Bone Material Strength as Measured by Microindentation In Vivo Is Decreased in Patients With Fragility Fractures Independently of Bone Mineral Density

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Context: Bone mineral density (BMD) does not fully capture fracture risk as the majority of fractures occur in patients with osteopenia, suggesting that altered bone material properties and changes in microarchitecture may contribute to fracture risk.

Objective: This study aimed to evaluate the relationship between bone material strength (BMS), measured by microindentation in vivo, and fracture in patients with low bone mass.

Methods: BMS was measured in 90 patients (mean age, 61.0 y; range, 40.4–85.5 y) with low bone mass with or without a fragility fracture. Sixty-three patients had sustained one or more fragility fractures.

Results: There was a significant negative correlation between age and BMS ($r = -0.539$; $P < .001$) and with the 10-year fracture probability with and without inclusion of femoral neck BMD as calculated by FRAX ($r = -0.383$; $P < .001$ and $r = -0.426$; $P < .001$, respectively). BMS values were lower in patients with a fragility fracture compared with nonfracture patients (79.9 ± 0.6 vs 82.4 ± 1.0; $P = .032$) despite similar BMD. BMS was comparable in patients with a fragility fracture whether they had osteopenia or osteoporosis (79.8 ± 0.8 vs 78.7 ± 1.1; $P = .456$). In patients with osteopenia, BMS was significantly lower in fracture patients than in nonfracture patients (80.3 ± 0.7 vs 83.9 ± 1.2; $P = .015$).

Conclusion: These data suggest that patients with fractures have altered material properties of bone that are not captured by BMD. Additional studies are required to establish the value of BMS in the prediction of fracture risk, especially in patients with osteopenia. **(*J Clin Endocrinol Metab* 100: 2039–2045, 2015)**

Osteoporotic fractures are common and their incidence increases with age, regardless of sex (1–3). All fractures represent a significant cause of morbidity and decreased quality of life, but fractures have also been shown to be associated with increased mortality (4, 5). There is mounting evidence that bone mineral density (BMD) measurements using dual-energy x-ray absorptiometry (DXA) only partially capture fracture risk, given that a majority of fragility fractures have been shown to occur in patients with osteopenia (6, 7). This strongly suggests that determinants of bone strength other than bone mass may contribute to bone fragility in these patients. Such determinants would include changes in microarchitecture and of material properties of bone. Up until recently this hypothesis was, however, difficult to test in humans due to lack of appropriate techniques for evaluation of these determinants of bone strength.

Recent studies examining structural changes of bone in patients with fractures demonstrated a deterioration in bone microarchitecture (8, 9), as well as an association

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Abbreviations: BMD, bone mineral density; BMI, body mass index; BMS, bone material strength; DXA, dual-energy x-ray absorptiometry; FN, femoral neck; FRAX, 10-year fracture probability; IDI, indentation distance increase; PMMA, polymethylmethacrylate.

between increased cortical porosity and distal forearm fractures in patients with osteopenia (10). Reference point indentation is a new tool that permits in vivo measurements of bone material properties in humans. This technique has been extensively validated in animal models (11–13). Diez-Perez et al (14) were the first to report data on microindentation in vivo in humans, showing a significantly higher indentation distance increase (IDI) in patients with osteoporotic fractures compared with nonfracture controls. Further development of the technique has led to the introduction of a handheld device to measure bone material strength (BMS), a parameter derived from the ratio of the mean IDI between the calibration phantom and bone, as a quantifiable parameter of the ability of bone to resist microindentation (15). This device is inserted in the skin of the tibia until it reaches the bone surface and indents it. Using this technique, Farr et al (16) recently reported that postmenopausal women with type 2 diabetes mellitus had lower BMS compared with age-matched nondiabetic controls, and suggested that this may contribute to the increased bone fragility observed in these patients.

The main objective of our study was to evaluate the relationship between BMS, as assessed by the microindentation in vivo technique, and fracture in patients with low bone mass.

Patients and Methods

Study design

This was a cross-sectional study evaluating BMS using the microindentation in vivo technique in men and women attending the outpatient clinic of the Center for Bone Quality or the regional Fracture Liaison Service of the Leiden University Medical Center between July 2013 and August 2014.

Patients

Patients were sequentially invited to take part in the study. Details of the recruitment process are shown in Supplemental Figure 1. Inclusion criteria included age between 40 and 85 years, low bone mass (osteopenia or osteoporosis as diagnosed by DXA), and willingness to be investigated using the microindentation in vivo technique. Exclusion criteria were a metabolic bone disorder other than osteoporosis, serum 25-OH vitamin D concentrations less than 25 nmol/L, pathological fractures, severe liver or kidney impairment (chronic kidney disease stage IV or V), current use of glucocorticoids, aromatase inhibitors, androgen deprivation therapy, or chemotherapy, and previous or current use of bone-acting agents (bisphosphonates, denosumab, selective estrogen receptor modulators, strontium ranelate, recombinant PTH), immobilization, local infection of the tibia at the site of examination, bilateral hip replacement, participation in other research studies, and inability to provide informed consent. Past use of glucocorticoids (>3 mo ago) was not an exclusion criterion as fracture risk has been shown to reverse quickly after discontinuation of treatment (17, 18). The Medical Ethics Committee of the Leiden University Medical Center approved the study and informed consent was obtained from all patients.

Methods

A full medical history including data on menopausal status, clinical risk factors for fractures for the calculation of the 10-year fracture probability (FRAX), a detailed fracture history with documentation of site and date of occurrence of the fracture, and information regarding use of medication were obtained from all patients. A fragility fracture was defined as any low-energy fracture, excluding those of the hands, feet, and skull. The FRAX probability for a major osteoporotic fracture and for a hip fracture was calculated using reference values for the Dutch population (19). Both fracture probabilities were computed with and without the inclusion of femoral neck BMD in the calculation. Fractures sustained less than 12 months before the investigation were not included as a previous fracture in the calculation of the FRAX (20–22).

Serum biochemistry

Blood samples were collected for the measurement of serum calcium, phosphate, albumin, creatinine, and liver enzymes using semiautomated techniques; serum 25-hydroxyvitamin D was measured using the 25-OH-vitamin D TOTAL assay (DiaSorin D.A./N.V., Brussels, Belgium) and plasma Intact PTH was measured by the immulite 2500 (Siemens Diagnostics).

Bone mineral density

Areal BMD was measured at the lumbar spine (L1–L4) and at both femoral necks using DXA with the Hologic QDR 4500 (Hologic Inc). Average values of the left and right femoral neck (FN) were used for analysis. T-scores were calculated using reference values of the National Health and Nutrition Examination Survey (NHANES) III and osteopenia and osteoporosis were diagnosed according to World Health Organization criteria.

Radiographs of the spine

Lateral radiographs of the thoracic and lumbar spine were performed for the detection of vertebral deformities. All radiographs were independently evaluated by two of the authors using the semiquantitative method of Genant (23).

Bone material strength

BMS was evaluated by microindentation in vivo using the Osteoprobe, a Reference Point Indenter (kindly provided by Active Life Scientific Inc) (11, 14, 15). After local anesthesia using a solution of 1% Lidocaïne, the hand-held Osteoprobe is inserted in the skin of the midshaft of the right tibia (mean distance between distal apex of the patella and medial malleolus) until it reaches the bone surface, which is indented upon activation of the instrument. During measurements, the Osteoprobe is maintained perpendicular to the surface of bone at the site of investigation. A minimum of five and up to 25 measurements were performed at the same site. During the procedure, the operator

classified the sequential measurements as poorly, adequately, or well performed before checking the obtained data, to avoid reporter bias in the interpretation of results. After at least five adequate measurements in each subject, five additional measurements are performed on a polymethylmethacrylate (PMMA) plastic calibration phantom. BMS is calculated as 100 times the ratio of the mean indentation distance increase from impact into the PMMA calibration phantom divided by the indentation distance increase from impact into bone. The probe induces a microfracture as it indents the surface of the cortical bone of the tibia. The more easily this occurs, the deeper the probe indents the bone, and thus the lower the BMS (15). Coefficient of variation of the method was less than 10% for different levels of BMS.

Statistical analysis

All analyses were performed using the SPSS software for Windows (Version 20.0; SPSS Inc). All data are expressed as mean ± SD unless otherwise stated. Normality assumptions were checked by normality plots and by inspection of histograms of residuals from the various regression models. Between-group differences in baseline characteristics were assessed using a Student *t* test, a χ^2 test, or a Mann-Whitney *U* test for nonnormally distributed variables. Pearson's correlations were used to assess correlations between patients' parameters and BMS. Spearman's correlations were used to assess correlations between parameters that were not normally distributed and BMS values. ANOVA models with BMS as outcome variable, adjusted for covariates, were used to compare BMS values between groups. Binary logistic regression analysis was used to assess the separate contributions of BMS and femoral neck BMD (variables) to fracture (outcome). A probability level of random difference of .05 was considered significant.

Results

Ninety of 125 eligible patients with low bone mass agreed to take part and were included in the study (Supplemental Figure 1). Forty-nine of them, all with fractures, were recruited from the Fracture Liaison Service whereas 41, with or without fractures, were attending the outpatient clinic. Patients' characteristics and laboratory values are shown in Table 1. These were 53 women and 37 men; mean age, 61.0 years; range, 40.4–85.5 years; 61% of whom had osteopenia. Sixty-three patients (24 men) had sustained a low-energy fracture (vertebral n = 8; hip n = 10; nonhip/nonvertebral n = 45), in 43 of whom the fracture was recent. Microindentation was performed at a median time of 4.0 months after a fracture. Patients without history of a clinical fracture had also no radiological evidence for vertebral deformities on spinal radiographs.

BMS was significantly inversely related with age (r = −0.539; $P < .001$; Figure 1) and with the 10-year fracture probability with and without inclusion of femoral neck BMD in the calculation of FRAX (r = −0.383; $P < .001$ and r = −0.426; $P < .001$, respectively). BMS values were inversely and significantly related with age and with the 10-year fracture probability in both sexes (age: women, r = −0.422, $P = .001$; men, r = −0.570, $P < .001$; FRAX: women, r = −0.286, $P = .038$; men, r = −0.393, $P = .016$). Because of the relationship between BMS and age all further reported values of BMS were adjusted for age. Unadjusted values are shown in Supplemental Table 1.

Table 1. Characteristics of 90 Patients With Low Bone Mass

Characteristic	Fracture (n = 63)	No Fracture (n = 27)	*P* Value
n	63	27	
Age, y	62.6 ± 9.6	57.1 ± 9.5	.015
Male/female	24/39	13/14	.374
BMI, kg/m^2	24.3 ± 3.5	25.3 ± 4.7	.725
Parental hip fracture, n (%)	9 (14%)	4 (15%)	.948
Smoking, n (%)	14 (22%)	2 (7%)	.092
Alcohol use >3 IU/d, n (%)	14 (22%)	1 (4%)	.031
Glucocorticoids, n (%)	4 (6%)	6 (22%)	.028
FRAX probability			
Major fracture, %	6.9 ± 1.0	4.0 ± 0.8	.001
Hip fracture, %	2.0 ± 0.8	1.0 ± 0.3	.003
PTH[a], pmol/L	3.8 ± 1.9	3.5 ± 1.5	.570
Calcium[b], mmol/L	2.41 ± 0.08	2.41 ± 0.10	.826
25-OH D, nmol/L	67.4 ± 28.6	79.6 ± 26.5	.062
Creatinine[c], μmol/L	73.5 ± 13.1	78.4 ± 15.0	.163
LS BMD, g/cm^2	0.87 ± 0.13	0.86 ± 0.12	.402
T-score LS	−1.7 ± 1.2	−1.9 ± 1.1	.431
FN BMD, g/cm^2	0.67 ± 0.09	0.69 ± 0.08	.303
T-score FN	−1.8 ± 0.7	−1.6 ± 0.6	.329

Abbreviation: LS, lumbar spine. Values are expressed as mean ± SD FRAX is expressed as median ± SEM.

[a] PTH reference range, 0.7–8.0 pmol/L.

[b] Calcium reference range, 2.15–2.55 mmol/L.

[c] Creatinine reference range, 64–104 μmol/L for males; 49–90 μmol/L for females.

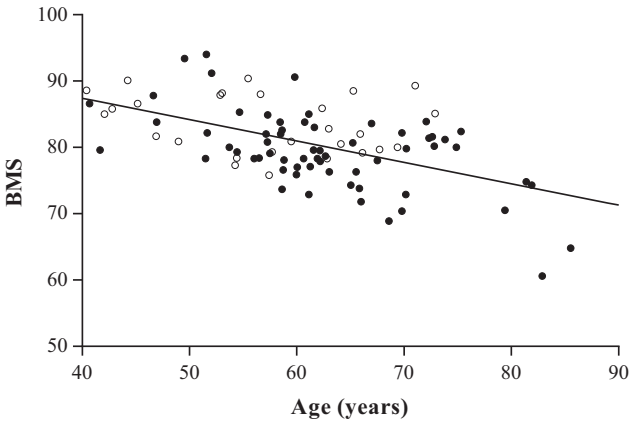

Figure 1. Relationship between age and BMS in 90 patients with osteoporosis or osteopenia. Closed circles represent patients with fragility fractures, open circles represent patients without fragility fractures. r = −0.539; $P < .001$.

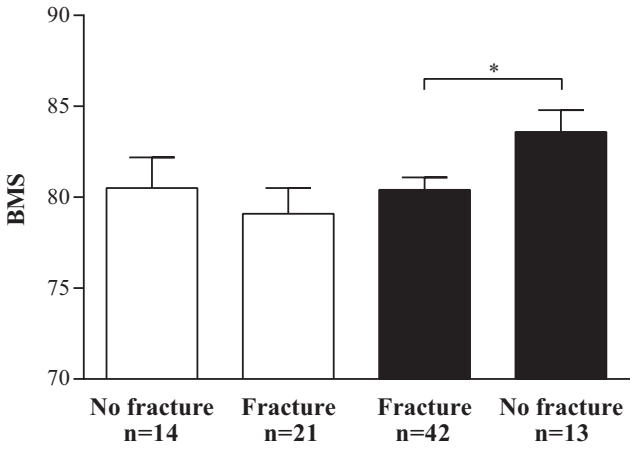

Figure 2. BMS in patients with osteoporosis (open bars) and osteopenia (closed bars), with and without fragility fractures. Mean ± SEM are shown. *, $P = .015$.

BMS values did not differ between women and men (80.0 ± 0.7 vs 81.6 ± 0.8; $P = .147$). There was no significant relationship between BMS and BMD (lumbar spine, r = 0.129, $P = .157$; femoral neck, r = 0.134, $P = .143$), body mass index (BMI; r = 0.075, $P = .413$) or any of the biochemical parameters measured.

BMS in patients with low bone mass

BMS was comparable in patients with osteoporosis and those with osteopenia (79.9 ± 0.8 vs 81.2 ± 0.7; $P = .230$). Patients with osteoporosis were predominantly women, had significantly lower BMI and significantly lower lumbar spine and femoral neck BMD than patients with osteopenia.

Patients with osteoporosis and a history of fragility fracture (n = 21) were significantly older than those without a fragility fracture (n = 14) (65.8 ± 10.5 y vs 53.7 ± 10.0 y; $P = .002$) and were more likely to be active smokers and/or to consume more than 3 units of alcohol per day (29% vs 0%; $P = .028$ for either). All other measured parameters, including BMS (79.3 ± 1.3 vs 80.7 ± 1.6, $P = .540$) did not differ between the two groups; Figure 2.

In patients with osteopenia, there was no significant difference in clinical characteristics, serum biochemistry, or BMD between fracture (n = 42) and nonfracture (n = 13) patients. However, BMS values were significantly lower in patients with fragility fractures compared with those without a fragility fracture (80.3 ± 0.7 vs 83.9 ± 1.2; $P = .015$; Figure 2). This difference remained significant also after exclusion of patients with a hip fracture (80.4 ± 0.8 vs 83.8 ± 1.2; $P = .027$).

BMS in patients with fragility fractures

BMS values were significantly lower in patients with fragility fractures compared with those who had never sustained a fracture (79.9 ± 0.6 vs 82.4 ± 1.0, $P = .032$), despite similar lumbar spine and femoral neck BMD values between the two groups; Figure 3. A lower BMS was associated with a higher odds for fractures (odds ratio, 1.15 [95% confidence interval 1.05–1.27], $P = .004$) whereas this was not the case for femoral neck BMD (odds ratio, 6.17 [95% confidence interval 0.02–2124.51], $P = .542$).

Among patients with a fragility fracture (n = 63), 42 had osteopenia and 21 osteoporosis; details shown in Table 2. BMS values were comparable in all patients with fragility fractures whether they had osteopenia or osteoporosis (79.8 ± 0.8 vs 78.7 ± 1.1; $P = .456$); Figure 2. There was no significant difference in BMS between pa-

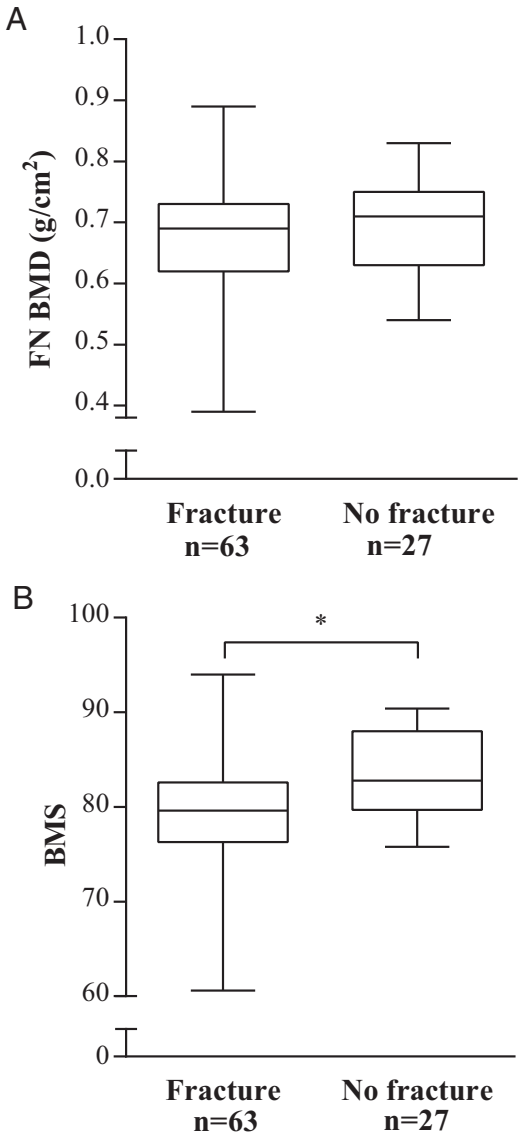

Figure 3. A, FN BMD; and B, BMS in patients with and without fragility fractures. Data are shown in box-whisker plots and statistical differences are displayed for BMS. Boxes indicate median and interquantile range. Bars indicate minimum and maximum values. *, $P = .032$.

Table 2. Characteristics of 63 Patients With Fragility Fractures

Characteristic	Osteopenia (n = 42)	Osteoporosis (n = 21)	*P* Value
n	42	21	
Age, y	61.0 ± 8.8	65.8 ± 10.5	.058
Male/female	20/22	4/17	.028
BMI, kg/m^2	25.0 ± 3.2	22.8 ± 3.8	.046
Parental hip fracture, n (%)	6 (14%)	3 (14%)	1.000
Smoking, n (%)	8 (19%)	6 (29%)	.391
Alcohol use >3 IU/d, n (%)	8 (19%)	6 (29%)	.391
Glucocorticoids, n (%)	3 (7%)	1 (5%)	.715
FRAX probability			
Major fracture, %	6.5 ± 0.6	9.8 ± 2.6	.004
Hip fracture, %	1.4 ± 0.4	4.0 ± 2.2	.001
PTH, pmol/L	3.8 ± 1.8	3.9 ± 2.2	.913
Calcium, mmol/L	2.41 ± 0.08	2.41 ± 0.09	.975
25-OH D, nmol/L	69.2 ± 29.3	63.7 ± 27.5	.474
Creatinine, μmol/L	74.6 ± 12.1	71.5 ± 14.9	.159
LS BMD, g/cm^2	0.92 ± 0.12	0.77 ± 0.09	<.001
T-score LS	−1.3 ± 1.1	−2.6 ± 0.9	<.001
FN BMD, g/cm^2	0.71 ± 0.07	0.60 ± 0.09	<.001
T-score FN	−1.5 ± 0.5	−2.3 ± 0.8	<.001

Abbreviation: LS, lumbar spine. Values are expressed as mean ± SD. FRAX is expressed as median ± SEM.

tients who had sustained a leg fracture of the ipsilateral side of the measurement (n = 8) compared with those with a fracture of the contralateral side (n = 10) (77.6 ± 1.8 vs 78.3 ± 1.6; $P = .777$). Compared with patients with osteoporosis, patients with osteopenia comprised relatively more men and had higher BMI and BMD and lower 10-year fracture probability. All other clinical characteristics and biochemical measurements were similar between the two groups.

Discussion

We show here that patients who had sustained a fragility fracture demonstrate a significantly lower BMS, as measured by the microindentation in vivo technique, compared with patients who did not fracture. More importantly, our data also demonstrate that there was no difference in BMS in patients with fragility fractures whether they had osteopenia or osteoporosis. Our findings thus suggest that bone material properties are altered in patients with a fragility fracture and that the microindentation in vivo–derived BMS measurement captures elements of bone fragility independently of BMD. Analysis of data on bone turnover markers, which may also be associated with an increase in fracture risk independently of BMD (24), were not undertaken in this study because reliable interpretation of the data was precluded by the large number of patients with a recent fracture and the influence of this on serum levels of these markers.

Microindentation in vivo is a new technique, designed to measure the resistance of bone to fracture by separating mineralized collagen microfibers and thus, locally inducing microcracks. In the first human studies, the material properties of bone were quantified by total indentation distance, indentation distance increase (IDI), and creep indentation distance (14, 25). Of these parameters, IDI differentiated best between bone that was easily susceptible to fracture and bone that did not easily fracture, and the parameter was found to correlate best with toughness of bone (11, 13). This has led to the development of the derived parameter of BMS, which is calculated by the ratio of the IDI of the calibration material PMMA to the IDI of bone (15).

In our study, we found a strong relationship between BMS and age, which may play an important role in the increased fracture risk observed in elderly patients, in whom deteriorated bone microarchitecture has also been demonstrated (8). Several ex vivo studies have shown an inverse relationship between age and toughness of bone (26, 27). Bone toughness is best predicted by the Indentation Distance Increase and thus by BMS (13). As bone strength is inversely correlated with the density of microcracks in bone tissue, the observed alteration in bone material properties in the elderly might well be explained by the previously demonstrated age-related accumulation of microcracks (26, 27).

Having established that BMS reflected bone fragility independently of BMD, we went on to test the association between BMS and the clinical risk factors used in the FRAX algorithm without inclusion of BMD measurements in the calculation. We found a significant relationship between BMS and the 10-year fracture probability calculated by FRAX without BMD, probably reflecting the lack of correlation between BMS and BMD values. These observations suggest that microindentation in vivo is able to capture an element of the contribution of clinical risk factors used in the FRAX algorithm to altered material properties of bone, and thus to increased fracture risk.

Our data complement and extend those of Diez-Perez et al (14), who, using the microindentation technique, showed that bone material properties, as measured by IDI, were poorer in 27 postmenopausal women who had sustained mainly a hip fracture (n = 25) or vertebral fracture (n = 2), compared with age-matched controls who had not sustained a fracture, with the caveat that women in the control group had a higher BMD than the fracture patients.

Postmenopausal women have altered bone microarchitecture, and more recent data showed that osteopenic women who sustained a fracture had worse bone microarchitecture than nonfracture controls (8, 9). Increased cortical porosity has also been suggested to contribute to the risk of distal forearm fractures in postmenopausal women with osteopenia (10). Our data provide evidence that bone material properties are altered in patients with osteopenia who have sustained a fracture, most whom are currently not being offered treatment with bone-modifying agents.

Our study has strengths as well as limitations. We sequentially investigated patients of both sexes with a wide age range and low bone mass reflecting everyday clinical practice. The frequency of osteopenia and osteoporosis within the group of patients with a fragility fracture is further consistent with previous reports in fracture patients (6, 7) and all measurements were performed by two dedicated operators. Furthermore, age and sex of patients enrolled onto the study did not differ from those who were not investigated. A limitation of our study is that, whereas the main source of the fragility fracture patients included in the study was our regional Fracture Liaison Service, the nonfragility fracture controls were recruited from patients routinely attending our outpatient clinic, in whom BMD measurements were requested at the discretion of the treating physician, possibly creating a selection bias.

In conclusion we demonstrate in this study that BMS, as measured by the microindentation in vivo technique, captures elements of bone fragility such as the effect of aging and that of the cumulative effect of clinical risk factors as calculated by the FRAX algorithm, independently of BMD. Furthermore, we demonstrate that BMS is comparable in patients with a fragility fracture, whether they have osteoporosis or osteopenia. These data suggest an aspect of altered bone quality contributing to bone fragility which is not captured by BMD. Additional studies are required to establish the value of BMS as a predictor of fracture risk, especially in patients with osteopenia.

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