Bone xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Comparison of cyclic and impact-based reference point indentation measurements in human cadaveric tibia

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ARTICLE INFO

Article history:
Received 21 November 2014
Revised 20 February 2015
Accepted 31 March 2015
Available online xxxx

Edited by: David Fyhrie

Keywords:
Aging
Bone
Indentation

ABSTRACT

Although low bone mineral density (BMD) is strongly associated with increased fracture risk, up to 50% of those who suffer fractures are not detected as high-risk patients by BMD testing. Thus, new approaches may improve identification of those at increased risk for fracture by in vivo assessment of altered bone tissue properties, which may contribute to skeletal fragility. Recently developed reference point indentation (RPI) allows for the assessment of cortical bone indentation properties in vivo using devices that apply cyclic loading or impact loading, but there is little information available to assist with the interpretation of RPI measurements. Our goals were to use human cadaveric tibia to determine: 1) the associations between RPI variables, cortical bone density, and morphology; 2) the association between variables obtained from RPI systems using cyclic, slow loading versus a single impact load; and 3) the age-related differences in RPI variables. We obtained 20 human tibia and femur pairs from female donors (53–97 years), measured total hip BMD using dual-energy X-ray absorptiometry, assessed tibial cortical microarchitecture using high-resolution peripheral quantitative computed tomography (HR-pQCT), and assessed cortical bone indentation properties at the mid-tibial diaphysis using both the cyclic and impact-based RPI systems (Biodent and Osteoprobe, respectively, Active Life Scientific, Santa Barbara, CA). We found a few weak associations between RPI variables, BMD, and cortical geometry; a few weak associations between measurements obtained by the two RPI systems; and no age-related differences in RPI variables. Our findings indicate that in cadaveric tibia from older women RPI measurements are largely independent of age, femoral BMD, and cortical geometry. Furthermore, measurements from the cyclic and impact loading RPI devices are weakly related to each other, indicating that each device reflects different aspects of cortical bone indentation properties.

Introduction

Skeletal fractures are associated with increased disability and mortality and are highly prevalent among the elderly. Although low bone mineral density (BMD) is strongly associated with increased fracture risk, there are many who suffer from fractures despite having normal bone density. Up to 50% of those who experience a fracture are not identified as having osteoporosis by BMD testing [1]. It has been proposed that there are several other factors that contribute to skeletal fragility including altered bone microarchitecture and changes in tissue-level mechanical properties. A few techniques are available for non-invasive assessment of bone morphology and microstructure, and several clinical studies have demonstrated the contribution of bone microarchitecture to bone strength and fracture risk assessment using these methods [2]. However, there is little in vivo information available on the contribution of altered bone matrix properties to skeletal fragility in humans because until recently the biomechanical properties of the bone tissue could not be assessed non-invasively. Prior studies have demonstrated altered bone matrix composition in those with a history of fracture, but required bone biopsies for analysis by Fourier transform infrared spectroscopy [3–6].

Recently developed reference point indentation (RPI) is a minimally-invasive technique that allows for the assessment of cortical bone indentation properties via cyclic or impact based loading [7–10]. The bench-top Biodent system (Active Life Scientific, Santa Barbara, CA) measures the distance a test probe indents into bone using a specified load over multiple cycles, with a maximum load of 10 N (Fig. 1). Several variables, which are based on the force applied and indentation distance into the bone across one or all cycles, are calculated from these measurements [11]. Few data exist on this novel technique. One study by Gallant et al. combined the indentation data collected from cyclic indentation of rat femurs, rat vertebrae, and dog ribs to demonstrate that indentation distance increase (increase in the indentation distance in the last cycle relative to that in the first cycle) is negatively correlated with apparent toughness estimated

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http://dx.doi.org/10.1016/j.bone.2015.03.021
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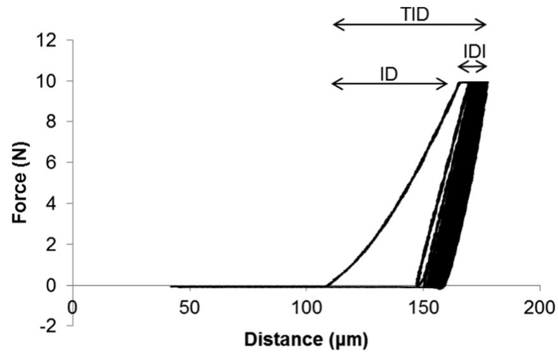

Fig. 1. Force versus distance graph for a cyclic-based RPI test with 20 loading cycles. Variables derived from these curves include indentation distance (ID), total indentation distance (TID), indentation distance increase (IDI), loading and unloading slopes, and energy dissipation (ED). Average ED is calculated as the area within the test's hysteresis loop from the third to last cycle. The average slopes during loading and unloading are measured from the third to last cycle.

from whole bone biomechanical testing ($r^2 = 0.51$) [12]. Two clinical studies using cyclic indentation of the mid-tibia demonstrated greater indentation distances in postmenopausal women with hip fractures compared to women without fractures [13,14].

In comparison, the hand-held Osteoprobe (Active Life Scientific, Santa Barbara, CA), designed for in vivo use in humans and large animals, measures the indentation distance following a single 30 N impact load (preceded by a 10 N preload, Fig. 2) [9]. A single variable, bone material strength index (BMSi), defined as the average indentation distance into bone due to the impact load normalized to the indentation distance measured on a polymethyl methacrylate (PMMA) reference phantom, is obtained from these measures. The Osteoprobe has been used to show that postmenopausal women with type 2 diabetes have approximately 10% lower BMSi than those without diabetes [15]. However, as emphasized in a commentary by Jepsen and Schlecht [16], the two RPI systems have completely different loading profiles, and no studies have reported whether the variables acquired from these devices are comparable.

Moreover, there are limited data regarding the factors that may affect RPI measures and the age-related changes in RPI measures. For example, one study showed positive correlations between matrix mineralization measures assessed by Raman spectroscopy and indentation distances and energy dissipation assessed by RPI in diabetic rats [17], while contrastingly, another study indicated that tissue composition did not account for differences in RPI measures in a rat

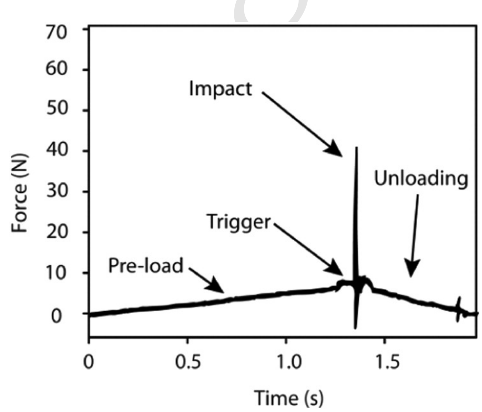

Fig. 2. Force versus time graph for an impact-based RPI test. Indentation distance is measured at the time of impact (on the order of 1 ms duration) from impact-based RPI tests and is normalized to the indentation distance into a PMMA reference phantom ∗ 100 to assess BMSi. Figure reprinted with permission from Bridges et al. [9].

model of chronic kidney disease versus controls [18]. One investigation showed that indentation distances and energy dissipation assessed by RPI decrease with age in porcine bone [11], whereas another showed that indentation distances were greater in old human bone compared to young bone [19]. Altogether, there is limited information on what factors influence RPI measurements in human bone, and how these properties change with age.

Hence, the goals of this study were to use human cadaveric tibias to determine: 1) the associations between RPI measurements and cortical bone density and morphology; 2) the association between indentation properties measured by the two systems and the inter-correlations between the multiple parameters derived from the cyclic indentation testing; and 3) age-related differences in RPI measurements. We hypothesized that indentation properties will be associated with cortical tissue mineral density and morphology, that cyclic and impact-based RPI measurements will be correlated with each other, and that RPI measurements will worsen with age.

Methods

Specimen collection

We obtained 20 human tibia and femur pairs from female donors with an age range of 53 to 97 years (average: 74.2 ± 14.6 years) (Anatomic Gifts Registry, Hanover, MD). Specimens were harvested fresh and frozen at −20 °C until testing. None of the donors had any history of diabetes, bone metabolic disorders, or bisphosphonate use.

Bone mineral density and geometry assessment

Total hip bone mineral density (BMD, g/cm^2) was measured using dual-energy X-ray absorptiometry (DXA, QDR Discovery, Hologic Inc., Bedford, MA). During the scanning procedure, femurs were submerged in a water bath and fixed in a position similar to that used during in vivo DXA scans. We measured cortical tissue mineral density (Ct.TMD, mg/cm^3), cortical thickness (Ct.Th, mm), and cortical porosity (Ct.Po, %) at the mid-tibia using high-resolution peripheral quantitative computed tomography (HR-pQCT, XtremeCT, Scanco Medical AG, Bassersdorf, Switzerland). Briefly, 110 slices were obtained at 82 μm nominal resolution (X-ray tube current 95 mA, effective energy 60 kVp). The scan region was centered at the site of RPI measurements at the midshaft, defined as the exact midpoint between proximal and distal ends of the bone.

Reference point indentation

Tibias were thawed overnight, kept hydrated with saline, and indented at the mid-diaphysis using both cyclic and impact loading devices (Active Life Scientific, Santa Barbara, CA). Indentations were made within a ~0.25 cm^2 region to minimize site-based variation. For the cyclic loading device (Biodent), five separate indentation tests were performed ≥1 mm apart on the anterio-medial surface of the tibia at 10 N maximum force, 2 Hz, for 20 cycles, and results from the five separate tests were averaged, following a protocol similar to other studies [13,14]. Indentations were made using a probe assembly consisting of a beveled reference probe with blunted end (~5 mm cannula length) and test probe with spherical tip (2.5 μm radius point) that tapers from a 90° cone shape to cylindrical shaft (BP2 probe, Active Life Scientific, Santa Barbara, CA). The following variables were measured (Fig. 1): indentation distance (ID, indentation distance measured in the first cycle [μm]), creep indentation distance (CID, total indentation distance during the hold step of the first cycle [μm]), average creep indentation distance (avg CID [μm]), total indentation distance (TID, total indentation distance across all cycles [μm]), indentation distance increase (IDI, increase in the indentation distance in the

Please cite this article as: Karim L, et al, Comparison of cyclic and impact-based reference point indentation measurements in human cadaveric tibia, Bone (2015), http://dx.doi.org/10.1016/j.bone.2015.03.021