboratory test results grouped by iOC and ucOC levels. Similar trends of laboratory test results were observed among the quintile groups for iOC and ucOC levels. Highly significant inverse associations were seen for FPG, HbA1c, FSI, HOMA-IR, and triglyceride levels with iOC and ucOC ranks.

Correlations between iOC, ucOC, or TRACP5b levels and glucose metabolism indices

Table 3 shows correlation coefficients between iOC, ucOC, ucOC/iOC ratio, or TRACP5b levels and glucose metabolism indices. Levels of iOC and ucOC, and the ucOC/iOC ratio showed significant inverse correlations with all glucose metabolism indices, even after adjusting for potential confounding factors. In contrast, TRACP5b levels did not show significant correlations. The correlation coefficients of iOC with glucose metabolism indices turned out to be insignificant when further adjusted for ucOC; however, correlation coefficients of ucOC with glucose metabolism indices remained significant when further adjusted for iOC levels.

Serum iOC and ucOC levels and mean levels of glucose metabolism indices

The adjusted mean levels of glucose metabolism indices are shown by quintile group of serum iOC or ucOC levels in Fig. 1. Adjustment was made for potential confounding factors, as well as ucOC for the analysis of iOC and iOC for the analysis of ucOC. Significant decreasing trends were observed for all glucose metabolism indices in higher ucOC quintiles, and similar trends were also seen for the ucOC/iOC ratio (data not shown), but the corresponding trends for iOC were not significant. These associations were considerably attenuated when 176 participants on drug therapy for T2DM, including 17 men on insulin and 30 on thiazolidinediones, were excluded from the analysis, but were still significant for FPG with ucOC [mg/dl, mean (95% confidence interval): 100.7 (98.7, 102.8), 97.7 (95.8, 99.6), 97.3 (95.4, 99.2), 96.6 (94.6, 98.6), and 95.6 (93.4, 97.8) for lowest to highest quintile groups of ucOC, respectively; p for trend=0.0041], and HbA1c with ucOC [%, mean (95% confidence interval): 5.66 (5.59, 5.73), 5.51 (5.45, 5.58), 5.50 (5.43, 5.57), 5.51 (5.44, 5.58), and 5.45 (5.38, 5.53); p for trend=0.0025].

Prevalent T2DM and serum iOC and ucOC levels

In participants with T2DM, both iOC and ucOC levels, and the ucOC/iOC ratio were significantly lower than in participants without T2DM, even after adjusting for confounders [ng/ml, mean (95% confidence interval): 4.4 (4.2, 4.7) vs. 5.0 (4.9, 5.2) for iOC, $p<0.0001$; 2.2 (2.0, 2.4) vs. 3.0 (2.9, 3.1) for ucOC, $p<0.0001$; 0.56 (0.52, 0.59) vs. 0.66 (0.64, 0.68) for ucOC/iOC, $p<0.0001$]. The difference remained significant for ucOC when further adjusted for iOC levels [2.4 (2.3, 2.5) vs. 2.9 (2.8, 3.0), $p<0.0001$], whereas the difference in iOC levels between participants with and without T2DM was not significant after ucOC adjustment [4.9 (4.7, 5.1) vs. 4.8 (4.7, 5.0), $p=0.6032$].

Serum iOC and ucOC levels and ORs of prevalent T2DM

Figure 2 illustrates ORs of prevalent T2DM among the quintile groups of iOC or ucOC levels compared with the lowest quintile group after adjusting for potential confounding factors and further adjusting for ucOC for iOC analysis or iOC for ucOC analysis. A significant dose-dependent decreasing trend in ORs was observed for ucOC (OR [95% confidence interval]: 0.487 [0.330, 0.719], 0.441 [0.291, 0.669], 0.298 [0.181, 0.493], and 0.208 [0.113, 0.381] for second lowest to highest quintile groups, respectively; p for trend<0.0001), but not for iOC quintile groups.

Discussion

In the present study, serum ucOC and iOC levels were inversely associated with FPG, HbA1c, FSI, and HOMA-IR levels in elderly Japanese men; associations with iOC were principally explained by ucOC levels. While these associations were relatively modest, they were statistically significant. Our findings are consistent with results from animal and cell-based studies [16, 17] demonstrating that ucOC functions as a hormone that enhances responsiveness to insulin and glucose tolerance. These findings are also supported by a recent study [35] reporting that mice lacking the insulin receptor only in osteoblasts exhibited low circulating ucOC levels and decreased bone formation, and

Table 1 Anthropometric and lifestyle characteristics of participants in quintile groups of serum iOC or ucOC levels

	Total	Quintiles of serum iOC concentration (range in ng/ml)					*P* for trend
		Q1 (1.1–3.5)	Q2 (3.5–4.6)	Q3 (4.6–5.5)	Q4 (5.5–6.9)	Q5 (6.9–17.0)	
N	1,597	329	304	330	313	321	
Age (year)	73.0±5.2	72.2±4.7	72.8±5.4	73.0±5.1	72.9±5.2	74.1±5.4	0.0017
Height (cm)	162.8±5.7	162.7±5.7	162.5±5.2	163.0±6.0	162.7±6.0	163.2±5.5	0.1947
Weight (kg)	61.2±8.4	61.6±8.6	61.8±8.5	60.9±8.5	60.9±8.5	60.0±8.5	0.0123
BMI (kg/m2)	23.1±2.7	23.2±2.7	23.4±2.8	23.3±2.6	23.0±2.7	22.5±2.7	0.0002
Current smoker (%)	17.5	19.2	17.8	18.2	14.1	18.7	0.3833
Ex-smoker (%)	60.2	59.4	63.8	58.8	63.9	56.7	0.7245
Smoking (pack year)	28 (5, 49)	30 (3, 52)	33 (11, 50)	28 (5, 49)	24(5, 46)	26 (0, 46)	0.0416
Habitual drinker (%)	48.8	60.8	51.0	48.2	47.0	37.1	<0.0001
Ethanol intake (g/week) [a]	88 (0, 224)	151 (0, 291)	91 (0, 245)	87(0, 224)	52 (0, 182)	9.8 (0, 91)	<0.0001
Physical activity (MET·min/day)[a]	174 (75, 361)	167 (67, 301)	174 (78, 322)	164 (86, 377)	193 (75, 386)	183(79, 370)	0.0808
Milk consumption ≥200 ml/day (%)	52.1	52.3	53.6	55.8	47.6	51.1	0.3523
Natto consumption ≥1 pack/week (%)	43.4	44.7	48.7	45.8	39.0	38.9	0.0172
Prevalence of T2DM (%)	17.9	25.2	22.4	19.1	11.5	10.9	<0.0001
		Quintiles of serum ucOC concentration (range in ng/ml)					*P* for trend
		Q1 (0.4–1.8)	Q2 (1.8–2.4)	Q3 (2.4–3.2)	Q4 (3.3–4.5)	Q5 (4.5–21.4)	
N		319	317	323	318	320	
Age (year)		72.6±4.9	72.7±5.2	73.0±4.9	72.7±5.1	74.0±5.5	0.0009
Height (cm)		162.9±5.4	162.9±5.4	162.4±5.9	163.4±5.7	162.5±6.0	0.7756
Weight (kg)		61.7±8.4	61.2±8.3	61.2±8.3	62.4±8.5	59.4±8.3	0.0079
BMI (kg/m2)		23.2±2.7	23.1±2.7	23.4±2.7	23.4±2.7	22.5±2.7	0.0035
Current smoker (%)		21.3	15.1	17.0	17.9	15.9	0.2341
Ex-smoker (%)		60.2	62.2	62.5	55.4	60.9	0.5432
Smoking (pack-year)[a]		30 (11, 52)	28 (3, 47)	30 (7, 50)	24 (0, 46)	25 (2, 49)	0.1318
Habitual drinker (%)		57.7	53.9	49.2	47.2	36.3	<0.0001
Ethanol intake (g/week)[a]		133 (0, 301)	91 (0, 231)	88 (0, 242)	52 (0, 198)	10 (0, 140)	<0.0001
Physical activity (MET·min/day)[a]		189 (86, 375)	183 (86, 341)	172(64, 359)	158 (75, 375)	163 (75, 346)	0.8092
Milk consumption ≥200 ml/day (%)		57.1	57.4	53.9	48.4	43.8	<0.0001
Natto consumption ≥1 pack/week (%)		61.8	51.7	40.3	38.4	25.0	<0.0001
Prevalence of T2DM (%)		31.7	18.3	18.0	12.3	9.1	<0.0001

Data are expressed as mean±SD or proportion (%)

Q1–Q5 lowest to highest quintile groups, *BMI* body mass index, *Natto* Japanese traditional fermented soy bean product, *T2DM* type 2 diabetes mellitus

[a] Median (first and third quartiles)

Table 2 Laboratory test results of participants in quintile groups of serum iOC or ucOC levels

	Total	Quintiles of serum iOC concentration (range in ng/ml)					*P* for trend
		Q1 (1.1–3.5)	Q2 (3.5–4.6)	Q3 (4.6–5.5)	Q4 (5.5–6.9)	Q5 (6.9–17.0)	
iOC (ng/ml)	4.9 (1.5)	2.6 (1.3)	4.0 (1.1)	5.0 (1.1)	6.2 (1.1)	8.4 (1.2)	–
ucOC (ng/ml)	2.8 (1.8)	1.7 (1.8)	2.1 (1.5)	2.7 (1.5)	3.5 (1.5)	5.1 (1.5)	<0.0001
ucOC/iOC	0.67 (1.60)	0.63 (1.9)	0.52 (1.5)	0.53 (1.5)	0.56 (1.5)	0.61 (1.4)	0.9628
TRACP5b (mU/dl)	209.6 (1.7)	140.4 (1.8)	181.2 (1.7)	211.1 (1.5)	246.9 (1.5)	307.1 (1.5)	<0.0001
FPG (mg/dl)	101.4 (1.2)	105.9 (1.3)	103.7 (1.3)	102.2 (1.2)	98.0 (1.2)	97.4 (1.2)	<0.0001
HbA1c (%)	5.3 (1.1)	5.4 (1.2)	5.4 (1.1)	5.3 (1.1)	5.2 (1.1)	5.1 (1.1)	<0.0001
FSI (mU/l)[a]	5.0 (2.0)	5.3 (2.0)	5.5 (2.1)	4.9 (2.0)	4.8 (2.0)	4.5 (2.1)	<0.0001
HOMA-IR[a]	1.2 (2.3)	1.4 (2.3)	1.4 (2.4)	1.2 (2.3)	1.2 (2.1)	1.1 (2.2)	<0.0001
Triglyceride (mg/dl)	116.4 (1.6)	126.1 (1.7)	122.1 (1.6)	110.8 (1.6)	114.6 (1.6)	109.4 (1.6)	<0.0001
Total C (mg/dl)	204.3 (1.2)	204.6 (1.2)	202.2 (1.2)	205.5 (1.2)	208.0 (1.2)	201.4 (1.2)	0.5710
HDL-C (mg/dl)	53.5 (1.3)	53.7 (1.3)	53.3 (1.3)	53.9 (1.3)	53.8 (1.3)	52.8 (1.3)	0.4433
LDL-C (mg/dl)	119.7 (1.3)	118.6 (1.3)	117.7 (1.3)	121.9 (1.3)	123.0 (1.3)	117.4 (1.3)	0.7763
	Conversion factor for SI units	Quintiles of serum ucOC concentration (range in ng/ml)					*P* for trend
		Q1 (0.4–1.8)	Q2 (1.8–2.4)	Q3 (2.4–3.2)	Q4 (3.3–4.5)	Q5 (4.5–21.4)	
iOC (ng/ml)	0.1709 to nmol/l	3.4 (1.4)	4.1 (1.4)	4.6 (1.4)	5.9 (1.4)	7.2 (1.4)	<0.0001
ucOC (ng/ml)	0.1718 to nmol/l	1.2 (1.5)	2.1 (1.1)	2.8 (1.1)	3.8 (1.1)	6.2 (1.3)	–
ucOC/iOC	–	0.34 (1.5)	0.51 (1.4)	0.60 (1.4)	0.65 (1.4)	0.86 (1.4)	0.0001
TRACP5b (mU/dl)	–	168.0 (1.7)	179.8 (1.7)	199.6 (1.7)	239.1 (1.6)	280.3 (1.6)	0.0001
FPG (mg/dl)	0.05551 to mmol/l	109.9 (1.3)	101.4 (1.2)	101.3 (1.2)	98.4 (1.2)	96.6 (1.2)	0.0001
HbA1c (%)	–	5.6 (1.2)	5.3 (1.1)	5.3 (1.1)	5.2 (1.1)	5.1 (1.1)	0.0001
FSI (mU/l)[a]	6.0 to pmol/l	5.6 (2.1)	5.1 (2.0)	4.9 (2.0)	4.9 (1.9)	4.6 (2.1)	0.0001
HOMA-IR[a]	–	1.5 (2.5)	1.3 (2.3)	1.2 (2.2)	1.2 (2.1)	1.1 (2.3)	0.0001
Triglyceride (mg/dl)	0.01129 to mmol/l	125.9 (1.7)	114.9 (1.6)	113.2 (1.6)	117.8 (1.6)	110.7 (1.6)	0.0040
Total C (mg/dl)	0.02586 to mmol/l	203.2 (1.2)	205.2 (1.2)	204.8 (1.2)	204.4 (1.2)	204.1 (1.2)	0.7780
HDL-C (mg/dl)	0.02586 to mmol/l	53.6 (1.3)	54.2 (1.3)	54.0 (1.3)	53.0 (1.3)	52.6 (1.3)	0.2531
LDL-C (mg/dl)	0.02586 to mmol/l	116.8 (1.3)	120.6 (1.3)	120.2 (1.3)	120.6 (1.3)	120.4 (1.3)	0.1418

Data are expressed as geometric mean and SD in parenthesis. Arithmetic mean+SD and mean−SD values correspond to geometric mean×SD and mean÷SD, respectively

Q1–Q5 lowest to highest quintile groups, *TRACP5b* tartrate-resistant acid phosphatase isoenzyme 5b, FPG: fasting plasma glucose, *HbA1c* hemoglobin A1c, *FSI* fasting serum insulin, *HOMA-IR* homeostatic model assessment for insulin resistance, *total C* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

[a] For 1,580 participants without current insulin treatment

developed hyperglycemia accompanied by severe glucose intolerance and insulin resistance. Notably, these metabolic abnormalities were rescued by ucOC infusion, suggesting that osteoblast-insulin signaling presents a closed-loop feedback that regulates bone formation and glucose metabolism via the hormonal actions of ucOC.

Bone turnover is also regulated by many other factors, including sex hormones, parathyroid hormone, calcium and vitamin D intake, and mechanical stress, which are all independent of glucose metabolism. Furthermore, many factors regulate glucose metabolism independently of bone turnover. Osteoblast-insulin signaling would present one of the bone and glucose metabolism regulating pathways if it exists in humans. Furthermore, circulating ucOC levels are clearly vitamin K-dependent [36], but glucose levels are not. In fact, vitamin K supplementation had no significant effect on HOMA-IR and plasma insulin levels in a RCT, and its subgroup analysis showed a modest but statistically significant decreasing effect on the same indices in men [37]. This suggests that vitamin K has no effect or even a decreasing effect on blood glucose levels, despite its serum ucOC reducing activity. These results would be difficult to explain given the hormonal effects of ucOC on osteoblast-insulin signaling. Accordingly, the complete loss of function of a specific gene in an animal study may not necessarily equate to a pathological change in bone and

Table 3 Correlation coefficients between iOC, ucOC, ucOC/iOC ratio, or TRACP 5b, and glucose metabolism indices.

		FPG	HbA1c	FSI[a]	HOMA-IR[a]	TG
iOC	Crude	−0.125	−0.142	−0.076	−0.098	−0.101
		$p<0.0001$	$p<0.0001$	$p=0.0025$	$p=0.0001$	$p<0.0001$
	Adjusted	−0.112	−0.144	−0.063	−0.085	−0.072
		$p<0.0001$	$p<0.0001$	$p=0.0121$	$p=0.0008$	$p=0.0044$
	Further adjusted for ucOC	0.016	0.007	0.003	0.007	−0.045
		$p=0.5262$	$p=0.7748$	$p=0.9105$	$p=0.7819$	$p=0.0769$
ucOC	Crude	−0.204	−0.228	−0.108	−0.144	−0.086
		$p<0.0001$	$p<0.0001$	$p<0.0001$	$p<0.0001$	$p=0.0006$
	Adjusted	−0.193	−0.233	−0.102	−0.141	−0.059
		$p<0.0001$	$p<0.0001$	$p<0.0001$	$p<0.0001$	$p=0.0198$
	Further adjusted for iOC	−0.159	−0.185	−0.080	−0.113	−0.017
		$p<0.0001$	$p<0.0001$	$p=0.0014$	$p<0.0001$	$p=0.5103$
ucOC/iOC	Crude	−0.155	−0.172	−0.072	−0.101	−0.021
		$p<0.0001$	$p<0.0001$	$p=0.0040$	$p<0.0001$	$p=0.4083$
	Adjusted	−0.151	−0.173	−0.076	−0.107	−0.012
		$p<0.0001$	$p<0.0001$	$p=0.0026$	$p<0.0001$	$p=0.6362$
TRACP 5b	Crude	−0.029	−0.069	−0.150	−0.139	−0.073
		$p=0.2533$	$p=0.0061$	$p<0.0001$	$p<0.0001$	$p=0.0034$
	Adjusted	0.041	0.022	−0.051	−0.030	0.027
		$p=0.1022$	$p=0.3859$	$p=0.0431$	$p=0.2318$	$p=0.2820$

Adjusted adjusted for age, height, weight, weekly alcohol consumption, smoking (pack-years), physical activity (MET·min/day), milk intake and fermented soy bean product '*natto*' intake

FPG fasting plasma glucose, *HbA1c* hemoglobin A1c, *FSI* fasting serum insulin, *HOMA-IR* homeostatic model assessment for insulin resistance, *TG* triglyceride

[a] 1,580 participants without current insulin treatment

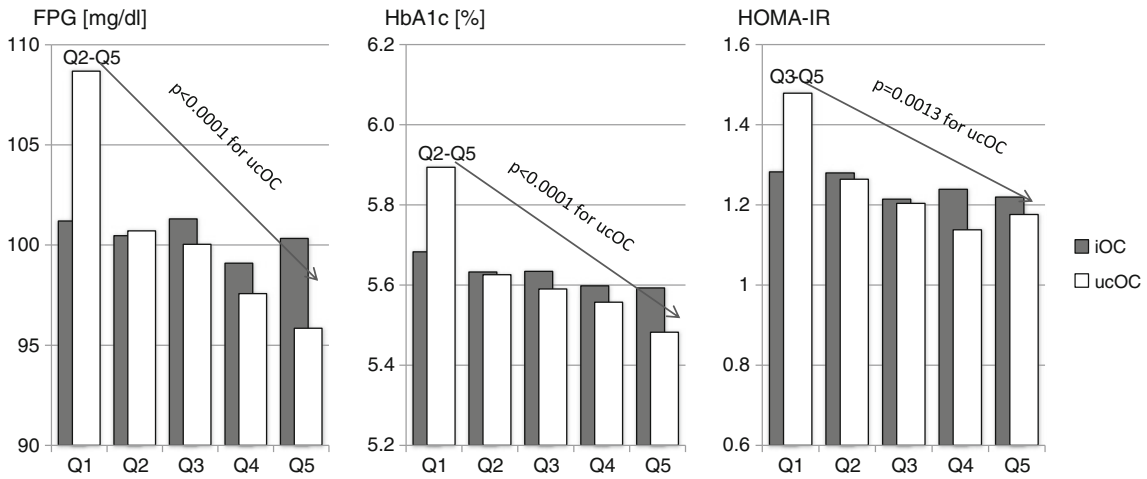

Fig. 1 Adjusted mean values of glucose metabolism indices among quintile groups of intact osteocalcin (iOC) and undercarboxylated osteocalcin (ucOC) levels. For the analysis of iOC, means were adjusted for ucOC; for the analysis of ucOC, means were adjusted for iOC. In addition, adjustments were made for age, height, weight, weekly ethanol consumption, pack-years of smoking, physical activity in MET·min/day, milk intake, and fermented soy bean product '*natto*' intake. *FPG* fasting plasma glucose, *HbA1c* glycated hemoglobin A_{1c}, *HOMA-IR* homeostasis model assessment of insulin resistance, *Q1–Q5* lowest to highest quintile groups of iOC or ucOC, *Q2–Q5*, *Q3–Q5* significantly different from Q2–Q5 or Q3–Q5 quintile groups ($p<0.05$). The *arrow* indicates a significant linear relationship between ucOC quintile groups and levels of glucose metabolism indices in the regression analysis, with adjustment for the confounding variables listed above

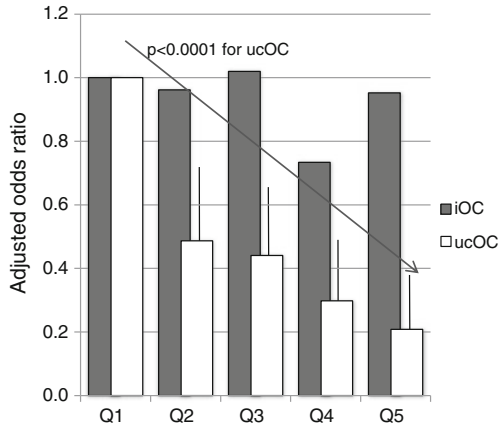

Fig. 2 Adjusted odds ratios of prevalent type 2 diabetes mellitus (T2DM) for quintile groups of intact osteocalcin (iOC) or undercarboxylated osteocalcin (ucOC) levels, with the lowest quintile used as reference. Odds ratios were adjusted for ucOC to analyze iOC and adjusted for iOC to analyze ucOC. In addition, adjustments were made for age, height, weight, weekly ethanol consumption, pack-years of smoking, physical activity in MET·min/day, milk intake, and fermented soy bean product '*natto*' intake. *Q1–Q5* quintile groups of iOC or ucOC. *Vertical line on each open column* denotes the upper 95% confidence limit for OR for the ucOC quintile group. The *arrow* indicates a significant linear relationship between ucOC quintile groups and odds ratios of T2DM in the logistic regression analysis, with adjustment for the confounding variables listed above

glucose metabolism in humans. Nonetheless, it will be informative to determine whether osteoblast-insulin signaling functions as a pathway that reciprocally regulates bone turnover and glucose metabolism in humans.

Although circulating OC levels are inversely associated with HOMA-IR and FPG levels as previously reported in human studies, the form of OC—ucOC or carboxylated OC—associated with glucose metabolism indices is unclear. Shea et al. [22] reported that only carboxylated OC was inversely correlated with HOMA-IR and FPG. The same study group reported supporting data from a cross-sectional study showing that men and women with higher dietary phylloquinone (vitamin K_1) intake, indicating lower ucOC levels, had lower HOMA-IR values [38]. On the other hand, Kanazawa et al. reported that serum ucOC and total OC levels were inversely correlated with FPG and HbA1c levels in Japanese men and women with T2DM [24]. While these findings are partially consistent with our results, the ucOC/total OC ratio was neither correlated with FPG nor HbA1c levels in that study. This implies that ucOC levels were proportionate to total OC levels and, therefore, that the remainder of total OC, mostly carboxylated OC, was also proportionate to total OC and could have been inversely associated with FPG and HbA1c. Thus, our study and those by Shea et al. [22] and Kanazawa et al. [24] report different results. Further research is needed to determine the form of OC that is associated with glucose metabolism indices in humans.

Another issue that needs to be addressed is whether osteoblast-insulin signaling in bone and glucose metabolism also applies to T1DM and T2DM in humans. Serum insulin levels decrease in T1DM, but increase in T2DM. Despite this, reduced serum OC [39–42] and an inverse correlation between circulating OC and HbA1c levels [40, 42] were observed in T1DM patients as well as T2DM patients. In T1DM, decreased insulin secretion from pancreatic β-cells may directly reduce the production of ucOC and OC from osteoblasts, which leads to a further reduction in insulin secretion. In T2DM, insulin resistance results in elevated blood glucose levels, and this prolonged hyperglycemia has been reported to decrease bone formation by osteoblasts [39, 43, 44]. This in turn results in the reduced production of ucOC and OC, which may lead to further increase in insulin resistance by decreasing adiponectin formation in adipocytes [16]. These findings support the possibility that osteoblast-insulin signaling could function in T1DM and T2DM in humans, but this remains to be confirmed.

The present study has several strengths. This population-based study had a sufficiently large sample size, which may reflect the health status of a general elderly male population in Japan. We evaluated a range of lifestyle and geriatric factors with validated methods at baseline, some of which were used to adjust for potential confounding factors. The present study is a part of an ongoing cohort study that plans a 10-year follow-up with three waves of clinical surveys at a university hospital, where incident cases of T2DM can be confirmed. In addition, it is a single-center study free of inter-center variations.

However, we should acknowledge several limitations. Participants of the FORMEN Study were community-dwelling volunteers, thus patients with severe or symptomatic T2DM may have been less likely to participate in the study. This potential bias may have resulted in underestimation of the association of interest. Second, participants were restricted to elderly Japanese men, thus caution should be used in generalizing the results. Third, we measured only intact molecules of OC, levels of which would be lower than total OC levels, whereas the assay for ucOC may have overestimated the real value because the antibody used in the assay recognizes N-terminal fragments of OC [45]. This prevents the calculation of carboxylated OC levels by subtracting ucOC levels from total OC levels, and directly confirming the association between carboxylated OC and glucose metabolism indices. Fourth, the diagnosis of T2DM was based on self-reported data, FPG and HbA1c levels were determined only once, and glucose tolerance was not tested. Therefore, misclassification of patients may have occurred, but this also underestimated the strength of the association. Fifth, thiazolidinedione therapy for T2DM, which has been reported to decrease bone formation and serum OC levels [46], may have confounded the association. Despite this, the association was attenuated and

remained significant after excluding subjects on drug therapy (including thiazolidinediones) for T2DM. Finally, all results were obtained from cross-sectional analyses; therefore, we cannot determine at this time whether OC regulates glucose metabolism or hyperglycemia suppresses bone formation and OC production.

Conclusions

In the present study, serum ucOC levels showed significant inverse associations with glucose metabolism indices (e.g., FPG, FSI, HOMA-IR, and HbA1c) in a population of community-dwelling elderly Japanese men; however, iOC, after adjusting for ucOC levels, was not significantly associated with these indices. These findings, which are consistent with results from animal-based studies, should be confirmed by longitudinal studies in humans.

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Conflicts of interest None.

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