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Anterior and Posterior Variations in Mechanical Properties of Human Vertebrae Measured by Nanoindentation

Hugo Giambini¹, Hua-Jun Wang^{1,2}, Chunfeng Zhao¹, Qingshan Chen¹, Ahmad Nassr¹, and Kai-Nan An¹

¹Biomechanics Laboratory, Division of Orthopedic Research Mayo Clinic, Rochester, Minnesota USA

²Department of Orthopedics, School of Traditional Chinese Medicine, Southern Medical University, Guangzhou, China

Abstract

Osteoporotic spinal fractures are a significant global public health issue affecting more than 200 million people. Local degradation of the mechanical properties of bone and changes in global spine curvature increase fracture risk. However, a gap in knowledge exists relating material properties of trabecular bone in different regions of the spine. The purpose of our project was to measure the intrinsic mechanical properties of the anterior and posterior regions of human vertebral bodies in the thoracic and lumbar spine. Nanoindentation was used to evaluate Young's modulus (E) and hardness (H) of anterior and posterior trabecular bone regions from each vertebra (T7, T8 and L4). One-way ANOVA and the Turkey-Kramer test were used to analyze significance between vertebrae and t-test was used to test for significance within vertebrae. There was no difference in (E) and (H) within vertebrae. Young's modulus in the anterior regions of T7 (19.8 ± 1.3) and T8 (19.6 ± 1.4) were statistically greater than that in L4 (17.6 ± 0.5). There was no difference between the posterior regions of all vertebrae. There was a statistical significant difference in hardness between the anterior regions of T7 and T8 compared to L4, while the posterior regions demonstrated no difference. The results presented in this study, for the first time, reveal the differences in bone properties between the kyphotic thoracic spine and lordotic lumbar spine regions. This information will be helpful in understanding vertebral body remodeling and adaption in different regions of the spine which may be associated with spinal curvature and loading conditions.

Keywords

Nanoindentation; Vertebrae; Trabecular bone; Stiffness; Regions

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Corresponding Author: Kai-Nan An, Ph.D., Mayo Clinic, 200 First Street SW, Rochester, MN 55905, Phone: 507-266-0982, Fax: 507-284-5392, an.kainan@mayo.edu.

Conflict of Interest Statement

None of the authors have any professional or financial conflict of interests to report.

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Introduction

Osteoporotic spine fracture is a significant global public health issue affecting more than 200 million people with a prevalence of 14% in the general population (Samelson et al., 2006; Wang et al., 2012). These fractures are more common in the postmenopausal female population (16 to 24%), leading to a significantly negative impact in the quality of life and patient's function (Melton, 1997; Samelson et al., 2006). There are multiple factors affecting the etiology of vertebral fractures and bone fragility. Some of these factors relate to the local degradation of the intrinsic mechanical properties of the bone extracellular matrix, trabecular bone microarchitecture and to the global spinal alignment posture (Arlot et al., 2008; Chen et al., 2008; Cortet et al., 1999; Edmondston et al., 1994; Fields et al., 2009; Gong et al., 2006; Hengsberger et al., 2002; Homminga et al., 2001; Mosekilde, 1993; Routh et al., 2005; Sornay-Rendu et al., 2009; Stauber et al., 2006; Wood et al., 1996). In addition, it is known that an initial vertebral fracture is often associated with subsequent fractures, a phenomenon termed "vertebral fracture cascade" (Briggs et al., 2007; Briggs et al., 2006). However, there is an existing gap in knowledge relating the intrinsic material properties of trabecular bone in different regions of the spine. A better understanding of factors leading to vertebral fracture is required so that the uncertainty leading to subsequent vertebral failure can be reduced. A correlation between spinal alignment, bone mineral density (BMD), micro-architecture and intrinsic mechanical properties of bone should improve fracture risk prediction and reduce vertebral failure.

Regional variations and bone micro-architecture also have significant roles in the risk of fracture. Chen et al. found a decrease of bone volume in the central and anterosuperior regions of L4 vertebrae and an increase in perforated trabeculae and SMI (*Structural Model Index: ratio of plates and rods*) with age (Chen et al., 2008). Similarly, Gong et al. found that trabecular bodies with low mass tended to have high SMI (Gong et al., 2006). Mosekilde et al. showed a decrease in the thickness of horizontal trabeculae, perforations in the horizontal network and an increase in the distance between them with aging, while vertical trabeculae stays almost constant (Mosekilde, 1993). Finally, a finite element study by Fields et al. concluded that BMC (bone mineral content) and SMI were highly correlated with strength (Fields et al., 2009).

Nanoindentation has been widely used in an effort to understand the intrinsic ultrastructural material properties of bone (Feng et al., 2012; Hengsberger et al., 2002; Oliver and Pharr, 1992; Rho et al., 1999; Rho et al., 1997; Turner et al., 1999; Wu et al., 2011; Zysset et al., 1999). Several studies have also investigated the effect of the degree of mineralization (Mulder et al., 2008; Tai et al., 2005; Zebaze et al., 2011) and indentation location within a bone surface (Brennan et al., 2009; Harrison et al., 2008; Norman et al., 2008) on elastic modulus and hardness. Our study was motivated by the lack of current knowledge related to the variation in intrinsic mechanical properties within the vertebrae and between vertebrae from different regions of the spine. The spine is composed of three columns, i.e. anterior (anterior region of vertebral body), middle (posterior region of vertebral body), and posterior (posterior elements) columns (Denis, 1983). The anterior and middle columns are considered as the primary load bearing structures. The load distribution between the anterior and middle column varies in different regions of the spine due to changes in spinal curvature (lordosis and kyphosis). This may change the intrinsic bony material properties in different regions of the vertebrae. The purpose of this project was to measure the mechanical properties of the anterior and posterior regions of human vertebral bodies in the thoracic and lumbar spine at the nano-material level. This data could provide further insight into the relationship between spinal curvature and intrinsic material properties at the nano scale. In addition, it could also be used as material properties estimations in finite element models or

as a validation for bone models (Adam and Swain, 2011; Chevalier et al., 2007; Feng et al., 2012; Goncalves Coelho et al., 2011; Harrison et al., 2008; Wolfram et al., 2010).

Materials and Methods

2.1 Specimen Preparation

Fifteen fresh frozen human vertebrae (five T7, five T8 and five L4) were obtained from five cadavers. Donors were all females ranging from 73 to 100 years old (85.2 ± 10 yr.) The vertebrae were dissected, surrounding soft tissue and posterior processes removed and kept frozen at -20°C . Cortical bone and endplates were removed using a high-speed diamond saw. Bone marrow and remaining soft tissue inside the vertebrae was carefully rinsed using a pulsed irrigation device (Waterpik, Fort Collins, CO) (Fig. 1). The vertebrae were dehydrated in a series of graded ethanol solutions and embedded in acrylic (Leco Corporation, St. Joseph, MI, USA). The embedded samples were then polished using the Ecomet® (Buhler, Lake Bluff, IL, USA) polisher with sandpaper of different grades (600 and 800 grit sizes) and non-crystallizing colloidal silica polishing suspension (Buhler, Lake Bluff, IL, USA). The specimens were then polished on microcloths (TexMet®, Bueheler) with $0.05\text{ }\mu\text{m}$ grit alumina powder followed by plain microcloth. They were then cleaned of any debris using the pulsed irrigation device and left to dry before testing.

2.2 Nanoindentation

A load-controlled nanoindenter (TI 950® Hysitron, Minneapolis, MN, USA) was used to evaluate Young's modulus (E) and hardness (H) of anterior and posterior trabecular bone regions from each vertebra. The nanoindenter has load and displacement resolutions of 1nN and 0.04nm, respectively, and allows for continuous monitoring of loads and displacements. A sharp Berkovich pyramidal diamond indenter with an elastic modulus (E_{tip}) of 1,141GPa and Poisson's (ν_{tip}) of 0.07 was used for all measurements as previously described (Brennan et al., 2009; Mulder et al., 2008; Nazarian et al., 2008; Oliver and Pharr, 1992; Rho et al., 1997). Trabecular junctions were identified under the optical microscope and automatically positioned beneath the indenter (Fig. 2). To obtain specimen-specific tissue modulus and hardness, multiple indentations were performed on each region. In each T7, T8 and L4 vertebrae the average number of trabecular junctions randomly tested were 10 and 11 in the anterior and posterior regions, respectively. The number of trabecular junctions tested depended on the total number available within the specimens. One to three indents were performed at each junction; this resulted in an average of 100 total indents in the anterior and posterior regions of the T7, T8 and L4 vertebrae. A permanent hardness impression was made to the samples by advancing the indenter at a constant rate of $333\text{ }\mu\text{N/s}$ to a maximum load of 1mN, holding for 5 sec. and then unloading the sample at the same rate.

A load-displacement curve was obtained from each indentation (Fig. 3) from which the Young's modulus (E) and Hardness (H) were calculated by applying the method of Oliver and Pharr (Oliver and Pharr, 1992). The slope of the unloading curve will experimentally determine the stiffness (S) of the sample from which the reduced modulus (E_r) can be obtained according to:

$$S(h_{\max}) = \frac{dp(h_{\max})}{dh} = \frac{2}{\sqrt{\pi}} \beta E_r \sqrt{A} \quad (1)$$

where $h_{\max}$ is the maximum contact depth, β is an empirical constant related to the indenter tip geometry and A is the projected contact area. The tip-specimen schematic in Fig. 4 shows all the parameters and geometric variables used for modulus and hardness

calculations. Equation 2 was used to obtain the Young's modulus of the material ($E_{specimen}$), taking into account the non-rigidity characteristics of the samples and the indenter tip,

$$\frac{1}{E_r} = \frac{1 - \nu_{specimen}^2}{E_{specimen}} + \frac{1 - \nu_{tip}^2}{E_{tip}} \quad (2)$$

where $\nu_{specimen}$ was taken to be 0.3 (Adam and Swain, 2011; Brennan et al., 2009; Chevalier et al., 2007; Guo and Goldstein, 2000; Harrison et al., 2008; Hengsberger et al., 2002; Mulder et al., 2008; Norman et al., 2008; Rho et al., 1997; Wolfram et al., 2010; Zysset et al., 1999). The hardness (H) was defined as the load at maximum indentation depth divided by the contact area (Brennan et al., 2009; Oliver and Pharr, 1992; Rho et al., 1997):

$$H = \frac{P(h_{max})}{A_c(h_{max})} \quad (3)$$

2.3 Statistical Methods

Data analysis was performed using the JMP version 9 software package for Windows (SAS Institute Inc., Cary, NC). Paired and unpaired t-tests were used to test for significance within vertebrae and one-way repeated-measures analysis of variance (ANOVA) and a post hoc test (Turkey-Kramer test) were used to test for significance between vertebrae. A difference of $P < 0.05$ was considered statistically significant.

Results

Typical force-displacement curves, as shown in Fig. 3, were obtained for each indentation from which the Young's modulus (E) and hardness (H) were calculated. There was no difference in hardness and Young's modulus between the anterior and posterior regions within vertebrae. The modulus in the anterior regions of T7 (19.8 ± 1.3) and T8 (19.6 ± 1.4) was statistically greater than in L4 (17.6 ± 0.5) (Fig. 5). There was no difference among the posterior regions of all vertebrae (T7: 17.8 ± 2.2 , T8: 18.8 ± 1.2 , L4: 17.5 ± 1.1).

The average hardness results demonstrated a similar trend (Fig. 6). The hardness of the anterior regions in T7 (0.74 ± 0.07) and T8 (0.74 ± 0.04) were statistically higher than that in L4 (0.64 ± 0.06), while the posterior regions showed no difference among vertebrae T7 (0.74 ± 0.03), T8 (0.73 ± 0.05), and L4 (0.65 ± 0.08).

Discussion

In this study, we successfully measured the intrinsic mechanical properties of the anterior and posterior regions of human vertebral bodies in the thoracic and lumbar spine. There was no significant difference in elastic modulus and hardness within vertebrae. On the other hand, the elastic modulus in the anterior regions of T7 and T8 were statistically greater than in L4, while no difference was found between the posterior regions of all vertebrae. The average hardness results showed a similar significance trend. There was a difference between the anterior regions of T7 and T8 compared to L4, while the posterior regions showed no difference between vertebrae.

Nanoindentation experiments have been shown to vary in results depending on age (Feng et al., 2012), trabeculae orientation and bone sample (Tai et al., 2005; Zysset et al., 1999). Rho et al. found the Young's modulus for transversely oriented vertebral trabeculae to have an average value of 13.4 GPa (Rho et al., 1997), similar to the range of 1–14 GPa reported by

Guo et al. (Guo and Goldstein, 1997). Also, Zysset et al. found moduli of trabecular bone in the neck of the femoral bone to be 11.4 ± 5.6 GPa (Zysset et al., 1999). Our study analyzed longitudinally oriented trabecular bone properties between vertebrae from the thoracic and lumbar spines. The results obtained for Young's modulus and hardness are in good agreement with previous work. Rho et al. found that samples of human trabecular bone, in the longitudinal direction, averaged 19.4 ± 2.3 GPa (Rho et al., 1999), while Brennan et al. found an average modulus across the width of the trabeculae of 20.78 ± 2.4 GPa (Brennan et al., 2009). Small differences in the obtained Young's modulus values compared to previous published literature can be explained by the Poisson's ratio of 0.3 used in this study. As previously shown (Guo and Goldstein, 2000; Rho et al., 1997) and as demonstrated by equation 2, the Poisson's ratio will have an effect in the final Young's modulus results. Studies have also shown moduli on wet specimens to decrease ~25% and hardness ~57% compared to its dry condition (Guo and Goldstein, 2000; Townsend et al., 1975; Wolfram et al., 2010). However, consistent with previous work on dry samples, our results demonstrated similar Young's modulus and hardness values. Performing nanoindentation experiments under wet or physiological conditions would add additional constraints related to thermal drifts and experimental protocols (Hengsberger et al., 2002).

The spine, composed of three columns, bears most of the load in the anterior and posterior vertebral body regions, corresponding to the anterior and middle columns respectively. This load varies in different spine regions due to different spine curvatures (lordosis vs. kyphosis) and loading conditions (Denis, 1983). Furthermore, sagittal spine curvature has been shown to be an independent predictor of vertebral fractures. Loss of lumbar lordosis has been shown to create a forward shift of the spine and distributes the load towards the anterior part of the vertebrae, increasing the chances of a wedge-type fracture (Kobayashi et al., 2008). In his review, Briggs et al. described that even though a relationship between an increased thoracic kyphosis and vertebral fracture is still debatable, as the severity or the number of fractures increases, the relationship becomes clearer (Briggs et al., 2007). Similarly, Huang et al. found that a hyperkyphotic spine is a significant and independent predictor of fracture (Huang et al., 2006). Thevenon et al. found kyphosis to be positively correlated to low bone mineral content and a study performed by Ettinger et al. suggested kyphosis to be associated with low BMD (Ettinger et al., 1994; Thevenon et al., 1987).

In osteoporosis, as vertebral BMD decreases, its strength and stiffness also decrease. Homminga et al. hypothesized that this reduction would cause a shift in loading from the trabecular core to the cortical shell of the vertebrae (Homminga et al., 2001). However, they showed that an osteoporotic vertebra will have, at the tissue level, the same amount of load as a control vertebra. This means that the osteoporotic tissue, supporting the same load as a control, will be at a higher risk of fracture, sustaining BMD as a strong factor in the risk of fracture. Bone tissue is composed primarily of collagen and mineral. At the tissue level, the degree of mineralization and the intrinsic properties of bone also contribute to its strength (Burr, 2002; Follet et al., 2004). Follet et al. showed highly mineralized bone to have higher stiffness and compressive strength compared to the less mineralized samples (Follet et al., 2004). Yet, it is not possible to measure the effect of mineralization on fracture risk as it cannot be measured in vivo, and only animal studies have been performed (Epstein, 2005). Although there exists a correlation between BMD measured by dual x-ray absorptiometry and fracture risk, this correlation is not consistent. A high increase in BMD does not correlate to a high reduction in fracture risk (Divittorio et al., 2006; Watts et al., 2004). Burr et al. showed in their dog study that raloxifene, an anti-remodeling drug, produced higher stiffness values in vertebral treated samples compared to controls, but more importantly, higher compressive strength independent of BMD measurements (Allen et al., 2006). Multiple factors contribute to bone quality and the risk of vertebral fracture. Our study showed the lumbar vertebrae (L4) to have smaller Young's modulus and hardness values in

the anterior region compared to the thoracic vertebrae (T7 and T8). These would represent a lower compressive strength threshold and the vertebrae being more susceptible to fracture. Nevertheless, there exist multiple factors that can compensate for the low hardness and Young's modulus values. Vertebral geometry, size, microarchitecture, microcracks, mineralization and/or muscle activity and strength in that region are some components that relate to fracture risk and vertebral strength. Also, it has been previously shown that an increase in SMI and regional variations such as trabeculae perforations and/or a decrease in horizontal trabeculae thickness affect vertebral strength and increase the risk of fracture (Chen et al., 2008; Fields et al., 2009; Gong et al., 2006; Mosekilde, 1993). In spite of the Young's modulus and hardness values being smaller in the lumbar vertebrae compared to the thoracic region, their influence and the role of degree of mineralization in fracture risk prediction remains unclear and needs to be further studied.

This study has several limitations. First, we did not measure BMD from each vertebra and thus we did not correlate the values to the mechanical parameters. These correlations and measurements would have provided a further understanding between BMD, intrinsic material properties and vertebrae location in the spine. Second, we did not measure the spine alignment; this may have provided an additional insight into mechanical properties and fracture risk related to spine posture. Finally, the measurements of Young's modulus and hardness show differences in intrinsic material properties, between regions of the spine, which can affect fracture risk and vertebral strength. However, these values do not directly reflect microarchitecture which also affects fracture risk.

Several research approaches have attempted to understand the relationship between spine posture and BMD, micro-architecture and vertebral strength or intrinsic bone material properties of single trabecula. However, there is limited knowledge regarding bone material properties at the nano level from different spine regions. In this study we hypothesized that bone from different spine regions, due to changes in alignment (kyphosis vs. lordosis) and loading, would present differences in their intrinsic bony material properties. Due to the natural kyphosis in thoracic spine, the anterior portion of vertebra may be subject to higher loads compared to the anterior region in the lordotic lumbar spine. For this purpose, we measured the mechanical properties of the anterior and posterior regions of human vertebral bodies in the thoracic and lumbar spine.

In summary, the results presented in this study, for the first time, reveal the differences in bone properties between the thoracic and lumbar spine regions. Young's modulus and hardness of trabecular bone in human vertebral bodies were quantified for the anterior and posterior regions. Trabecular material Young's modulus and hardness from the anterior column in the thoracic regions were higher than that in the lumbar vertebrae. These differences in the material properties of the anterior vertebrae in the thoracic relative to the lumbar spine may be related to changes in spinal curvature from the kyphotic thoracic spine to the lordotic lumbar spine and associated changes in loading in these different regions. This information will be helpful in understanding vertebral body remodeling and adaption in different regions of the spine which may be associated with spinal curvature and loading conditions.

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Fig. 1. Example of sample bone used for nanoindentation. **A)** Specimen with bone marrow and any remaining soft tissue inside the vertebrae. **B)** Bone marrow and tissue was removed using a pulsed water jet.

Fig. 2. Typical trabecular junction surface optical micrograph. Following polishing of the samples, trabecular junctions were located under the optical microscope and indents were performed at their core.

Fig. 3.

A representation of a typical nanoindentation load vs. displacement curve. S : initial unloading stiffness; P_{max} : peak load; h_f : final depth after sample deformation; h_{max} : maximum indenter depth and maximum load.

Fig. 4. Schematic representation of the contact geometry during loading and unloading of the indenter tip and parameters used to obtain the hardness and Young's modulus of the material. h : total depth during loading; A : initial projected contact area; h_f : final depth after sample deformation; h_c : contact depth; h_s : displacement of the perimeter next to tip-contact region

Fig. 5. Young's modulus (mean $\pm$ st. dev.) values for the anterior and posterior regions of T7, T8 and L4 vertebrae. Within vertebrae there was no significant difference between anterior and posterior regions. There was also no difference between vertebrae in the posterior regions. (*) denotes a significant difference ($P < 0.05$).

Fig. 6. Hardness (mean $\pm$ st. dev.) values for the anterior and posterior regions of T7, T8 and L4 vertebrae. Similar to the Young's modulus, there was only a significant difference between vertebrae in the anterior region ($P < 0.05$).