Bone tissue properties measurement by reference point indentation in glucocorticoid-induced osteoporosis

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Conflicts of interest
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Abstract

Glucocorticoids, widely used in inflammatory disorders, rapidly increase bone fragility and, therefore, fracture risk. However, common bone densitometry measurements are not sensitive enough to detect these changes. Moreover, densitometry only partially recognizes treatment-induced fracture reductions in osteoporosis. Here, we tested whether the reference point indentation, technique could detect bone tissue properties changes early after glucocorticoid treatment initiation.

After initial laboratory and bone density measurements, patients were allocated into groups receiving calcium + vitamin D (Ca+D) supplements or anti-osteoporotic drugs (risedronate, denosumab, teriparatide). Reference point indentation was performed on the cortical bone layer of the tibia by a handheld device measuring Bone Material Strength index (BMSi). Bone mineral density was measured by dual-energy x-ray absorptiometry (DXA).

While Ca+D-treated patients exhibited substantial and significant deterioration, risedronate-treated patients exhibited no significant change, and both denosumab- and teriparatide-treated participants exhibited significantly improved BMSi 7 weeks after initial treatment compared to baseline; these trends remained stable for 20 weeks. In contrast, no densitometry changes were observed during this study period.

In conclusion, our study is the first to demonstrate that reference point indentation is sensitive enough to reflect changes in cortical bone indentation following treatment with osteoporosis therapies in patients newly exposed to glucocorticoids.
Keywords: Reference point indentation; Biomechanics; Corticosteroids; Bone material strength; Anabolics; Antiresorptives.
Introduction

Glucocorticoid treatment is widely used and effective for treating a number of immune and inflammatory disorders (1), but it can have deleterious effects on the bone. These effects, which are complex and only partially understood (2-5), eventually lead to rapid deterioration of bone strength with a subsequent increase in fracture risk detectable very early after initiating therapy (6-10). Bone mineral density (BMD) measurements have been recommended for guiding clinical decisions (11). However, in glucocorticoid-treated individuals, bone fractures occur at higher BMD levels than in individuals with postmenopausal or senile osteoporosis; these fractures can also occur very early, well before BMD evaluation can detect any significant decline (12,13). Furthermore, BMD can only partially measure the reductions in fracture risk (i.e., the effect on bone strength) after treatment with different anti-osteoporotic drugs (14,15). Thus, more sensitive clinical measurements based on other contributions to bone strength are needed. The BMD-independent glucocorticoid effect on bone fracture resistance (i.e., increased bone fragility) is a prime example of deterioration in bone tissue quality (16,17), which is defined as a mass-independent change in intrinsic material properties that contributes to the bone fragility observed in osteoporosis (6,7) and other conditions (18,19). Despite this need for measuring the contribution of bone properties at a tissue level to bone fragility, direct assessment of bone tissue mechanical properties has not yet been used in patients because mechanical testing requires sampling of bone specimens, not feasible in clinical practice. Moreover, longitudinally monitoring the BMD-independent effect of most available anti-osteoporotic drugs on fracture reduction (i.e., bone fragility improvement) (13,14) is also unfeasible. On one hand, repeated measurements of tissue components cannot be performed because of the invasive nature of the methods. On the other, imaging techniques for measuring bone microarchitecture and assessing bone strength are
restricted to research centers with advanced technologies, and these techniques have the disadvantages of long response periods \(^{(20-23)}\) or a low sensitivity for detecting changes \(^{(24)}\).

Reference point indentation (RPI) is a recently developed microindentation technique (Fig. 1) for assessing bone mechanical characteristics at the tissue level \(^{(25)}\). The data obtained by this technique in animal studies has been correlated with bone toughness in some \(^{(26)}\) but not all \(^{(27)}\) experiments and can detect treatment-induced changes in bone material properties \(^{(28)}\). In patients, reference point indentation can discriminate between fracture cases and controls \(^{(29)}\) and can identify bone tissue deterioration in cases of atypical fracture \(^{(30)}\). A new hand-held RPI instrument has been developed for convenient clinical use \(^{(31)}\) and has successfully detected bone quality deterioration in diabetic patients independent of BMD \(^{(32)}\). Although one theoretical advantage of reference point indentation is the ability to kinetically monitor changes in bone mechanical properties, available data from cross-sectional studies in humans has not yet formally demonstrated this potential. Here, we addressed this potential by evaluating the efficacy of reference point indentation for longitudinal studies in a patient population receiving glucocorticoid therapy, which is well known to cause rapid deterioration in bone strength. We further tested whether reference point indentation could detect tissue-level responses to various anti-osteoporotic medications.

**Materials and Methods**

**Study population**

A clinical series of consecutive cases were included in the study within 4 weeks of initiating glucocorticoid treatment. The required glucocorticoid dose was at least 5 mg/day prednisone (or equivalent) during the observation period. Patients were
considered ineligible if they had been previously exposed to systemic glucocorticoids, anti-osteoporotic medications, radiation therapy, or other drugs that could potentially affect the bone. Additionally, patients were ineligible if they were previously diagnosed with chronic endocrine, hepatic, renal, or malabsorptive disease; Paget’s disease of bone; neoplasia; or any condition that, in the opinion of the investigator, might interfere with the study protocol. Table 1 details the baseline characteristics of the study cohort, including background conditions.

All groups received Ca+D supplementation. According to Spanish guidelines (33), patients of age below 65 and T-score >-1.5 were assigned to Ca+D only and teriparatide was indicated in severe osteoporosis cases (T-score of -3.5 or below with or without fractures, or a T-score of -2.5 or below plus a fragility fracture). The remaining patients were assigned to the risedronate group unless any contraindications or upper gastrointestinal complaints occurred, in which case patients were assigned to the denosumab group.

**Reference point indentation method**

At baseline, patients underwent a general laboratory workup, and BMD was measured at the lumbar spine and hip using DXA (Hologic QDR 4500 SR™, Hologic, Bedford, MA). Bone indentation measurements were carried out at baseline (visit 0 [V0]) and again at 7 weeks (V1) and 20 weeks (V2) later using an Osteoprobe™ instrument (Active Life Scientific, Santa Barbara, CA). After skin disinfection, local anesthesia (2–3 cc of 1% mepivacaine) was subcutaneously administered on the anterior face of the mid-shaft of the right tibia using a 3-cc syringe with a 28-gauge needle. The test probe was then inserted through the skin into contact with the bone surface using the dominant hand to stabilize the needle on insertion and during repositioning. Careful attention was taken to avoid touching above the luer lock/needle guide not only to maintain sterility but also to maintain the integrity of the measurement value obtained by the procedure. Importantly,
caution was exercised to ensure that the Osteoprobe needle had gone through the periosteum and was perpendicular \cite{30} to and in contact with the bone surface prior to taking measurements. The outer housing of the instrument was then lowered with the non-dominant hand over a 1–2-second period until the trigger mechanism was released. During compression, a 10 N force was generated to establish the initial reference point at the cortical surface, followed by an additional trigger force of 30 N to obtain the experimental measurement. A total of 8 reference point indentations, each separated by at least 2 mm, were obtained in 2 parallel lines of 4 indents each in the center of the long axis of the anterior surface of the tibial bone. In most cases, only one skin insertion was necessary, and the probe could be displaced for successive measurements without new piercings. The measurement from the first indentation was systematically disregarded because the insertion process could have affected it. The instrument software gave clear visual indication of aberrant readings, which occurred if normalcy to the cortical surface was not maintained. Following patient measurements, 5 indentations were performed for normalization on a poly(methylmethacrylate) (PMMA) cube using the same probe and the same observer. Results were expressed as BMSi units defined as 100 times the ratio of the harmonic mean of the 5 penetrations of the test probe into a PMMA calibration cube to each penetration of the test probe into the cortical bone. The software provided by the manufacturer detects if an individual measurement, either in the bone or in the PMMA, deviates from the average indentation value in the tibia as well as from the reference calibration value of 100 for the calibration cube. Outlier values detected this way are not introduced in the calculation of the results. BMSi units are dimensionless, distinguishing them from strength measurements in the specialized mechanical engineering sense, which is measured in units of force per unit area.
Both Bland-Altman plots\textsuperscript{(34)} and intraclass correlation coefficient (0.73, 95% CI 0.46 to 0.99, p for significant correlation = 0.0012) suggest good correlation and no significant departure from expected confidence limits. In addition, no trend bias is observed in Bland-Altman plots either. All measurements in the current study were obtained by the same investigator (LM) to minimize the possible effect of inter-observer variation. The procedure took less than 5 minutes and caused only minimal discomfort to the patient during administration of the local anesthetic. In our experience with this and other ongoing studies in our center on over 650 patients and volunteers, no complications occurred; moreover, patient discomfort after the procedure was mild and did not require administration of pain medication except in a single case of mild local skin infection in a kidney transplant recipient from another study, which was subsequently resolved after a short course of antibiotics. Local skin infection, significant local edema, or thick subcutaneous adipose tissue at the site of indentation were considered as contraindications for this technique.

For ethical reasons, the following rescue condition was stipulated for patients receiving Ca+D only: if a >10% decrease in BMSi was observed at V1, these patients were switched to an active treatment and excluded from further follow up.

\textit{Power calculation}

Assuming a standard deviation of 17.5% change in BMSi variation at 7 weeks (according to pilot data), 43 subjects were necessary to recognize a statistically significant difference greater than or equal to 8.75% (0.5 standard deviations) in paired measurements with 90% power, accepting an alpha risk of 0.05 in a two-sided test.

\textit{Statistical analysis}

Wilcoxon tests for non-parametric related samples were used to evaluate significant changes in BMSi between visits. Non-parametric unrelated sample tests were used to
evaluate unadjusted differences in BMSi changes between treatment groups. Multivariate linear regression models were fit to estimate the association between a given anti-osteoporotic drug and BMSi changes after adjusting for the following potential confounders: age, gender, and cumulative dose of systemic glucocorticoids.

Study approval

The study protocol was approved by our institution’s Committee of Ethics and Investigation (Reference number: 2013/5141/I), and signed informed consent was obtained.

Results

A total of 52 patients were enrolled within 4 weeks of initiating glucocorticoid treatment. Following Spanish guidelines (33), 19/52 (36.5%) patients received 1000 mg/day calcium plus 800 IU/day 25-hydroxy (OH) vitamin D supplementation only (Ca+D); 14/52 (26.9%) received risedronate; 14/52 (26.9%) received denosumab; and 5/52 (9.6%) received teriparatide, all at approved and commercially available doses. The mean ± standard deviation (SD) glucocorticoid dose/day at baseline ranged from 33.4 ± 17.1 mg prednisone (or equivalent) in the Ca+D group to 43.9 ± 21.2 in the risedronate group. Similarly, the cumulative dose during the entire observation period ranged from 2.9 ± 2.0 g in the Ca+D group to 5.5 ± 4.2 g in teriparatide group (Table 1). Patients in the active treatment groups (risedronate, denosumab, and teriparatide) were older and exposed to a higher initial oral glucocorticoid dose than those in the Ca+D group. As expected, at baseline, in an unadjusted linear regression model, using Ca+D users as the reference group, bisphosphonate users had similar BMSi (p for a difference = 0.64), whilst both teriparatide and denosumab users had significantly lower BMSi by 12.97 (95%CI 4.34 to 21.60, p=0.004) and 7.08 (95%CI 1.04 to 13.13, p=0.023) units respectively.
Compared to baseline, BMSi significantly declined in the Ca+D-treated patients (median [inter-quartile range] = -11.4% [-19.3% to -6.2%]; \( P = 0.002 \)), did not significantly change in risedronate-treated patients (-1.1% [-8.1% to +11.0%]; \( P = 0.83 \)), and significantly increased in both the denosumab (+9.4% [+4.9% to +16.0%]; \( P = 0.001 \)) and teriparatide-treated patients (+16.8% [+10.7% to +26.2%]; \( P = 0.043 \)) at the first follow-up visit (V1) after 7 weeks. These differences incorporated multivariate adjustments for potential confounders (Table 2) and remained stable thereafter until the second follow-up visit (V2) at 20 weeks (Fig. 2). Of the Ca+D-treated patients, 10/19 (52.6%) were switched to bisphosphonate treatment at V1 after meeting the rescue condition stipulated in the Methods section. A total of 9, 14, 5, and 14 patients in the Ca+D, risedronate, teriparatide, and denosumab groups, respectively, reached the V2 follow up. In contrast to the changes observed by reference point indentation measurements, no changes in BMD were observed between baseline and V1 or V2 for any group (data not shown). Based on the data in Fig. 2 showing the evolution of BMSi in each treatment group relative to baseline, glucocorticoid treatment led to obvious bone indentation properties decline in Ca+D-treated patients. Risedronate exhibited a mere stabilizing effect on glucocorticoid treatment, and both teriparatide and denosumab demonstrated a positive effect on bone BMSi.

Discussion

Taken together, this study demonstrates for the first time that 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 glucocorticoids, well before BMD imaging by dual-energy x-ray absorptiometry (DXA) can detect any
alteration. Our findings are consistent with the early increase in fracture incidence observed in glucocorticoid-treated patients.

This observation builds a strong case for using RPI and BMSi to longitudinally assess bone tissue properties. Additionally, the observed rapid response is intriguing. Although our present observations cannot provide mechanistic explanation for this finding, we might speculate on some influence of glucocorticoids in the propensity of the bone to open microscopic cracks (35), which is ultimately the mechanism underlying the generation of clinical fractures at the tissue level, perhaps affecting bone matrix (35) although no data support this.

These results reveal striking BMSi changes in patients receiving various anti-osteoporotic treatment regimens. Such responses, ranging from stabilization to clear improvement, suggest that currently available drugs maintain or enhance bone tissue mechanical strength. Our data also show that monitoring these responses is clinically feasible using the reference point indentation method. These findings open the possibility of developing new therapies that more selectively affect bone tissue properties; thus, more personalized treatment can be achieved by targeting different components of bone strength.

Results are reported in Bone Material Strength index (BMSi) units, a direct measure of the in vivo bone tissue mechanical competence to applied mechanical force, and is calculated as the inverse of the normalized indentation distance. Exactly how BMSi relates to the specialized quantities measured by conventional mechanical testing is a current research focus, although clinical trials will ultimately determine its relevance.

Since the present work constitutes a proof-of-concept study, our findings have several limitations as an open-label, non-randomized design. For instance, effectively comparing the efficacy of the different drugs is not possible. Baseline values were, given the design of the study and the treatment allocation following the guidelines, much lower in the
teriparatide-treated patients than in the other groups. Phenomena like, for example, regression to the mean cannot be ruled out. Nonetheless, we demonstrate here the feasibility of measuring BMSi in patients, indicating that the reference point indentation technique is suitable for use in research and eventually in clinical practice. In particular, we show that the technique is minimally invasive, safe, and convenient for both the patient and physician. Fig. 1 depicts the footprint of a microindentation mark on the bone surface, which is equivalent in magnitude to naturally occurring features. Furthermore, changes in bone tissue properties can be detected as soon as 7 weeks after treatment initiation and remain stable thereafter, suggesting that most changes observed at the tissue level occur very early during treatment. Finally, measurements are taken in cortical bone; while trabecular deterioration, as a consequence of glucocorticoids, has been extensively assessed, cortical bone also suffers from the deleterious effect of these drugs\(^{36-40}\). Therefore, the effect of the drugs at a tissue level can also be traceable in cortical bone as, in fact, our results suggest.

In conclusion, reference point indentation can measure very early changes in BMSi in patients initiating glucocorticoid treatment as well as the differential effects of various pharmacologic therapies. These results open new possibilities to explore the effects of various interventions on bone mechanical properties at the tissue level in the clinic, either for diagnosis and monitoring or developing new therapies.

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Authors’ roles: Study design: LM, DPA, XN and ADP. Study conduct: LM, and ADP. Data collection: LM, RGF and XN; Data analysis: DPA and FM; Data interpretation: LM, DPA, FM, CR, PKA and ADP. Drafting manuscript: LM, DPA and ADP. Revising manuscript content: LM, DPA, FM, RGF, XN, CR, PKH and ADP. Approving final version of manuscript: LM, DPA, FM, RGF, XN, CR, PKH and ADP. DPA takes responsibility for the integrity of the data analysis.
References


27 Carriero A, Bruse JL, Oldknow KJ, Millán JL, Farquharson C, Shefelbine SJ.


Figure legends

**Figure 1** Osteoprobe indentation in bone to measure BMSi. (a) Clinical test with the Osteoprobe on a patient performed on the mid-diaphysis of the tibia. (b) Schematic illustrating the representative indentation depth of the test probe in bone. (c) Cross-section of an indentation on a cadaver bone scanned on the microtomography beamline at the Advanced Light Source, Lawrence Berkeley National Lab (Berkeley, CA) (courtesy of John Jameson). Note that the size of the indentation is comparable to naturally occurring irregularities on the bone surface.

**Figure 2** Bone Material Strength index (BMSi) values at V0 (baseline), V1 (7 weeks), and V2 (20 weeks) for the 4 groups expressed as the percentage change vs. baseline. Error bars represent the 25th, 50th, and 75th percentiles of BMSi normalized by baseline. † $P = 0.001$; $\# P = 0.002$; $\| P = 0.043$; $\& P = 0.043$; $\$ P=0.043$; $\& P = 0.028$; $\divideontimes P$ = 0.043; *: ns ($P$ values vs. baseline). For the Ca+D group, the change between V1 and V2 (dashed line) is biased by the exclusion of those patients (10/19) that suffered a decline, according to the rescue condition.
Table 1 Patient characteristics at baseline.

<table>
<thead>
<tr>
<th></th>
<th>Ca+D</th>
<th>Ris</th>
<th>TPTD</th>
<th>Dmab</th>
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</thead>
<tbody>
<tr>
<td><strong>Age (yrs)</strong></td>
<td>55.3 (17.9)</td>
<td>66.1 (17.0)</td>
<td>69.8 (8.0)</td>
<td>58.9 (12.8)</td>
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<tr>
<td><strong>Gender (men)</strong></td>
<td>11 (57.9%)</td>
<td>10 (71.4%)</td>
<td>1 (20.0%)</td>
<td>5 (35.7%)</td>
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<tr>
<td><strong>Background disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horton’s Arteritis</td>
<td>3 (18%)</td>
<td>1 (7%)</td>
<td>0 (0%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>PMR</td>
<td>2 (11%)</td>
<td>5 (36%)</td>
<td>1 (20%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>3 (18%)</td>
<td>1 (7%)</td>
<td>1 (20%)</td>
<td>3 (21%)</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>1 (6%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Adult Still’s disease</td>
<td>2 (11%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>1 (6%)</td>
<td>3 (21%)</td>
<td>0 (0%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Others</td>
<td>7 (39%)</td>
<td>4 (29%)</td>
<td>3 (60%)</td>
<td>9 (65%)</td>
</tr>
<tr>
<td><strong>Height (m)</strong></td>
<td>1.68 (0.11)</td>
<td>1.65 (0.08)</td>
<td>1.59 (0.10)</td>
<td>1.62 (0.10)</td>
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<tr>
<td><strong>Weight (kg)</strong></td>
<td>69.9 (9.5)</td>
<td>71.9 (12.9)</td>
<td>70.8 (13.7)</td>
<td>74.5 (14.9)</td>
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<tr>
<td><strong>BMI</strong></td>
<td>24.7 (2.8)</td>
<td>26.3 (4.5)</td>
<td>27.8 (2.4)</td>
<td>28.2 (4.5)</td>
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<td><strong>Fragility fracture</strong></td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>2</td>
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<tr>
<td>Lumbar spine BMD (g/cm²)</td>
<td>1.06 (0.13)</td>
<td>1.04 (0.23)</td>
<td>0.83 (0.19)</td>
<td>0.93 (0.21)</td>
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<tr>
<td>Femoral neck BMD (g/cm²)</td>
<td>0.83 (0.13)</td>
<td>0.75 (0.14)</td>
<td>0.62 (0.12)</td>
<td>0.72 (0.15)</td>
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<td>Total hip BMD</td>
<td>0.98 (0.14)</td>
<td>0.91 (0.14)</td>
<td>0.79 (0.13)</td>
<td>0.89 (0.29)</td>
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<tr>
<td>Initial GC dose (mg/d)</td>
<td>33.4 (17.1)</td>
<td>43.9 (21.2)</td>
<td>41.0 (17.5)</td>
<td>36.8 (15.9)</td>
</tr>
<tr>
<td>25 OH vit D (ng/mL)</td>
<td>20.6 (11.0)</td>
<td>19.8 (14.6)</td>
<td>42.2 (2.2)</td>
<td>28.2 (9.3)</td>
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<tr>
<td>Cumulative GC dose (g)</td>
<td>2.9 (2.0)</td>
<td>4.8 (2.3)</td>
<td>5.5 (4.2)</td>
<td>4.1 (1.4)</td>
</tr>
</tbody>
</table>

Ca+D = calcium + vitamin D; Ris = Risedronate; TPTD = Teriparatide; Dmab = Denosumab; Horton’s = Horton’s arteritis; PMR = Polymyalgia Rheumatica; BMI = Body Mass Index; BMD = Bone Mineral Density; GC = Glucocorticoids; 25 OH vit D = 25-hydroxy vitamin D.