Accepted Manuscript

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PII: S8756-3282(16)30328-3
DOI: doi: [10.1016/j.bone.2016.11.003](https://doi.org/10.1016/j.bone.2016.11.003)
Reference: BON 11175

To appear in: *Bone*

Received date: 12 September 2016
Revised date: 31 October 2016
Accepted date: 2 November 2016

Please cite this article as: Herrera Sabina, Diez-Perez Adolfo, Clinical experience with microindentation *in vivo* in humans, *Bone* (2016), doi: [10.1016/j.bone.2016.11.003](https://doi.org/10.1016/j.bone.2016.11.003)

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Review

Clinical experience with microindentation *in vivo* in humans

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Abstract

Densitometry and imaging techniques are currently used in clinical settings to measure bone quantity and spatial structure. Recently, reference point indentation has opened the possibility of directly assessing the mechanical characteristics of cortical bone in living individuals, adding a new dimension to the assessment of bone strength. Impact microindentation was specifically developed for clinical studies and has been tested in several populations where there are discrepancies between bone density and fracture propensity, such as type 2 diabetes, atypical femoral fracture, stress fractures, glucocorticoids treatment, patients with osteopenia and fragility fractures, and individuals infected with HIV, among others. Microindentation will complement, not replace, existing bone analysis methods, particularly where bone mineral density does not fully explain fracture propensity. The available evidence provides solid proof of concept; future studies will fully define the role of microindentation for the assessment of bone health both in clinics and in research.

Highlights

- Reference Point Indentation techniques for direct measurement of bone tissue mechanical properties have been tested in humans.
- Low microindentation values have been found in situations where bone density does not explain fracture propensity.
- Microindentation may complement available techniques for a full assessment of bone health.

Keywords

Bone microindentation. Bone mechanics. Cortical bone assessment. Reference Point Indentation

Abbreviations and Glossary of terms

BMD: Areal bone mineral density measured by DXA.

BMSi: Bone Material Strength index. Ratio of the penetration of a probe into the bone compared to a reference standard of polymethyl methacrylate, measured by impact microindentation. Expressed in absolute units [1].

CID: Creep indentation distance. Calculated as a constant force during the 1st indentation cycle in cyclic microindentation. Expressed in microns [2].

Cyclic microindentation: Application to the bone of a series of cycles of force, using the BioDent™ laboratory instrument [2].

IDI: Indentation Distance Increase. Difference in distance penetrated between the 1st and last indentation cycle in cyclic microindentation. Expressed in microns [2].

Impact microindentation: Application of a single, high-speed force on the outer cortical bone using a hand-held instrument (Osteoprobe™) [1].

RPI: Reference Point Indentation. Generic term that encompasses both impact and cyclic microindentation.

TID: Total Indentation Distance. Difference in penetration achieved between the beginning of the 1st and the end of the last indentation cycle in cyclic microindentation. Expressed in microns [2].

1. Foreword

Reference Point Indentation (RPI), a novel technique for direct measurement of mechanical characteristics of cortical bone at a tissue level, was first published in a clinical series of patients in 2010 [2] and since then a number of preclinical and clinical studies have been performed. The present study aimed to review the published clinical experience in different groups of patients and to discuss how RPI can contribute to the assessment of bone health as well as potential development of this new technique. Preclinical results were not included in the analysis.

2. Measurement of bone strength

From a clinical perspective, bone strength could be understood as the ability of bone to resist trauma without breaking. According to the currently accepted definition of osteoporosis [3], bone strength reflects the integration of bone quantity and bone quality. Bone quantity can be measured in the clinical setting by various techniques. Among them, dual-energy x-ray densitometry (DXA) is the most widely used and a standard for the diagnosis and management of osteoporosis. DXA is an excellent predictor of fracture risk [4] and efficiently captures the influence of treatments on bone propensity to fracture [5] in the average patient with osteoporosis.

However, two other aspects contributing to the mechanical strength of bone are important for estimating mechanical competence. The first is the spatial distribution of bone tissue, either at a macroscopic (geometric) or microscopic (microarchitectural) level, and the second is the composition of the bone tissue. Spatial distribution of bone tissue can be assessed in clinical practice by a variety of imaging techniques, from radiography to high-definition computed tomography [6] or magnetic resonance imaging [7]. The higher the definition of the images, the better the possibility of accurately measuring microscopic structures. The bone tissue components are also relevant for bone strength. A very long list of tissue constituents contributes to better or worse mechanical behavior of the bone tissue,

including, among others, collagen characteristics [8], crystallinity [9], degree of mineralization and homogeneity [10] and non-collagen proteins [11].

Ultimately, bone mechanical performance is the final result of the integration of bone density, macro/microarchitecture and tissue composition, which can be calculated by building image-based algorithms. Finite-elements analysis [4], the most widely used technique, can estimate overall mechanical competence and also simulate vector forces applied to the bone and their ability to resist them. This strategy integrates the quantitative and architectural components of bone strength, although tissue composition is based on the optical density of the voxel unit. However, its feasibility in clinical practice is restricted due to the limited availability of CT (or MRI) devices. Only the DXA-based TBS (Trabecular Bone Score) is an easy and practical technique for measuring structure in the vertebrae [12].

Direct measurement of the mechanical characteristics of bone requires destructive testing, in which force is applied to a small bone or a sample of bone until it breaks. These tests require sophisticated technology only available in a few biomechanics laboratories. Moreover, invasive bone sampling is required in living patients, either a bone biopsy or a specimen obtained during a surgical procedure. This makes the study cumbersome, expensive and not feasible in daily clinical practice.

Therefore, there is a need for a convenient technique for the assessment of the mechanical characteristics of bone tissue in clinical settings. This was the basis for the development of the Reference Point Indentation (RPI) techniques. We review below the clinical experience published to date.

3. Reference Point Indentation Techniques

Two different approaches have been developed for measuring RPI parameters. Both are based on the principle that the deeper a test probe penetrates into a cortical bone's outer surface (at the anterior midtibia), the less resistant is the bone tissue to a mechanical challenge. The early development of RPI was based on a cyclic indentation technique (Figure 1a); more recently, impact microindentation (Figure 1b) has been developed for clinical use. Details on both techniques have been previously published [13]. Cyclic

microindentation parameters are expressed primarily in terms of indentation distances, although other biomechanical parameters can be calculated, such as the slopes of the loading-unloading curves or the calculation of dissipated energy (Figure 2a) [2]. Impact microindentation results are expressed as Bone Material Strength index (BMSi) units representing the ratio between the penetration of the probe into the bone and its penetration in a methyl methacrylate reference phantom (Figure 2b) [14]. The technical protocol for measuring patients with impact microindentation has been recently described [15].

4. Early clinical studies: Cyclic microindentation

The first two studies on microindentation in living humans were performed with a cyclic microindentation device. They are summarized in Table 1.

4.1. Discriminant ability between fractures and controls

The first clinical study that validated RPI, using cyclic microindentation [2], was performed in 27 women with osteoporosis-related fractures and 8 controls of comparable ages. Two parameters, Total Indentation Distance (Total ID) and Indentation Distance Increase (IDI), were significantly greater in patients with fracture than in controls, with good (above 90%) areas under the receiver operating characteristic (ROC) curve. The study showed that this technique is capable of discriminating cases with and without fractures, independently of BMD.

4.2. Atypical femoral fractures

The second study using cyclic RPI was done in patients with atypical femoral fractures (AFF) after long-term bisphosphonates treatment [16]. Four groups of patients were included: AFF exposed to bisphosphonates (n=6), typical osteoporotic fractures with no previous treatment (n=38), patients on long-term bisphosphonates with no complications (n=6), and fracture-free controls (n=20). Significant differences were observed between control, typical fracture and AFF groups for Total ID and IDI; the deterioration in bone material properties at a tissue level in patients with AFF was similar to that of the osteoporotic fracture group. Moreover, RPI parameters in the group on long-term treatment with no complications did not differ from controls, suggesting that bisphosphonate therapy itself does not deteriorate the microindentation properties of bone tissue.

5. Clinical studies with impact microindentation.

Several studies have been performed with impact microindentation in living humans. These are summarized in Table 2.

5.1. *Type 2 diabetes mellitus.*

The first clinical study using impact microindentation [17] studied 60 postmenopausal women, 30 diagnosed with type 2 diabetes for more than 10 years and 30 age-matched non-diabetic controls. Compared to controls, patients had significantly lower bone material strength index (BMSi) although their bone mineral density (BMD) was similar to age-matched, non-diabetic women. Moreover, the average glycated hemoglobin level over the previous 10 years was negatively correlated with BMSi.

A second study in postmenopausal women with type 2 diabetes mellitus [18] has found that BMSi was significantly reduced in these patients and inversely associated with the duration of the diabetes. Advanced glycation end products accumulation was inversely associated with BMSi and lower bone formation biochemical markers in patients with diabetes but not in controls, further indicating the contribution of BMD-independent factors to the bone disease in these patients.

5.2. *Racial differences in material properties*

Given that previous studies had failed to identify differences in bone mass or calcium metabolism that could explain the high risk of hip fractures in Norwegian women, Duarte Sosa et al [19] compared 42 Norwegian with 46 Spanish women, a population with a 50% lower rate of hip fracture. The participants had normal BMD values, without vertebral fractures or secondary osteoporosis. The BMSi value of Norwegian women was significantly lower than in Spanish women, but total hip BMD was significantly higher in the Norwegian participants. Regression analysis revealed that indentation values did not vary with BMD. The researchers concluded that impaired bone material properties and geometrical factors linked to higher stature provide a plausible hypothesis for the higher risk of fractures in the Scandinavians.

5.3. *Patients with osteopenia and fragility fractures*

It is well known that, numerically, most fragility fractures occur in patients with osteopenia assessed by DXA [20][21]. Malgo et al [22] carried out a cross-sectional study of 90

patients with low bone mass with or without fragility fractures; 63 of the patients had sustained one or more fragility fractures. BMSi values were lower in patients with a fragility fracture compared with non-fracture patients, despite similar BMD, and were comparable in patients with a fragility fracture whether they had osteopenia or osteoporosis. In patients with osteopenia, BMSi was significantly lower in patients with fracture than in the non-fracture group. The fact that BMSi values were significantly decreased in patients with fragility fractures regardless of osteopenia or osteoporosis suggests that bone material properties are compromised in these patients and play an important role in the development of fractures regardless of BMD.

5.4. Glucocorticoid-induced bone fragility

Mellibovsky et al [23] analyzed a series of patients who were within 4 weeks of initiating glucocorticoid treatment. As a bone-protective therapy, they received either calcium + vitamin D alone or were treated with risedronate, denosumab or teriparatide. BMSi was measured at baseline, 7 weeks and 20 weeks. There was no association between initial individual glucocorticoid dose and BMSi change. After adjustments, BMSi significantly decreased in the Ca+D-alone group, did not significantly change in the risedronate group and significantly increased in both the denosumab and teriparatide-treated groups, with significant changes already detectable at the first follow-up visit (after 7 weeks of therapy). No changes were detected in BMD in such a short period of time. This was the first study to show early changes in cortical bone indentation properties after systemic glucocorticoid treatment with microindentation, at a time when BMD imaging by DXA cannot detect any alteration.

5.5. Patients with HIV

Patients with HIV have an increased risk of fracture [24]. This led to the first study designed to measure BMSi in a group of individuals infected with the HIV virus [25]. Impact microindentation results showed a significantly lower BMSi in patients with HIV than in controls; the study groups had no significant differences in BMD at any of the sites examined.

5.6. Chronic atrophic gastritis

Men, but not women, with chronic atrophic gastritis [26] have been found to have lower lumbar BMD, a higher frequency of osteoporosis at the lumbar spine and osteopenia at

total hip, compared to controls. A trend toward a lower trabecular bone score was observed in the patient group, compared to controls. BMSi did not differ by sex or between patients and controls, probably as a result of the small number of patients included in the study.

5.7. Kidney transplant

In a long-term (>10 years) series of patients who received a kidney transplant [27], BMD was lower at lumbar spine, total hip and femoral neck, compared to controls. A decrease in BMSi was also observed, although this difference disappeared in the adjusted model (for age, gender and body mass index (BMI)). These results suggest that bone tissue mechanical characteristics are restored in the long term after kidney transplant, even if there is not a complete normalization of BMD.

5.8. Stress fractures

Mean BMSi in a group of women with stress fracture was significantly lower than in controls [28]. The fracture group also had a significantly lower mean BMD than the controls. There was no correlation between BMSi and BMD or bone turnover. This study suggests that a deterioration of material properties of bone is associated with –or contributes to– stress fractures.

5.9. Fractures in the elderly

In a cohort of elderly women with and without fractures, Rudäng et al [29] found that BMSi was positively associated with BMD of the total hip, non-dominant radius, and lumbar spine, although there was no association between crude or adjusted BMSi and prevalent fractures. In this study, BMSi was associated with areal BMD but was not related to fracture.

5.10. Correlation with subcutaneous fat

A study by Sundh et al [30] measured bone parameters and subcutaneous fat at the tibia in a group of Scandinavian women. BMSi was inversely correlated to body mass index, whole body fat mass and, in particular, to subcutaneous fat. This tibia fat was found to be independent of covariates and associated with BMSi, cortical porosity (Ct. Po) and cortical volumetric (Ct. v) BMD. BMSi was independent of covariates associated with cortical porosity and cortical volumetric BMD at the distal tibia. In this first study to associate fat mass with BMSi, fat was independently and inversely associated with BMSi and Ct.v BMD

but positively associated with Ct.Po. This would suggest a possible adverse effect of adipose tissue on bone quality and bone microstructure.

6. Preliminary results of other clinical studies

Some clinical results have been communicated as abstracts to medical meetings and therefore can be only briefly discussed. Malgo et al [31] studied well-controlled patients with acromegaly and matched controls with microindentation, finding no differences in BMD but lower BMSi in the patient group. In patients with type 1 Gaucher's disease, Herrera [32] also reported decreased BMSi. A study by Rufus et al on the feasibility of impact microindentation found excellent patient tolerance and acceptance in a population-based sample and controls [33]. In patients with diabetes, Karim et al [34] found that individuals with T2D had increased cortical porosity, a nonsignificant trend for increased Advanced Glycation Endproducts (AGE) and compromised biomechanical properties in femoral neck specimens. Similar results were reported by Nilsson et al [35], showing that microindentation (BMSi) was significantly lower in elderly women with T2D, compared to controls. In older adults with T2D, Barnouin [36] assessed the relationship between aerobic fitness and bone material strength and suggested a correlation of aerobic fitness with higher BMSi. Sundh et al [37] tested 20 healthy but inactive post-menopausal women, finding that intense mechanical loading resulted in a rapid improvement of bone material properties before any increase in bone mass.

7. Discussion

Bone RPI is emerging as a feasible technique for the clinical settings. After the initial validation study using cyclic microindentation [2], clinical development has focused on impact microindentation, which is much more convenient for clinicians and for patients. Although both techniques follow the same general principle, it is important to note that because the mechanical challenge is different they do not measure the same mechanical properties [13]. Therefore, the results obtained with the former cannot be extrapolated to the latter. Cyclic microindentation is used in laboratory experiments, while clinical studies are now performed with impact microindentation. Again the extrapolation of preclinical RPI results to the clinical measurements must be done with great caution. Exactly what we measure with RPI is not fully understood. Both techniques show a weak or nonexistent

association with BMD and cortical geometry, but there is also a weak correlation between RPI impact and cyclic variables [39]. What RPI does, when penetrating the outer cortical bone, is to estimate the propensity for the opening of microscopic cracks. The cyclic approach is more similar to a creep test or a low-cycle fatigue test while the impact technique is more similar to a microhardness test [40]. However, the translation of the classical biomechanical test results to RPI variables, and to BMSi in particular, is an unresolved issue.

From a clinical perspective, the beginning of the interest in RPI was the demonstration of its ability to discriminate between individuals with established osteoporosis and controls [2]. Not by chance, later experiments were centered on situations where the risk of fracture is only partially explained by decreased BMD, as in patients with atypical femoral fractures (using cyclic microindentation) [16], patients with type 2 diabetes mellitus (using impact microindentation) [17], or patients with osteopenia and fragility fractures [22]. The common basis for these results was that bone density does not fully account for the increased fracture risk and, on the other hand, that the correlation between the indentation parameters and BMD was poor. Similar results with decreased BMSi and “preserved” BMD are present in the treatment-naïve HIV-infected population. The discrepancies between BMD and fracture risk are important when comparing Scandinavian and Mediterranean women; here again, lower BMSi can account for at least a part of the increased fracture rates in Norwegians [19]. Stress fractures, another clinical situation where bone fragility is not explained by bone density, also are associated with deterioration in BMSi [28]. Altogether, the evidence supports the idea that, independently of BMD, tissue-level properties captured by RPI may help to explain the increased fragility of bone. Returning to the classical definition of osteoporosis [3], elements other than BMD are relevant for bone health. For example, in type 2 diabetes, the accumulation of AGE correlates with BMSi [18].

Our approach to osteoporosis has been based mainly on the quantitative aspect of how much bone there is, very likely because we have a method to accurately and conveniently measure this dimension: bone densitometry. Only recently have we begun incorporating imaging techniques to assess the architectural aspects of the osteoporotic bone [41][42]. What RPI can add is the direct measurement of tissue-level characteristics of bone, which is not captured by the clinically available methods to assess quantitative or spatial distribution. Osteoporosis can result from the deterioration of any of the three components

of bone strength –density, microarchitecture and tissue properties– and likely by a combination of all three, in different proportions for different pathological situations. The poor discrimination capacity of RPI to identify fracture risk in the study performed in the elderly [29] suggests that when certain components are very dominant, such as bone density and microarchitecture in the elderly, tissue assessment by RPI may add very little to the explanation of increased bone fragility, although further studies are needed to assess this particular population. Similarly, patients with chronic atrophic gastritis have a major decrease in BMD and/or architecture [26] probably limiting the discriminant ability of RPI to differentiate between patients and controls. On the opposite, in Type 2 diabetes both of these components are relatively preserved and BMSi is the most deteriorated component of bone strength [17].

Finally, just as our understanding of osteoporosis has been centered on bone density, so has our assessment of its treatment. Drug trials have focused on the selection of participants using BMD criteria and have measured efficacy by increases in bone density, which is of course relevant but may not sufficiently capture BMD-independent effects of the drugs [43][44]. This might explain why some treatments show anti-fracture efficacy largely independent of the bone density effects [45]. RPI might contribute to explaining some of the effects of commonly used antiosteoporosis medications, as shown by the very rapid increase in BMSi induced by teriparatide or denosumab in patients initiating glucocorticoids [23]. This rapid variation, after only 7 weeks, in the response of bone to indentation suggest that BMD-independent (and, plausibly, remodeling-independent effects) might be induced by the treatments. Our hypothesis is that non-collagenous proteins that constitute the elements that absorb energy in bone during the process of separation of mineralized collagen fibrils and initiation of microcracks, called sacrificial bonds, might play a role in the indentation properties of bone and in the effects of drugs [46]. Preclinical results also support the effectiveness of treatments on RPI properties, independently of BMD changes [47].

8. Future development needs and opportunities

Reference Point Indentation is at the beginning of its development as a tool for assessing the skeletal health. Although the available evidence shows the technique's potential, large prospective studies are needed to determine whether the technique can independently

predict incident fractures. Reference values are also needed, similar to what is available for BMD, and reference intervals for normal, intermediate and pathological BMSi must be tested for validity according to race, gender and geographical region. It is also important to establish how much useful information RPI can contribute to the clinician's assessment of different pathological situations and of patient response to treatment interventions. The available information suggests that most of the potential lies in the scenarios where BMD does not fully explain a patient's skeletal fragility or the anti-fracture effect of treatments. Development and implementation of an automated system in the device's software to identify which measurements are outliers and should be excluded from the calculation of results is an additional technical need; this would exclude the potential for observer bias that exists when the operator makes this decision.

Cortical assessment in the tibia has been pointed out as a limitation of RPI, as the main clinical fragility fractures occur in other areas of the skeleton where trabecular bone also plays a determinant role. Ultimately the clinical evidence can overcome this issue if the prediction of fractures is good enough on its own or adds precision to the combination of various bone assessment techniques. This has been experimentally demonstrated in cadaver experiments [48] for cyclic microindentation, but must also be studied for the impact microindentation tests appropriate for clinical use.

The opportunities for the future are significant. In approaching the individual patient, the clinician currently chooses a treatment strategy supported by the available evidence, obtained in standard populations selected by strict inclusion and exclusion criteria. However, very often the case to be managed does not match that particular study population [49]. Patients with osteoporosis commonly suffer comorbidities and receive multiple drugs that jeopardize the effect expected from a treatment [50]. Access to more exhaustive information about the pathophysiological determinants of fracture propensity would help the clinician to better refine interventions and measure their effects. In other areas of medicine, like heart diseases, the clinician assesses the patient using clinical examination, electrocardiography, sophisticated ultrasounds, angiography and/or isotopes. Although RPI will not replace the available clinical measurements, it has the potential to add information to bone density and structure data and contribute to a comprehensive understanding of the individual patient.

A comprehensive evaluation of the different contributors to bone strength has excellent potential for the development of drug therapies. First, it will help to better identify the BMD-independent effects of the available drugs and translate this into the specific needs of each type of patient. Second, new drugs can be developed that more specifically account for the various deteriorated components of bone health, beyond the one-size-fits-all approach we currently adopt. Third, if we can integrate different surrogates that very accurately analyze the effect of drugs on bone fragility, developing new drugs may become much more accessible. Substantially shorter efficacy studies in reduced populations would then be able to certify the therapeutic benefits, making the pivotal trials much more affordable, with the consequent benefit in exploring new treatment opportunities.

Finally, the present review shows that RPI is a clinically feasible technique with high potential for the evaluation of patients with different type of osteoporosis and for the assessment of intervention effectiveness. A wide range of research opportunities is presented by this direct *in vivo* assessment of the mechanical properties of bone tissue.

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Conflict of interest

A Diez-Perez owns shares of Active Life Scientific, a manufacturer of microindentation devices. S Herrera has no conflict of interest.

Acknowledgments

Sponsored in part by the RETICEF, Instituto Carlos III (Grant RD12/0043/0022), Spanish Ministry of Economy and Competitiveness and FEDER Funds

Figure legends

Figure 1. RPI devices. A) Cyclic Reference Point Indenter (BioDent[®]); B) Impact Reference Point Indenter (Osteoprobe[®]).

Figure 2. Schematic representation of the screen captures for A) Cyclic RPI with the force/distance curve and; B) Impact RPI with the distance/time curve (early version of the device).

Figure 1a

Figure 1b

Figure 2B

Table 1. Published studies of clinical experience with cyclic microindentation

Study authors and year	Study design	Study population	Outcomes
Díez-Pérez A, et al. <i>J Bone Miner Res</i> 2010;25:1877–85.	Series of cases and controls.	27 women with osteoporosis-related fractures and 8 controls of comparable ages	Measured Total Indentation Distance and Indentation Distance Increase were significantly greater in fracture patients than in controls. Areas under the receiver operating characteristic curve for the two measurements were above 90%.
Güerri-Fernandez R, et al. <i>J Bone Miner Res</i> 2013;28:162–8.	Series of cases and controls.	Four groups of patients (n=70): 6 atypical femoral fractures, 38 typical osteoporotic fractures, 6 taking long-term bisphosphonates, and 20 controls without fracture	After adjusting by age, significant differences were observed between controls and typical and atypical fractures for Total Indentation Distance and for Indentation Distance Increase.

Total published studies: 14.
Total Patients Indented. 1244

Table 2

Study authors and year	Microindentator used	Study design	Study population	Outcomes
Farr JN et al. <i>J Bone Miner Res</i> . 2014 Apr;29(4):787-95		Series of cases and controls.	60 postmenopausal women including 30 patients diagnosed with T2D for >10 years and 30 age-matched, nondiabetic controls	T2D patients had significantly lower BMS, adjusted $p=0.022$. In patients with T2D, the average glycated hemoglobin level over the previous 10 years was negatively correlated with BMS ($r=-0.41$; $p=0.026$)
Duarte Sosa D, et al. <i>J Bone Miner Res</i> 2014;30:1784–9	impact microindentation	Series of cases and controls??	42 Norwegian and 46 Spanish women with normal BMD values, without vertebral fractures or secondary osteoporosis. 88	BMSi value of Norwegian women was significantly inferior when compared to Spanish women $p < 0.001$
Malgo F, et al. <i>J Clin Endocrinol Metab</i> 2015;100:2039–45	impact microindentation	Cross-sectional study	90 patients with low bone mass with or without a fragility fracture. Sixty-three patients had sustained one or more fragility fractures.	BMS values were lower in patients with a fragility fracture compared with nonfracture patients $P = 0.032$) despite similar BMD. In patients with osteopenia, BMS was significantly lower in fracture patients than in nonfracture $p = 0.015$
Mellibovsky L, et al. <i>J Bone Miner Res</i> 2015;30:1651–6.	impact microindentation	Series of cases	52 consecutive cases were included in the study within 4 weeks of initiating glucocorticoid treatment.19	Changes in cortical bone indentation properties, at the tissue level, can be tracked longitudinally using the reference point indentation technique in patients exposed to systemic glucocorticoid treatment. These changes occur very early (within the first few weeks) after starting

			patients received treatment with calcium plus 25 (OH) vitamin D; 14 risedronate; 14 denosumab; and 5 received teriparatide.	glucocorticoids, well before BMD imaging by DXA can detect any alteration.
Güerri-Fernandez R, et al. <i>JAIDS</i> . 2016 Jul 1;72(3):314-8	impact microindentation	Series of Cases and controls.	50 infected with HIV and 35 controls. 85	HIV infection is associated with bone damage, independently of BMD. $p < 0.001$
Sundh D, et al. <i>J Bone Miner Res</i> 2016;31:749–57	impact microindentation	Series of cases	202 women, bone parameters and subcutaneous fat were measured at the tibia	BMSi was inversely correlated to body mass index (BMI) ($r = -0.17$, $p = 0.01$), whole body fat mass ($r = -0.16$, $p = 0.02$), and, in particular, to tibia s.c. fat ($r = -0.33$, $p < 0.001$).
Aasarød KM et al. <i>Scand J Gastroenterol</i> 2016 Feb 8	impact microindentation	Cross-sectional study	17 chronic atrophic gastritis 41 sex- and age-matched controls. 58	Lower lumbar BMD, increased frequency of osteopenia and osteoporosis in male, but not female patients with CAG. BMS did not differ between the groups.
Pérez-Sáez MJ et al. <i>Transplantation</i> 2016. Pub ahead of print	impact microindentation	Series of cases and controls.	40 long-term after kidney transplant and 94 sex-age matched controls. 134	BMD was lower at lumbar spine ($p=0.025$), total hip ($p<0.001$) and femoral neck ($p<0.001$) in KTR than in controls. BMSi was also lower in KTR ($p=0.012$) although this difference disappeared after adjusted model by age, gender and body mass index ($p=0.145$).
Duarte Sosa D, et al. <i>Acta Orthop</i> . 2016 Jun 20:1-6.	impact microindentation	Series of cases and controls.	30 women with previous stress fractures and in 30 normal controls. 60?	BMSi was inferior in patients with previous stress fracture, but was unrelated to BMD and bone turnover. $p = 0.02$
Rudäng R et al <i>Osteoporosis int</i> 2016 APR; 27(4): 1585-92	impact microindentation	Cohort	211 older women	BMSi was positively associated with aBMD of the total hip ($\beta = 0.14$, $p = 0.04$), non-dominant radius ($\beta = 0.17$, $p = 0.02$), and lumbar spine (L1-L4) ($\beta = 0.14$, $p < 0.05$). No association in crude or adjusted BMSi with prevalent fractures.

Malgo F et al. abstract al ENDO2016, Boston, Massachusetts	impact microindentation	Cross- sectional study	32 well-controlled acromegalic patients and 32 age-matched controls. 64	Adjusted for BMI, LS BMD was not significantly different between acromegaly patients and controls, but BMSi was lower than in controls p= 0.004
Furst JR et al J Clin Endocrinol Metab 2016 JUN;101(6):2502- 10.	impact microindentation	Cross- sectional study	16 postmenopausal women with T2D and 19 matched controls. 35	BMSi was reduced by 9.2% in T2D (P = .02) and was inversely associated with the duration of T2D (r = -0.68, P = .004). Increased SAF was associated with reduced BMSi (r = -0.65, P = .006) and lower bone formation marker procollagen type 1 amino-terminal propeptide (r = -0.63, P = .01) in T2D, whereas no associations were seen in controls

Highlights

- Reference Point Indentation techniques for direct measurement of bone tissue mechanical properties have been tested in humans.
- Low microindentation values have been found in situations where bone density does not explain fracture propensity.
- Microindentation may complement available techniques for a full assessment of bone health.