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Bone tissue mechanical strength is independent of age in healthy individuals

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Summary

Objective: Impact microindentation (IMI) is a technique that allows the measurement of mechanicalbone tissue resistance *in vivo*. IMI has proven to provide useful information on the evaluation of skeletal diseases, but the effect of age on the bone property that is measured by this technique is unknown. This study aims to analyzethe relationship between age and MIH.

Material and methods: Bone Material Strength index (BMSi), IMI's output variable, was measured in 69 healthy women (median age: 49 years, range: 30-81 years) and 19 healthy men (median age: 34 years, range: 24-98 years). The correlation between BMSi and age was analyzed by linear regression. The association between BMSi and age was evaluated by ANOVA after adjusting for body mass index. The potential effect of postmenopausal estrogenic depletion on BMSi was studied by comparing the younger vs the older subset of women through a t-student test.

Results: Linear regression analysis showed that BMSi was not correlated with age in either men (R^2=0.0016, p=0.74) or women (R^2=0.076, p=0.25). Similarly, the BMI-adjusted ANOVA model revealed a lack of association of BMSi with age in men (p=0.78) and women (p=0.73). Finally, there were not significant differences on BMSi detected between the younger and the older subset of women (p=0.8).

Conclusions: Bone tissue mechanical resistance in healthy individuals is independent of age and postmenopausal estrogenic depletion.

Key words: *impact microindentation, Bone Material Strength index (BMSi).*

Introduction

Osteoporotic fractures pose a serious public health problem given their high prevalence and enormous impact in terms of morbidity, mortality and economic cost[1]. Hence there is considerable interest in understanding the underlying pathophysiology of bone fragility, which, from a mechanical standpoint, is determined by bone strength. Bone resistance, in turn, comes from the integration of bone mineral quantity, bone architecture, and the material properties of bone.

The mineral quantity of the bone is usually measured by bone densitometry (DXA), the most commonly used, standardized method for assessing bone mass and fracture risk[2]. Bone architecture, both at the micro- and macroscopic level, is examined using different imaging techniques, including high-resolution peripheral quantitative tomography, bone magnetic resonance and the more accessible Trabecular Bone Score[3]. However, the material properties of bone are difficult to assess due to its high complexity, reflected in its multiple constituents including non-collagenous proteins, crystallinity, hydration of bone tissue, and the characteristics of mineralization and collagen, among others[4,5]. Furthermore, as researchers need bone tissue samples for analysis, the study of these properties has traditionally been restricted to a few centers specialized in bio-mechanics.

Microindentation has been developed as a technique to measure the material properties of bone easily and non-invasively. However, the property specifically measured has not yet been determined, so for the time being, the mechanical strength of the bone is evaluated globally. This technique involves measuring the penetration distance of a needle in the cortical bone to gauge its mechanical resistance[6]. The procedure is usually carried out on the anteromedial side of the tibia in a practical, safe and painless way[7]. There are currently two types of clinical microindentation: the cyclic microindentation, using the BioDent® instrument (Active Life Scientific Inc., Santa Barbara, USA). The other is impact microindentation (IMI), carried out with the OsteoProbe®. Several clinical studies can provide relevant information on bone strength and risk of fracture with both types[6,8]. However, given its greater manageability, OsteoProbe® has replaced BioDent® in clinical studies. Despite its increasing use, there are still many unresolved basic issues surrounding IMI implementation including the effect of age on bony material properties. We examined the influence of age on bone tissue mechanical strength in a cohort of healthy men and women.

Material and methods

Participants

Healthy volunteers older than 18 years of age were recruited consecutively from Internal Medicine outpatient lists without bone metabolism-related diseases.

Those individuals with the following criteria were excluded from the study:

- History of fragility fractures or traumatic fractures of the tibia.
- History of primary bone diseases (including osteoporosis), secondary bone diseases, deformities in the lower extremities of congenital or acquired origin, and bone metastasis.
- History of diabetes mellitus, chronic kidney disease and severe liver failure.
- Previous or concurrent treatment with glucocorticoids, aromatase inhibitors, androgen deprivation therapy, chemotherapy and antiresorptive agents or osteo formers (bisphosphonates, teriparatide, denosumab, strontium ranelate and selective modulators of the estrogen receptor).

Participants' height and weight were measured to calculate the body mass index (BMI, kg/m^2).

The study protocol was approved by the Ethics Committee of the Mar Health Park and written informed consent of all the participants obtained.

Impact Microindentation

Impact microindentation (IMI) was evaluated using OsteoProbe®, a hand-held device with an impact mechanism, a disposable probe with a conical tip (radius of tip sharpness: <10 µm) and a displacement transducer. The procedure has been described in detail previously[7]. Prior to microindentation, a local anesthetic (2% mepivacaine) is applied to the anteromedial part of the non-dominant tibia. The probe is then inserted perpendicular to the bony cortex in the anesthetized region until it reaches the bone surface. The device is slowly compressed until it reaches a pre-load resistance of 10 Newtons (N), after which an impact load of 30 N is automatically activated. The displacement transducer measures indentation depth. The operator can eliminate the measurements that are considered incorrect.

After 8 valid indentations separated by approximately 2 mm, 5 additional indentations are made with the same probe in a polymethyl methacrylate (PMMA) block for calibration. The value obtained in the IMI is the Bone Mineral Resistance Index (or BMSi, from Bone Material Strength index), which is defined as 100 times the relation between the harmonic mean of the distance of the 8 bony indentations and that of the 5 indentations in the PMMA block. Nine different operators with experience in the technique carried out the measurements in our study.

Statistical analysis

Separate analyzes were carried out for women and men. Descriptive values are shown using mean and standard deviation, as well as median and total range, as appropriate. The correlation between age and BMSi was represented by a linear regression, and its association with BMI-adjusted ANOVA evaluated. Due to the lack of clinical information on the menstrual status of the participants, the potential effect of estrogen deprivation on the mechanical resistance of bone tissue was analyzed by comparing the BMSi of women between 20-39 years (most likely premenopausal) with women >60 years (most likely postmenopausal) using Student's t test.

The study figures were obtained through the Prism 7 program (GraphPad Software, La Jolla, California, USA). The statistical analyzes were performed with the SPSS program version 23 (IBM Corp®, Armonk, New York, USA), accepting as significant the results with $p<0.05$.

Results

For our study, 69 women and 19 men of Caucasian origin were recruited. The participants' characteristics and the BMSi measurements are shown in table 1. The coefficient of inter-operator variation was less than 5%.

Linear regression analyzes showed that BMSI does not correlate with age in women (R^2=0.076, p=0.25) nor in men (R^2=0.0016, p=0.74) (Figure 1). Likewise, no significant associations were detected between the BMSi and the age in the ANOVA analysis adjusted for BMI neither in women (p=0.73) nor in men (p=0.78). Finally, no significant differences were observed in the BMSi between the subgroup of women aged 20-39 years and those older than 60 years (p=0.8) (Figure 2).

Discussion

In the present study, the influence of age on the mechanical resistance of bone tissue measured by IMI in a cohort of healthy men and women was evaluated. The results indicate that bone tissue resistance is not determined by age in women or men, and that therefore, it is not affected by aging. Furthermore, no BMSi differences were found between the subset of younger women versus the subset of older women which would indicate that the depletion of estrogen that accompanies menopause does not exert a significant effect on the mechanical resistance of the bone tissue.

Bone microindentation has emerged as a promising new tool to evaluate bone mechanical resistance in living individuals[6-8]. Although it is still unclear which physical properties are specifically measured, several clinical studies reveal that this technique has a good discriminant capacity between patients with and without fragility fractures[9-11], although studies in geriatric populations with osteoporotic fractures show discrepancies[12].

The measurements that result in an altered BMSi seem to be especially informative in those conditions associated with an increased fracture risk that are not explained by abnormal BMD values[13-16].

Given the increasing use of IMI as a complementary technique for assessing bone health in clinical research and its potential future role in clinical practice, it is imperative to clarify the possible effects of physiological factors, such as age, on the mechanical resistance of the bone tissue.

Our study results indicate that BMSi is not significantly affected by aging or estrogenic depletion. Mirzaali MJ, et al. observed through micromechanical studies in cadaver bone that the properties of microindentation in the elderly were constant with age[17] which concurs with our study. On the contrary, aging and estrogenic depletion reportedly exert a negative effect on BMD and bone architecture[18,19]. This reinforces the notion that the microindentation technique measures a very specific characteristic of bone strength or bone quality completely different from other techniques available to date. Microindentation may cause the separation of the protein-based "glue" proteins, which hold together the mineralized collagen fibrils, a property that would constitute the first barrier of resistance to fracture[20].

Currently, bone tissue resistance is considered to be genetically determined, but, at the same time, clinical studies show that it can be negatively influenced by non-genetic factors, such as a deficient glycemic control[13,15,21], an excess of tissue local adipose[16], treatment with glucocorticoids[22], excess growth hormone[23], chronic kidney disease[24,25] and HIV infection[26]. In addition, alterations in certain signaling pathways and in the intracellular storage of lipids also seem to affect the mechanical resistance of bone tissue as has been observed in studies on the diseases of Camuratti-Englemann[27] and Gaucher type 1[28], respectively.

Our study has several limitations. First, the small number of subjects included in the study limits the generalization of our findings. Another limitation is that the factors that can affect the mechanical resistance of the bone tissue are not yet fully known. Therefore, these factors have not been introduced as co-variates in the statistical model for the adjustment of the confounding factor. This has been tried to compensate by means of the strict exclusion criteria used at the moment of the recruitment of the individuals and thus control the possible heterogeneity of the studied cohort.

Table 1. Characteristics of the participants

	Women (n=69)	**Men (n=19)**
Age, years (median, range)	49, 30-81	34, 24-98
BMI, kg/m² (mean ± SD)	24.3±4.5	24.6±3.1
BMSi (mean ± SD)	82±7.4	88±7.6

BMI: body mass index; DE: standard deviation; BMSi: Bone Material Strength index.

Figure 1. Linear regression analysis performed to evaluate the correlation between the Bone Material Strength index (BMSi) and age in healthy men and women

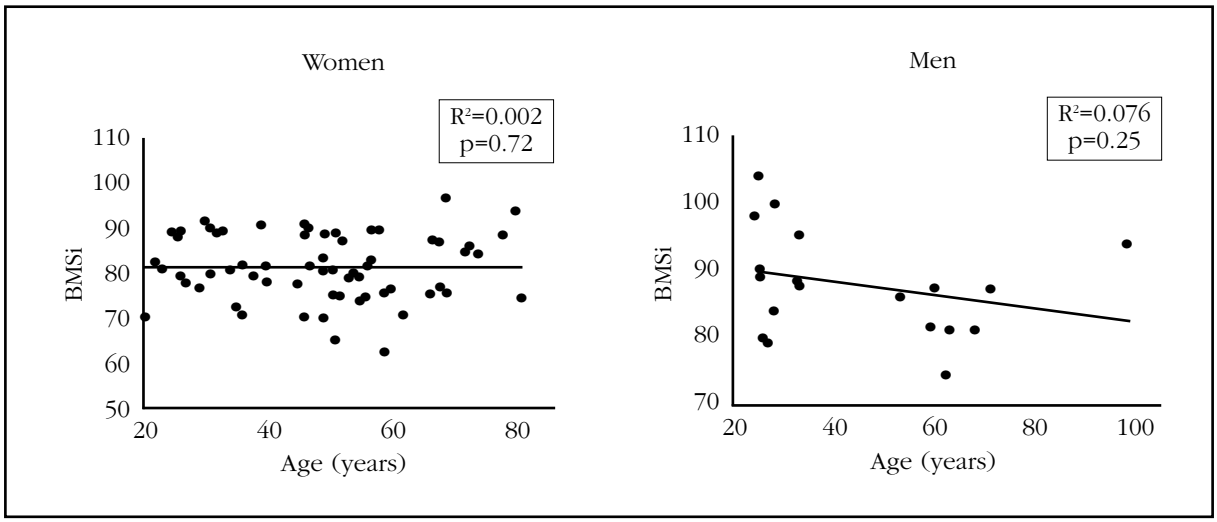
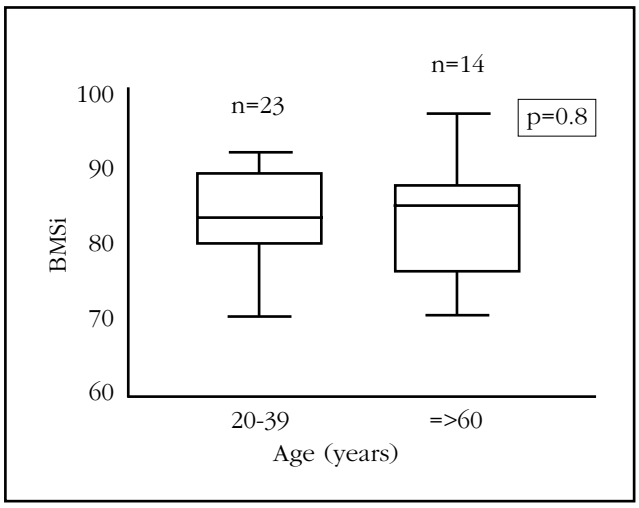


Figure 2. T-test carried out to compare the BMSi between women aged 20-39 years and women >60 years of age as an indirect measure of pre and postmenopausal status, respectively



Another limitation lies in the fact that this technique is performed exclusively on the cortical bone of the anteromedial tibia, so the generalization of BMSi results to other skeletal sites is debatable. However, we believe that the values obtained by microindentation in the tibia reflect the mechanical strength of the bone globally, since clinical studies have shown an inverse correlation between BMSI values and the incidence of osteoporotic fractures in other skeletal locations such as hip, and even in bones with a greater trabecular component, such as the vertebrae[10,11]. Finally, the data on the menstrual status were not collected, thus limiting the evaluation of the effects of menopause on the mechanical resistance of the bone tissue. This problem was counteracted by categorizing the subgroup of younger women as premenopausal and the subset of older women as postmenopausal.

In conclusion, the mechanical resistance of the bone tissue does not seem to be affected by aging and estrogen-related depletion related to menopause. Additional studies are needed to corroborate these findings in order to facilitate the implementation of the IMI in research and clinical practice.

Conflict of interests: Adolfo Díez-Pérez declares that he owns shares of Active Life Scientific, the manufacturer of microindentation devices. The remaining authors declare that they have no conflicts of interest.

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