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Hyperglycemia is associated with increased bone mineral density and decreased trabecular bone score in elderly Japanese men: The Fujiwara-kyo osteoporosis risk in men (FORMEN) study

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ABSTRACT

Purpose: Patients with type 2 diabetes mellitus (T2DM) have an increased fracture risk despite having higher areal bone mineral density (aBMD). This study aimed to clarify the association between glycemic and insulin resistance status and bone microarchitecture, and whether pentosidine and bone turnover markers play any roles in the association.

Methods: A total of 2012 community-dwelling men aged ≥65 years completed baseline measurements of spine aBMD, fasting plasma glucose (FPG) and serum insulin, hemoglobin A1c (HbA1c), osteocalcin, type I procollagen N-terminal propeptide, type I collagen C-terminal crosslinking telopeptide, tartrate-resistant acid phosphatase isoenzyme 5b, pentosidine, height and weight and an interview regarding past disease history. Homeostasis model assessment-insulin resistance (HOMA-IR) was also calculated. T2DM was defined as physician-diagnosed middle age or elderly-onset diabetes mellitus, or according to biochemical test results. To evaluate bone microarchitecture, trabecular bone score (TBS) was calculated at the same vertebrae as those used for aBMD measurement.

Results: After excluding participants who had a disease history and/or were taking medications affecting bone metabolism, 1683 men (age, 72.9 ± 5.2 years) were analyzed. Men with T2DM had significantly higher aBMD compared to those without T2DM. There was no significant difference in TBS. However, FPG, HbA1c and HOMA-IR levels were significantly inversely correlated with TBS after adjusting for age, BMI and aBMD. Multivariate linear regression analyses revealed that glycemic indices (FPG and HbA1c) were significantly associated with increased aBMD and decreased TBS, and that HOMA-IR was associated only with TBS. These associations did not change after further adjusting for bone turnover makers and pentosidine levels.

Conclusions: Hyperglycemia and elevated insulin-resistance were associated with low TBS independently of bone turnover and pentosidine levels.

1. Introduction

Osteoporosis and diabetes mellitus are both on the rise due to an increase in the elderly population, posing great public health and economic burdens on society [1] [2]. Although they represent different disease entities, diabetes mellitus, either type 1 or type 2, has been reported to increase the risk of fracture [3–7]. Interestingly, patients with type 2 diabetes mellitus (T2DM) often show significantly higher areal bone mineral density (aBMD) compared to non-diabetic controls [4]. Therefore, non-bone mass features representing bone strength, or bone quality, may play a substantial role in increasing fracture risk in patients with T2DM.

Bone quality includes several different elements such as material property, turnover and microarchitecture [8]. To date, many studies have examined effects of advanced glycation end-products (AGEs) [9] [10] or bone turnover markers [11] [12] [13] on increased fracture risk in diabetic patients, but only a few studies on bone microarchitecture

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have been published [14] [15], especially for axial bones. Differences in microarchitecture of axial bones between diabetic patients and non-diabetic controls had only been examined in a small-scale study using relatively low-resolution quantitative computed tomography (QCT) [15].

Trabecular bone score (TBS) quantifies local variation in the gray level distribution in dual-energy X-ray absorptiometry (DXA) images [16] [17] [18]. Although TBS is not a direct physical parameter of bone microarchitecture, it is significantly correlated with three-dimensional parameters of bone microarchitecture from micro CT imaging of cadaver vertebrae [17] and iliac bone biopsy specimens [19], and compression stiffness of human vertebrae [20]. Decreased TBS was associated with an elevated risk for osteoporotic fractures independently of aBMD in cohort studies [21] [22] [23] [24] [25]. These results were confirmed by a recent meta-analysis of prospective cohort data [26] and adopted as evidence in position papers [27,28].

Canadian women with diabetes have been reported to show a significantly higher aBMD and lower TBS than those without diabetes [29]. Similar results have been obtained in other studies [30,31]. In addition, TBS was inversely correlated with fasting plasma glucose (FPG) and glycated hemoglobin A_{1C} (HbA1c) levels [30,32], and was significantly lower in T2DM patients with poor glycemic control than in those with good control [33]. These reports suggest that prolonged hyperglycemia may cause the deterioration of bone microarchitecture, thereby increasing fracture risk. However, no study has ever examined whether insulin resistance or beta-cell function is involved in the association between T2DM and TBS or whether this association is mediated by other bone quality indices, such as AGEs or bone turnover. Accordingly, we aimed to clarify associations between glycemic and insulin-resistance indices and TBS in community-dwelling elderly Japanese men, and whether pentosidine, an AGE, and bone turnover marker levels affect these associations.

2. Materials and methods

2.1. Study setting

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 comprehensive strategies to prevent frailty, increase the number of healthy life years and enhance the 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 and FORMEN studies have been described elsewhere [34].

2.2. Study participants

Participants of the Fujiwara-kyo study were enrolled in four cities of Nara Prefecture, Japan, on a voluntary basis. Inclusion criteria were age ≥ 65 years at enrollment, living at home, ability to walk without assistance from another person and ability to provide self-reported information and written informed consent. Of the 4427 participants of the Fujiwara-kyo study, 2174 men were included in the FORMEN study. Exclusion criteria were incomplete test results in the FORMEN study, and past/current illnesses and 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 at any dose, bisphosphonate therapy for >-6 months and activated vitamin D use for >2 years).

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

2.3. Medical history

Participants completed a questionnaire survey consisting of 250 items that covered medical history of fracture, malignant diseases, hypertension, diabetes mellitus, coronary heart disease, dyslipidemia, asthma, kidney disease, prostate disease, medications due to these diseases and items related to the exclusion criteria of the present study. 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. With respect to history of fractures, those at skeletal sites other than the head, phalanx and lower legs that occurred without strong external force were treated as osteoporotic fractures.

2.4. Definition of T2DM

T2DM was defined as physician-diagnosed T2DM or non-insulin-dependent diabetes mellitus, physician-diagnosed middle age or older-onset diabetes mellitus without specification of type 1 or type 2 or by one of the biochemical test results obtained in the present study (FPG level ≥ 126 mg/dl or HbA1c level ≥ 6.5%) according to guidelines of the American Diabetes Association [35] and the Japan Diabetes Society [36].

2.5. Laboratory measurements

We drew blood from each participant after an overnight fast and obtained plasma and serum samples for the following conventional biochemical tests: FPG, HbA1c, serum insulin (FSI), creatinine, triglycerides and cholesterol. We stored remaining serum samples at −80 °C until the time of measurements for bone turnover markers and other bone-related indices.

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 [37]. To estimate insulin resistance and beta-cell function, homeostasis model assessment-insulin resistance (HOMA-IR) and HOMA-β, respectively, were calculated using FPG and FSI values for participants who were not on insulin therapy and whose FPG levels were ≤140 mg/dl [38].

Serum creatinine levels (mg/dl) were measured using an enzymatic method (L-type CRE·M; Wako Pure Chemical Industries, Ltd., Osaka, Japan). To evaluate renal function, estimated glomerular filtration rate (eGFR) was calculated with the Modification of Diet in Renal Disease Study equation modified for the Japanese population by the Japanese Society of Nephrology as follows: eGFR (ml/min/1.73m^2) = 194 × serum Cr$^{-1.094}$ × age$^{-0.287}$ [39].

We measured intact osteocalcin (OC) (ng/ml) by a two-site immunoradiometric assay (BGP IRMA kit Mitsubishi, Mitsubishi Kagaku Iatron Inc., Tokyo, Japan) with a sensitivity of 1 ng/ml [40], 4.9% intraassay coefficient of variation (CV), 3.7% interassay CV and 6.1% overall CV. Type I procollagen N-terminal propeptide (P1NP) was measured by a radioimmunoassay (Procollagen Intact PINP, TFB Inc., Tokyo, Japan) [41] with a sensitivity of 5 ng/ml, 3.6% intraassay CV and 4.2% interassay CV. Tartrate-resistant acid phosphatase isoenzyme 5b (TRACP5b) was measured by a fragment-absorbed immunocapture enzyme assay (Osteolinks-TRAP-5b, Nitto Boseki, Kooriyama, Japan) with a sensitivity of 19.2 mU/dl [42], 4.9% intraassay CV, 7.3% interassay CV and 8.8% overall CV. Serum type 1 collagen cross-linking C-terminal

telopeptide (sCTX) was determined by ELISA (FRELISA β CrossLaps R-N, FUJIREBIO Inc., Tokyo, Japan) [41] with a sensitivity of 0.075 ng/ml, 5.6% intraassay CV and 9.4% interassay CV. In addition, we measured serum pentosidine levels (μg/ml) as a bone-quality marker by a competitive enzyme-linked immunosorbent assay (FSK pentosidine ELISA kit; Fushimi Pharmaceutical Co., Marugame, Japan) with a 3.9% intraassay CV, 2.4% interassay CV and 4.6% overall CV, and with sensitivity of 0.00915 μg/ml. Pentosidine levels measured by this method were highly correlated with values determined by high performance liquid chromatography (HPLC) ($r = 0.936$) [43].

2.6. Bone mass measurements

aBMD (g/cm^2) was measured by DXA at the lumbar spine (aBMD) in a posteroanterior projection (QDR4500A, Hologic Inc., Bedford, MA, USA). The region of interest (ROI) was set as the second to fourth vertebrae, in accordance with Japanese guidelines for diagnosing osteoporosis at the time of the survey [44]. We excluded vertebrae with fractures or degenerative changes causing >1 SD greater aBMD from the immediately adjacent vertebrae in accordance with the International Society for Clinical Densitometry guidelines for individual vertebrae exclusion [45]. Consequently, the baseline study included data from 1036 men with three assessable vertebrae and 836 men with two assessable vertebrae. The short-term precision of aBMD measurements calculated from five measurements on different days from five male volunteers (age range, 21–41 years) was 1.2% [46]. Quality assurance was conducted using a spine phantom throughout the study period, and no significant drift in measurements was detected.

2.7. TBS measurement

TBS was calculated using TBS iNsight software (Version 2.1, Medimaps, Mérignac, France) for the same ROI used for aBMD measurements by one of the authors (RW), who was blinded to the participants' clinical data. TBS values were calibrated to standard values using the TBS calibration phantom (17 cm thickness and 25% fat mass equivalent), and were adjusted for body mass index (BMI) to 21.78. Participants with BMI >35 were excluded since BMI adjustments for TBS are not valid in such obese men. The short-term precision of TBS calculations was calculated as 1.5% (CV) from the same set of DXA scans used to evaluate the precision of aBMD measurements.

2.8. Body size measurements

We measured the height (cm) and weight (kg) of participants using an automatic scale and calculated BMI (kg/m^2).

2.9. Statistical analyses

All statistical calculations were performed with SAS software (Version 9.4, SAS Institute, Cary, NC, USA) on a personal computer. Levels of biochemical markers for glucose metabolism and bone turnover were all distributed log-normally; therefore, these values were logarithmically converted and then statistically analyzed. These data were expressed as geometric means and SDs. Pearson's correlation coefficients were calculated to evaluate the association between glycemic and HOMA indices and aBMD or TBS. The association was further evaluated by estimating a general linear model for aBMD or TBS with glycemic and HOMA indices as predictors and TBS (in a model for aBMD) or aBMD (in a model for TBS) in addition to age and BMI as covariates. Following this, effects of bone turnover markers and pentosidine on the association between glycemic and HOMA indices and aBMD or TBS were assessed by changes in regression coefficients for glycemic and HOMA indices when bone turnover markers and pentosidine levels were further entered into the models as covariates. The least square mean values of aBMD and TBS in participants with and without T2DM and in quartile groups of FPG and HbA1c were calculated after adjusting for TBS (in aBMD comparisons), aBMD (in TBS comparisons), age and BMI by the general linear model with the Tukey-Kramer multiple comparison procedure when appropriate.

3. Results

3.1. Anthropometric characteristics of participants with and without T2DM

Among 2174 male participants of the Fujiwara-kyo study, 2012 completed the study procedures of the FORMEN baseline study, and 1872 men had at least two assessable vertebrae. We excluded 184 men who met the exclusion criteria and 5 without blood samples for the present study. Among the remaining 1683 men, 313 had T2DM, including 198 men with a diagnosis of T2DM and a median duration of disease of 10.5 years prior to the study and 184 who have received pharmaceutical treatment.

Table 1 shows the basic characteristics of participants with and without T2DM. Age and height were similar among participants with or without T2DM, but those with T2DM weighed significantly more and had a significantly higher aBMD compared to those without T2DM. There was no significant difference in TBS between participants with and without T2DM. These results for aBMD and TBS did not change after adjusting for TBS (in aBMD comparisons) or aBMD (in TBS comparisons), age and BMI (data not shown). Frequency of past osteoporotic fractures did not differ between participants with and without T2DM. No significant difference in mean eGFR or in the prevalence of eGFR <60 was found between participants with and without T2DM.

Table 1 also shows laboratory test results according to T2DM prevalence. Significant differences in glycemic indices and levels of TG and HDL-C were observed between participants with and without T2DM. PTH, OC and P1NP were significantly lower, and pentosidine levels significantly higher, in participants with T2DM.

3.2. Correlation coefficients between glycemic and HOMA indices and aBMD and TBS

Table 2 shows correlation coefficients between glycemic and HOMA indices and aBMD and TBS. Significantly positive age- and BMI-adjusted correlations were observed between glycemic indices and aBMD. Although no significant correlation was initially observed between glycemic indices and TBS, further adjusting for aBMD yielded significant correlations between glycemic indices and TBS. HOMA-IR showed a significant inverse correlation with TBS after adjusting for BMI and aBMD, but HOMA-β did not.

When correlation coefficients were calculated in participants with and without T2DM separately, no correlation was significant in those with T2DM, whereas correlations between glycemic and HOMA-IR indices and TBS adjusted for age, BMI and aBMD remained significant in those without T2DM ($r = -0.096$, $p = 0.0004$ for FPG; $r = -0.075$, $p = 0.0056$ for HbA1c; $r = -0.093$, $p = 0.0007$ for HOMA-IR).

3.3. Association of glycemic and HOMA indices with aBMD and TBS after adjusting for bone turnover markers and pentosidine levels

In the general linear model for aBMD or TBS incorporating glycemic and HOMA-IR indices as predictors and age, BMI and TBS (in a model for aBMD) or aBMD (in a model for TBS) as covariates, FPG had a significant positive regression coefficient (0.012 [95% confidence interval (CI): 0.005, 0.020] for 1 SD increase) in the model for aBMD and a significant negative coefficient (−0.004 [95% CI: -0.007, −0.0003]) in the model for TBS. Similarly, HbA1c had a significant positive regression coefficient (0.012 [95% CI: 0.004, 0.019]) in the model for aBMD and a significant negative coefficient (−0.004 [95% CI: −0.007, −0.000]) in the model for TBS. HOMA-IR did not have a significant regression coefficient in

Table 1
Anthropometric and laboratory characteristics of participants with and without type 2 diabetes mellitus (T2DM) in the Fujiwara-kyo Osteoporosis Risk in Men study.

	Total	T2DM	non-DM	P for difference
N	1683	313	1370	
Age (years)	72.9 ± 5.2	72.8 ± 5.2	72.9 ± 5.1	$p = 0.6463$
Height (cm)	162.8 ± 5.7	163.0 ± 5.9	162.8 ± 5.7	$p = 0.5577$
Weight (kg)	61.2 ± 8.4	62.6 ± 8.8	60.8 ± 8.3	$p = 0.0006$
BMI (kg/m^2)	23.0 ± 2.7	23.6 ± 2.9	22.9 ± 2.7	$p = 0.0002$
LS-aBMD (g/cm^2)	1.015 ± 0.189	1.050 ± 0.196	1.007 ± 0.186	$p = 0.0002$
TBS	1.193 ± 0.083	1.193 ± 0.089	1.193 ± 0.082	$p = 0.9906$
FPG (mg/dl)	101.4$^{\times}/_{\div}$1.2	135.3$^{\times}/_{\div}$1.4	94.9$^{\times}/_{\div}$1.1	$p < 0.0001$
HbA1c (%)	5.7$^{\times}/_{\div}$1.1	6.5$^{\times}/_{\div}$1.2	5.0$^{\times}/_{\div}$1.1	$p < 0.0001$
FSI (mU/l)[A]	5.0$^{\times}/_{\div}$2.0	8.2$^{\times}/_{\div}$2.3	4.5$^{\times}/_{\div}$1.9	$p < 0.0001$
HOMA-IR[B]	1.1$^{\times}/_{\div}$2.0	1.8$^{\times}/_{\div}$2.1	1.0$^{\times}/_{\div}$1.9	$p < 0.0001$
HOMA-β[B]	52.0$^{\times}/_{\div}$2.0	52.0$^{\times}/_{\div}$2.2	52.0$^{\times}/_{\div}$1.9	$p = 0.9974$
Triglyceride (mg/dl)	116.3$^{\times}/_{\div}$1.6	130.0$^{\times}/_{\div}$1.6	113.4$^{\times}/_{\div}$1.6	$p < 0.0001$
Total C (mg/dl)	204.4$^{\times}/_{\div}$1.2	200.6$^{\times}/_{\div}$1.2	205.3$^{\times}/_{\div}$1.2	$p = 0.0197$
HDL-C (mg/dl)	53.5$^{\times}/_{\div}$1.3	51.2$^{\times}/_{\div}$1.3	54.0$^{\times}/_{\div}$1.3	$p = 0.0007$
LDL-C (mg/dl)	119.9$^{\times}/_{\div}$1.3	118.8$^{\times}/_{\div}$1.3	120.2$^{\times}/_{\div}$1.3	$p = 0.4633$
Creatinine (mg/dl)	0.88$^{\times}/_{\div}$1.2	0.89$^{\times}/_{\div}$1.3	0.87$^{\times}/_{\div}$1.2	$p = 0.2607$
eGFR (ml/min/1.73 m^2)	65.6$^{\times}/_{\div}$1.2	64.7$^{\times}/_{\div}$1.3	65.8$^{\times}/_{\div}$1.2	$p = 0.2838$
eGFR <60 n (%)	482 (28.6)	93 (29.7)	389 (28.4)	$p = 0.6920$
History of OPFx n (%)	44 (2.6)	8 (2.6)	36 (2.6)	$p = 1.0000$
PTH (pg/ml)	20.3$^{\times}/_{\div}$1.5	18.7$^{\times}/_{\div}$1.6	20.7$^{\times}/_{\div}$1.5	$p = 0.0014$
OC (ng/ml)	4.8$^{\times}/_{\div}$1.5	4.3$^{\times}/_{\div}$1.5	4.9$^{\times}/_{\div}$1.5	$p < 0.0001$
P1NP (ng/ml)[C]	34.1$^{\times}/_{\div}$1.5	29.4$^{\times}/_{\div}$1.5	35.0$^{\times}/_{\div}$1.5	$p < 0.0001$
TRACP5b (mU/dl)	207.0$^{\times}/_{\div}$1.7	198.0$^{\times}/_{\div}$1.7	209.2$^{\times}/_{\div}$1.7	$p = 0.1153$
sCTX (ng/ml)[D]	0.202$^{\times}/_{\div}$1.6	0.193$^{\times}/_{\div}$1.7	0.204$^{\times}/_{\div}$1.6	$p = 0.1163$
Pentosidine (mg/ml)	0.050$^{\times}/_{\div}$1.4	0.055$^{\times}/_{\div}$1.4	0.049$^{\times}/_{\div}$1.4	$p < 0.0001$

Data are expressed as mean ± SD or geometric mean $^{\times}/_{\div}$ SD.
BMI: body mass index.
aBMD: areal bone mineral density.
LS: lumbar spine.
TH: total hip.
FN: femoral neck.
TBS: trabecular bone score.
FPG: fasting plasma glucose.
HbA1c: glycated hemoglobin A_{1c}.
FSI: fasting serum insulin.
HOMA-IR: homeostasis model assessment-insulin resistance.
HOMA-β: homeostasis model assessment-beta cell function.
Total C: total cholesterol.
HDL-C: high-density lipoprotein cholesterol.
LDL-C: low-density lipoprotein cholesterol.
eGFR: estimated glomerular filtration rate.
OPFx: Osteoporotic fracture.
PTH: parathyroid hormone.
OC: osteocalcin.
P1NP: type I procollagen N-terminal propeptide.
TRACP 5b: tartrate-resistant acid phosphatase isoenzyme 5b.
sCTX: serum type I collagen cross-linking C-terminal telopeptide.
[A] For 1620 participants without current insulin treatment including 288 participants with T2DM.
[B] For 1519 participants without current insulin treatment and with FPG <140 mg/dl including 187 participants with T2DM.
[C] For 1196 participants with P1NP available including 189 participants with T2DM.
[D] For 1279 participants with sCTX available including 233 participants with T2DM.

the model for aBMD, but did have a significant negative coefficient in the model for TBS (−0.006 [95% CI: −0.011, −0.002]).

We further entered bone turnover markers and pentosidine into these models and the results are shown in Table 3. FPG and HbA1c still had significant positive regression coefficients for aBMD and significant negative coefficients for TBS. HOMA-IR had a significant negative coefficient for TBS. The association between glycemic indices and aBMD or TBS and the association between HOMA-IR and TBS appeared to be independent of bone turnover markers or pentosidine levels. These results did not change when we entered P1NP and sCTX into the models instead of OC and TRACP5b.

3.4. Adjusted mean aBMD and TBS in participants classified according to glycemic indices and HOMA-IR

The least square means of aBMD or TBS were compared across quartile groups of glycemic and HOMA-IR indices after adjusting for age, BMI and TBS for aBMD comparisons or aBMD for TBS comparisons. Results are shown in Fig. 1. aBMD was significantly higher, and TBS significantly lower, in the highest quartile group compared to other groups of glycemic indices with a significant increasing or decreasing trend, respectively, across quartile groups. However, a significant trend with HOMA-IR quartile groups was only seen for TBS.

4. Discussion

In the present study, community-dwelling Japanese elderly men with T2DM had a significantly higher aBMD compared to those without T2DM, but no significant difference in TBS. FPG and HbA1c levels showed significant positive correlations with aBMD and significant inverse correlations with TBS. HOMA-IR was inversely correlated only with TBS. These results were independent of serum pentosidine levels or bone turnover markers, suggesting that the deterioration of trabecular bone microarchitecture was possibly caused by prolonged hyperglycemic status due to insulin resistance, and that this association may not be mediated by low bone turnover or retention of pentosidine.

TBS did not significantly differ between T2DM and non-T2DM participants, unlike results from previous studies [29] [32] [33], but similar to another study [31]. The inconsistency with some previous studies may be attributed to the fact that participants of the present study were community-dwelling volunteers and may have included fewer patients with severe illnesses, such as uncontrolled T2DM. In fact, average HbA1c levels and HOMA-IR of the present study were lower than those reported in the previous studies (6.5% vs 7.7% [30], or 6.83% [32] for HbA1c, 1.8 vs 3.43 [32] for HOMA-IR), and our patients had relatively well-controlled T2DM. However, since TBS was significantly lower in the highest quartile groups of FPG and HbA1c relative to the lowest quartile groups, a prolonged hyperglycemic status leads to increased aBMD and may result in the deterioration of bone microarchitecture as suggested by previous studies [32]. In addition, the dose-response pattern of hyperglycemia between aBMD and TBS appeared to be different, as shown in Fig. 1, although the mechanism underlying this difference is unclear. One possibility is that hyperglycemia may have a threshold with regard to its effects on bone mass, but not with respect to its effects on trabecular microarchitecture.

A lower TBS suggests a lower trabecular number, greater spacing and lower connectivity in the trabecular bone [17], which reflect fracture-prone microarchitecture. However, Melton et al. reported results from a QCT study on the lumbar vertebrae, showing that trabecular volumetric BMD was greater in patients with T2DM compared to those without T2DM, and that no significant difference was found in the endocortical area or cortical thickness [15]. A more recent study by Patsch et al., who used HR-pQCT at the distal radius [47], also found no significant differences in microarchitecture indices of the trabecular bone between T2DM patients and non-DM controls. Deterioration of the trabecular bone microarchitecture, as suspected by TBS in the present study, is not consistent with these previous study results. However, the resolution of QCT used by Melton et al. [15] was not high enough to determine microarchitecture indices, and moreover, their sample size was small. Furthermore, the HR-pQCT study by Patsch et al. [47] examined the distal site of the radius, which may not have represented the status of the axial bone. Therefore, large-scale, high-resolution QCT studies on the lumbar spine will be necessary to confirm the present study results.

Prolonged hyperglycemia deteriorates osteoblast function, resulting in decreased bone formation and mineralization [48,49]. This extends the length of the bone turnover cycle, resulting in an elevated proportion of old bone tissue with increased retention of AGEs, which

Table 2
Correlation coefficients between glycemic and HOMA indices and aBMD or TBS in the Fujiwara-kyo Osteoporosis Risk in Men study.

	Age and BMI adjusted				Age, BMI and TBS or aBMD adjusted			
	FPG	HbA1c	HOMA-IR[A]	HOMA-β[A]	FPG	HbA1c	HOMA-IR[A]	HOMA-β[A]
aBMD	0.063	0.059	−0.031	−0.061	0.082[B]	0.077[B]	0.013[B]	0.043[B]
	$p = 0.0092$	$p = 0.0163$	$p = 0.2202$	$p = 0.0169$	$p = 0.0008$	$p = 0.0017$	$p = 0.6086$	$p = 0.0948$
TBS	−0.010	−0.011	−0.078	−0.047	−0.052[C]	−0.051[C]	−0.073[C]	−0.016[C]
	$p = 0.6772$	$p = 0.6403$	$p = 0.0022$	$p = 0.0687$	$p = 0.0323$	$p = 0.0381$	$p = 0.0044$	$p = 0.5290$

aBMD: areal bone mineral density at the spine.
TBS: trabecular bone score.
BMI: body mass index.
FPG: fasting plasma glucose.
HbA1c: glycated hemoglobin A_{1c}.
HOMA-IR: homeostasis model assessment-insulin resistance.
HOMA-β: homeostasis model assessment-beta cell function.
[A] For 1519 participants without current insulin treatment and with FPG < 140.
[B] Adjusted for age, BMI and TBS.
[C] Adjusted for age, BMI and aBMD.

decreases the elasticity of the bone, accumulates microcracks in the trabeculae and ultimately deteriorates bone microarchitecture. Therefore, AGEs and bone turnover are intermediate factors which play a significant role in the causal chain between hyperglycemia and compromised bone microarchitecture. However, the association between glycemic indices and TBS remained significant even after adjusting for pentosidine or bone turnover markers. Although no statistically significant effect does not always indicate no effect, this result suggests that the association is independent of pentosidine and bone turnover, and that the association may be mediated by a different mechanism in addition to AGE retention and low bone turnover, which has yet to be determined.

The present study has several strengths. This population-based study had a sufficiently large sample size that likely reflects the health status of a community-dwelling elderly male population in Japan. We evaluated a range of biochemical markers of bone turnover and common geriatric diseases using validated methods at baseline, some of which were used to adjust for potential confounding factors. The present study is a part of an on-going cohort study that plans a 10-year follow-up with three waves of clinical surveys at a university hospital, where incident fractures can be identified and the role of TBS in elevated fracture risk associated with T2DM can be evaluated. In addition, this is a single-center study and is free from inter-center variation.

Table 3
Association of glycemic and HOMA indices with aBMD or TBS adjusted for bone turnover markers and pentosidine levels in the Fujiwara-kyo Osteoporosis Risk in Men study.

		Outcome			
		aBMD		TBS	
Predictor	Covariates	Coefficient	95% confidence interval	Coefficient	95% confidence interval
FPG		0.010	(0.002, 0.018)	−0.004	(−0.008, −0.001)
	aBMD	–	–	0.046	(0.042, 0.050)
	TBS	0.097	(0.089, 0.104)	–	–
	Age	0.025	(0.018, 0.033)	−0.015	(−0.018, −0.012)
	BMI	0.066	(0.058, 0.074)	−0.023	(−0.026, −0.019)
	OC	−0.014	(−0.022, −0.005)	−0.001	(−0.005, 0.003)
	TRACP5b	−0.006	(−0.015, 0.002)	0.001	(−0.003, 0.005)
	Pentosidine	0.007	(0.000, 0.015)	−0.001	(−0.005, 0.002)
HbA1c		0.009	(0.001, 0.017)	−0.004	(−0.007, 0.000)
	aBMD	–	–	0.046	(0.042, 0.049)
	TBS	0.097	(0.089, 0.104)	–	–
	Age	0.025	(0.018, 0.033)	−0.015	(−0.018, −0.012)
	BMI	0.065	(0.057, 0.073)	−0.022	(−0.026, −0.019)
	OC	−0.014	(−0.023, −0.006)	−0.001	(−0.005, 0.003)
	TRACP5b	−0.006	(−0.015, 0.003)	0.001	(−0.003, 0.005)
	Pentosidine	0.007	(−0.001, 0.015)	−0.001	(−0.004, 0.003)
HOMA-IR		0.001	(−0.008, 0.010)	−0.007	(−0.011, −0.002)
	aBMD	–	–	0.046	(0.042, 0.050)
	TBS	0.097	(0.089, 0.104)	–	–
	Age	0.025	(0.017, 0.033)	−0.015	(−0.018, −0.011)
	BMI	0.066	(0.056, 0.075)	−0.019	(−0.024, −0.015)
	OC	−0.014	(−0.023, −0.006)	−0.001	(−0.005, 0.003)
	TRACP5b	−0.004	(−0.013, 0.005)	0.000	(−0.004, 0.004)
	Pentosidine	0.008	(0.000, 0.016)	−0.002	(−0.005, 0.002)

Coefficients: partial regression coefficients for one SD increase in variables except for age, which is presented for 5-year increase.
aBMD: areal bone mineral density at the spine.
TBS: trabecular bone score.
FPG: fasting plasma glucose.
HbA1c: glycated hemoglobin A_{1c}.
BMI: body mass index.
OC: osteocalcin.
TRACP5b: tartrate-resistant acid phosphatase isoenzyme 5b.
HOMA-IR: homeostasis model assessment-insulin resistance.
–: unavailable.

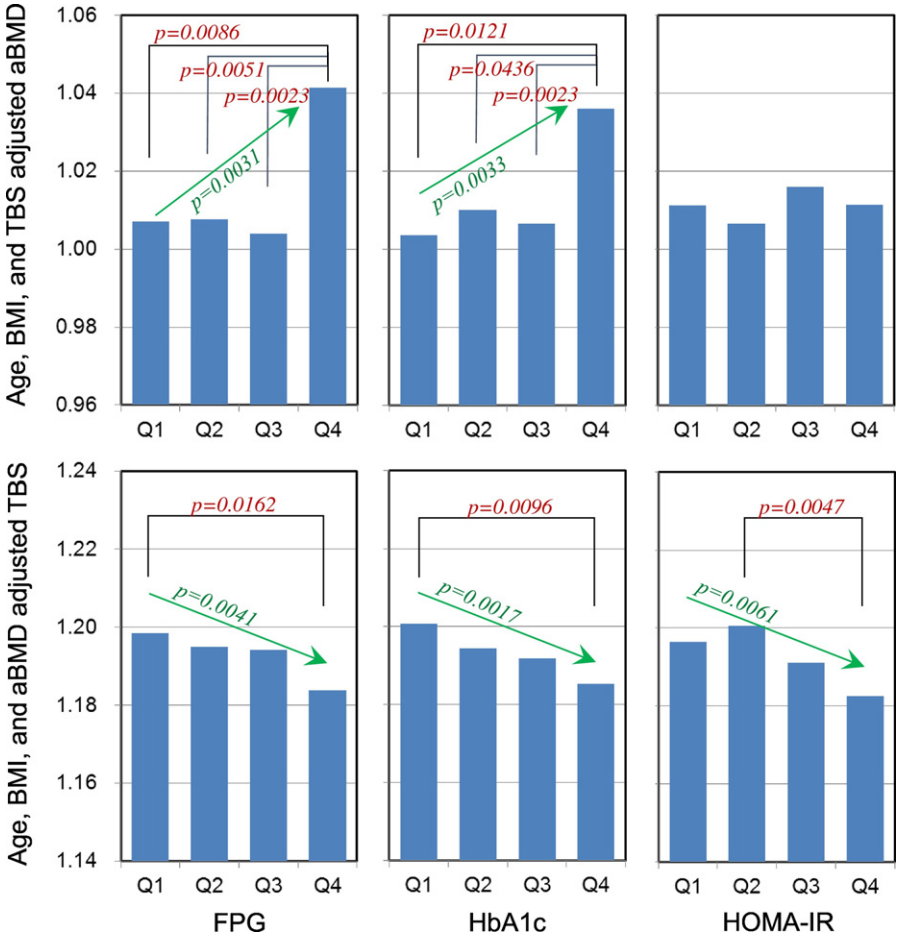

Fig. 1. Adjusted mean values of aBMD and TBS in participants classified according to quartile groups (Q1–Q4) of FPG, HbA1c or HOMA-IR. Mean values were adjusted for age, BMI and TBS in aBMD comparisons or for age, BMI and aBMD in TBS comparisons. aBMD: areal bone mineral density, TBS: trabecular bone score, FPG: fasting plasma glucose, HbA1c: glycated hemoglobin A_{1c}, HOMA-IR: homeostasis model assessment-insulin resistance, BMI: body mass index. The arrow indicates a significant linear trend between FPG, HbA1c or HOMA-IR quartile groups and aBMD or TBS values.

However, there are also several limitations worth noting. Since participants of the FORMEN study were volunteers, patients with severe or symptomatic T2DM may have been less likely to participate in the study. This potential bias may have resulted in an underestimation of the association of interest. Second, participants were restricted to elderly Japanese men; thus, caution should be exercised in generalizing the results. Third, the diagnosis of T2DM was based on self-reported data, and FPG and HbA1c levels were determined only once. Moreover, the oral glucose tolerance test was not performed. Therefore, misclassification of patients may have occurred, although this could have also underestimated the strength of the association. Fourth, we did not evaluate the effects of diabetic complications, such as CKD or hypogonadism, on TBS. While the prevalence of eGFR < 60 did not differ between participants with and without T2DM, serum testosterone levels were not determined in the present study. Fifth, we did not measure blood vitamin D levels. Vitamin D insufficiency may have increased the risk of T2DM [50] and decreased TBS simultaneously. Although vitamin D supplements did not significantly change TBS in a hospital-based cohort [51], this potential confounder remained unresolved in the present study. Finally, all results were obtained from cross-sectional analyses and the incidence of fracture was not determined.

5. Conclusions

Elderly Japanese men with a hyperglycemic status had a high aBMD and low TBS, and those with elevated insulin resistance showed low TBS. These associations were not explained by decreased bone turnover or retention of pentosidine.

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designing the study, collecting, analyzing and interpreting the data, writing the manuscript or deciding where to submit the manuscript for publication.

Conflicts of interest

Renaud Winzenrieth was a senior scientist at Medimaps at the time of study. Masayuki Iki, Yuki Fujita, Katsuyasu Kouda, Akiko Yura, Takahiro Tachiki, Junko Tamaki, Yuho Sato, Jong-Seong Moon, Nozomi Okamoto and Norio Kurumatani declare that they have no conflict of interest.

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