Bone 53 (2013) 301–305

Contents lists available at SciVerse ScienceDirect

Bone

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

Original Full Length Article

Reference-point indentation correlates with bone toughness assessed using whole-bone traditional mechanical testing

Maxime A. Gallant, Drew M. Brown, Jason M. Organ, Matthew R. Allen, David B. Burr *

Department of Anatomy and Cell Biology, Indiana University School of Medicine, 635 Barnhill Dr, MS-5035, Indianapolis, IN, 46202, USA

ARTICLE INFO

Article history:
Received 27 October 2012
Revised 11 December 2012
Accepted 13 December 2012
Available online 27 December 2012

Edited by: David Fyhrie

Keywords:
Bone
Mechanical testing
Material properties
Reference-point indentation
Toughness

ABSTRACT

Traditional bone mechanical testing techniques require excised bone and destructive sample preparation. Recently, a cyclic-microindentation technique, reference-point indentation (RPI), was described that allows bone to be tested in a clinical setting, permitting the analysis of changes to bone material properties over time. Because this is a new technique, it has not been clear how the measurements generated by RPI are related to the material properties of bone measured by standard techniques. In this paper, we describe our experience with the RPI technique, and correlate the results obtained by RPI with those of traditional mechanical testing, namely 3-point bending and axial compression. Using different animal models, we report that apparent bone material toughness obtained from 3-point bending and axial compression is inversely correlated with the indentation distance increase (IDI) obtained from RPI with r^2 values ranging from 0.50 to 0.57. We also show that conditions or treatments previously shown to cause differences in toughness, including diabetes and bisphosphonate treatment, had significantly different IDI values compared to controls. Collectively these results provide a starting point for understanding how RPI relates to traditional mechanical testing results.

Introduction

Mechanical testing of bones has become a standard outcome when investigating the effect of genetic manipulation or pharmacological intervention on bone properties. Many factors influence the outcome of mechanical testing of a bone sample: geometry, architecture, degree of mineralization, properties of the organic matrix, and hydration. Whole bone mechanical testing is routinely performed on rodent samples while microbeam testing and nanoindentation are mostly performed on smaller samples taken from larger animals. Whole bone mechanics can be transformed into material properties using standard equations accounting for size and shape [1]. These tissue properties provide intrinsic estimates of strength (stress), elasticity (modulus) and energy to fracture (toughness) (Fig. 1a). However, one of the downsides of mechanical testing is that whether it is 3 or 4-pt bending of whole bones or machined specimens, axial tension/compression or torsion testing, these tests can only be performed ex vivo and are destructive.

Recently, reference-point indentation (RPI) was introduced as a new way to test bone material properties using cyclic micro-indentation [2–4]. An advantage of this technique is that it does not critically damage the samples and theoretically can be performed repeatedly over time to sample longitudinal changes. RPI's main outcome is Indentation Distance Increase (IDI) [2], which is the absolute penetration depth increase from the 1st to the last cycle of each testing session (Fig. 1b). Other outcomes of interest are the 1st cycle indentation distance (ID), the total indentation distance (TID) and the 1st cycle unloading slope (US), an indicator of material stiffness. Because this is a relatively new technique, however, it is not clear how these variables are related to the standard material properties that define strength, modulus and toughness.

In this report, we investigate the relationship between RPI and traditional mechanical testing techniques using two animal models previously shown by our lab to have altered mechanical properties. Our results show that RPI measurements show group differences consistent with traditional mechanical testing techniques and significantly correlate to the apparent tissue-level properties measured by these standard techniques.

Material and methods

Bone samples

Bone samples were collected from previous studies conducted in our lab. All bones were kept frozen (−20 °C) in saline-soaked gauze until testing. Femora (n = 7–11/group), and 3rd and 4th lumbar vertebrae (n = 11–12/group) were used from type 2 diabetic ZDSD male rats (PreClinOmics, Indianapolis, IN) and from control CD male rats (CD® IGS, Charles Rivers). The ZDSD and CD rats were fed a high fat diet (start at 20 weeks of age) and only the ZDSD rats were clinically diabetic for 10 weeks. ZDSD rats and CD rats were sacrificed at 32 wk of age. We also used the 9th left ribs (n = 9–10/group) from skeletally mature

* Corresponding author. Fax: +1 317 278 2040.
E-mail addresses: maxgalla@iupui.edu (M.A. Gallant), drebrown@iupui.edu (D.M. Brown), jorgan@iupui.edu (J.M. Organ), matallen@iupui.edu (M.R. Allen), dburr@iupui.edu (D.B. Burr).

8756-3282/$ – see front matter
http://dx.doi.org/10.1016/j.bone.2012.12.015

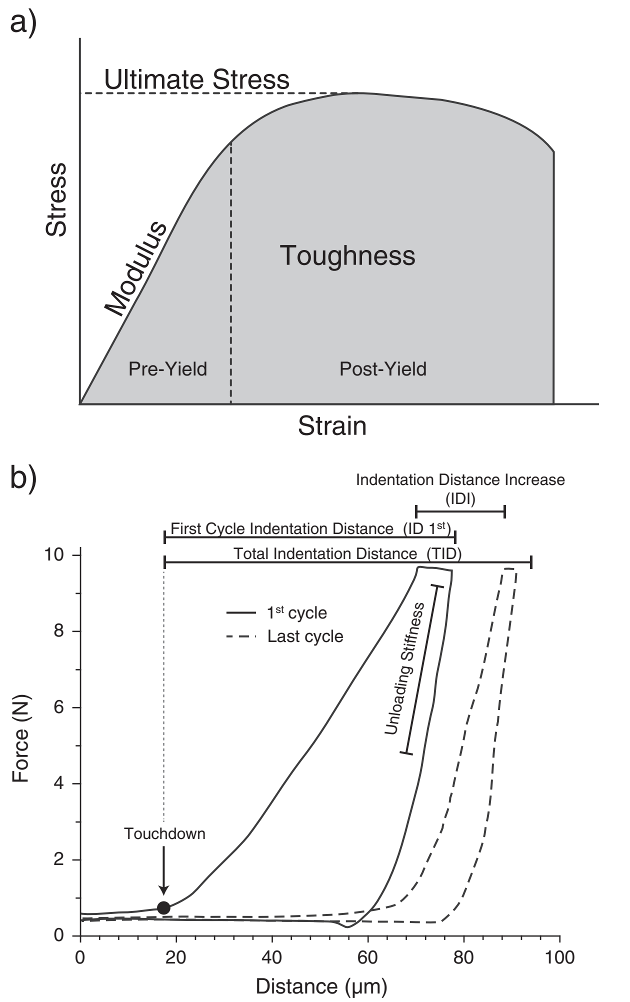

Fig. 1. Mechanical testing curves. a) Generic stress–strain curve of a long bone obtained from a 3-point bending test. b) Example of an RPI testing curve from the ZDSD rat femur group. To simplify the design, only the first and last cycle (20th) of the testing.

female beagles that had been treated daily with saline (1 ml/kg) or alendronate (1.0 mg/kg) for three years before sacrifice [5]. The rib samples were kept at −20 °C in saline-soaked gauze for ~3.5 yrs (time of sacrifice to time of RPI testing). All animal experiments were approved by our university's Animal Care and Use Committee.

Mechanical testing

Rat femoral mid-diaphyseal mechanical properties were measured via a three-point bending test using standard methods [1]. Briefly, bones were equilibrated to room temperature and placed posterior side down on the bottom support (18 mm span) of a servohydraulic test system (MTS Bionix II Test System, MTS Systems, Eden Prairie, MN). Bones were loaded centrally using a cross-head speed of 2 mm/min, and force vs. displacement data were collected at 10 Hz. The force–displacement points were converted to stress–strain data using standard beam bending equations [1], and material bone properties (ultimate stress, modulus, and modulus of toughness) were calculated using midshaft geometry from microCT scans.

LV_4 vertebral bodies were tested by axial compression loading to failure. Prior to testing, the LVs had their processes and end-plates removed (parallel cuts), leaving an intact vertebral body (±3 mm in height). Testing was performed at a rate of 0.5 mm/min, and the load-displacement data were used to directly measure the structural properties similar to those of the femoral diaphysis and analyzed up to the point of ultimate load. Material properties (ultimate stress, modulus, and modulus of toughness) were calculated using standard equations allowing for correction of bone volume (obtained by μCT) in the samples [1].

Mechanical testing of the dogs' 11th left ribs was performed previously by 3-pt bending [5].

MicroCT

Rat femur midshafts and lumbar vertebra bodies were scanned with a high-resolution μCT system (Skyscan 1172, Belgium) using 60 kV and 120 μA and 0.7° rotation steps, and then isotropic volume elements were reconstructed at 8 μm resolution. The scanning region was defined as a 1 mm region located at the femoral midshaft (determined using calipers between the inter-condyle region and the femoral neck) and the whole vertebral body prior to mechanical testing. Scans were reconstructed and analyzed using NRecon and CTAn, respectively (Skyscan). Outcome parameters from the 3D analyses included moment of inertia (I_x, mm^4) and cortical thickness (Ct.Th, mm) for the femur and bone volume (BV, mm^3) for the LV bodies.

Reference point indentation

Tissue mechanical properties of the anterior mid-diaphysis of the rat femur and the canine ribs were analyzed by cyclic micro-indentation using the BioDent 1000™ Reference Point Indentation instrument (Active Life Scientific, Inc., Santa Barbara, CA) using BP1 probes. Following published protocols [6], a series of pre-conditioning cycles (5 cycles, 2 N) was applied to the anterior mid-diaphysis of the rat femur, followed by 20 indentation cycles performed at 2 Hz, with a maximum force of 10 N. The left 9th ribs of the dogs were cut into 4 cm section at the point of largest curvature and RPI testing was performed on the anterior side using the same protocol as described above. For the 3rd lumbar vertebral cortical shells, a newer RPI instrument, the BioDent H and concentric BP3 probes were used. The left and right anterior regions of the LV_3 body were tested using a similar protocol as for the femur, but with reduced force (5 N for 10 cycles at 2 Hz) as the cortex is thinner at this location. The primary outcome measures are shown in Fig. 1b, and further described by Diez-Perez et al. [6]. The first cycle indentation distance is similar to a measurement obtained from a standard microindentation test, and is related to hardness, which will have a high correlation to density and tissue mineralization. The slope of the unloading portion of the first cycle (US) is considered to be a measure of elastic modulus. The increase in indentation distance (IDI) over the entire set of cycles has been shown to be related to the modulus of toughness [6]. Measurements were repeated 5 times per femur and rib and 4 times (2 on each lateral side) for the vertebrae and separated by 1–2 mm. Bones were kept wet prior to testing (wrapped in saline-soak gauze) and a saline drop was deposited at the test site. Prior to testing, probes were tested on a PMMA block according to manufacturer's indication to ensure proper function. Replicates were averaged for each sample and used to calculate the mean of each group. For the main outcome, IDI, the variance within a sample was less than 8%.

Statistical analysis

Statistical analyses were performed using GraphPad Prism v5.04 (San Diego, CA) and SYSTAT v11 (Richmond, CA). Data were found to be of Gaussian distribution, therefore *Student T-tests* were used to compare groups, and correlations were made using the *Pearson* product moment algorithm. Backward stepwise multiple regression models were constructed to explore the efficacy of predicting standard material properties obtained through traditional mechanical testing (i.e., toughness, post-yield toughness, and ultimate stress) from reference-point indentation material property data (i.e., IDI, ID, TID, and US). For all statistical tests, significance was set at $\alpha = 0.05$,