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Bone Properties by Nanoindentation in Mild and Severe *Osteogenesis imperfecta*

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# Abstract

## Background

*Osteogenesis imperfecta* is a heterogeneous genetic disorder characterized by bone fragility. Previous research suggests that impaired collagen network and abnormal mineralization affect bone tissue properties, however, little data is yet available to describe bone material properties in individuals with this disorder. Bone material properties have not been characterized in individuals with the most common form of *osteogenesis imperfecta*, type I.

## Methods

Bone tissue elastic modulus and hardness were measured by nanoindentation in eleven osteotomy specimens that were harvested from children with *osteogenesis imperfecta* during routine surgeries. These properties were compared between *osteogenesis imperfecta* types I (mild, n = 6) and III (severe, n = 5), as well as between interstitial and osteonal microstructural regions using linear mixed model analysis.

## Findings

Disease severity type had a small but statistically significant effect on modulus (7%, *P* = 0.02) and hardness (8%, *P* < 0.01). Individuals with *osteogenesis imperfecta* type I had higher modulus and hardness than did those with type III. Overall, mean modulus and hardness values were 13% greater in interstitial lamellar bone regions than in osteonal regions (*P* < 0.001).

## Interpretation

The current study presents the first dataset describing bone material properties in individuals with the most common form of *osteogenesis imperfecta*, i.e., type I. Results indicate that intrinsic bone tissue properties are affected by phenotype. Knowledge of the material properties of bones in *osteogenesis imperfecta* will contribute to the ability to develop models to assist in predicting fracture risk.

# Keywords

*Osteogenesis imperfecta,* Bone, Material properties, Elastic modulus, Bone microstructure, Nanoindentation

# 1. Introduction

*Osteogenesis imperfecta* (OI) is a heterogeneous collagen-related genetic disorder resulting in bone fragility ([Rauch and Glorieux, 2004](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0190), [Sillence et al., 1979](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0225)). Bone fragility in OI is thought to result from a combination of bone mass deficiency and compromised bone tissue material properties ([Rauch and Glorieux, 2004](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0190), [Roschger et al., 2008b](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0215), [Weber et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0280)). Little data, however, is yet available to describe bone material properties in individuals with OI ([Fan et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0060), [Fan et al., 2007a](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0065), [Fan et al., 2007b](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0070), [Weber et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0280)).

Severity of OI varies markedly, from mild to lethal in the perinatal period. OI types I–IV, representing the majority of individuals with OI, are dominant forms caused by defects in the quantity or structure of type I procollagen ([Forlino et al., 2011](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0080), [Smith, 1994](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0235)). Other less common forms of OI have been identified recently. These include another autosomal dominant form (OI type V) for which the gene defect is unknown, and six recessive forms attributed to deficiency of proteins that affect post-translational modification or folding of collagen ([Forlino et al., 2011](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0080)). In the current study, intrinsic bone material properties were compared between young individuals with two very distinct forms of OI: type I, the mildest form which tends to result in fewer fractures and near normal stature and physical function; and type III, a severe form that often leads to more frequent fractures and progressive skeletal deformities ([Sillence et al., 1979](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0225)).

Structural and material bone abnormalities have been noted in OI. From a structural perspective, individuals with OI tend to have low bone mass. Histomorphometric analyses have noted lower cortical thickness and decreased trabecular bone volume per tissue volume in children with OI ([Rauch et al., 2000](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0195), [Roschger et al., 2008a](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0210)).

At the ultrastructural level, the diameters of type I collagen fibrils in OI bone have been reported to differ from those of typical bone ([Cassella et al., 1994](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0035), [Stoss and Freisinger, 1993](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0240)). With respect to the inorganic content of bone, mineral content and the shape, size and composition of the mineral crystals can also be altered with OI pathologies ([Traub et al., 1994](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0250), [Vetter et al., 1991](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0265)). Calcium to phosphorus (Ca/P) ratio was observed to be lower than normal in OI bone ([Cassella and Ali, 1992](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0030), [Cassella et al., 1995](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0040)). It was also found that average bone mineralization density tends to be higher than normal in OI ([Boyde et al., 1999](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0010), [Roschger et al., 2008a](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0210), [Weber et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0280)).

These abnormalities in collagen and bone mineral crystals suggest that the intrinsic, i.e., tissue-level, bone material behavior is compromised in OI. Limited data, however, is yet available to describe bone material properties in individuals with OI. A few studies have used nanoindentation to characterize bone in severe (type III) and moderately severe (type IV) OI ([Fan et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0060), [Fan et al., 2007a](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0065), [Fan et al., 2007b](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0070), [Weber et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0280)). In nanoindentation, a diamond-tip indenter is pressed into the polished surface of a specimen, typically a few hundred nanometers deep, enabling local measurements of elastic modulus and hardness ([Lewis et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0145), [Weber et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0280)). These properties were found to be higher in children with severe OI than in age-matched controls ([Weber et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0280)), however, no significant differences were reported between moderate and severe OI ([Fan et al., 2007a](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0065), [Fan et al., 2007b](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0070)). No data is yet available to describe bone material properties in individuals with the most common form of OI, type I.

It is worth noting that wide ranges of values in elastic modulus (11–24 GPa) and hardness (0.3–0.9 GPa) have been reported for severe OI ([Fan et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0060), [Fan et al., 2007a](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0065), [Fan et al., 2007b](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0070), [Weber et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0280)). Variability between these studies may be attributed in part to the selection of indentation sites. Previous studies have indicated that these properties tend to be higher in interstitial than osteonal bone regions ([Hoffler et al., 2005](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0130), [Rho and Pharr, 1999](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0200)).

The primary question of this study was: do the material properties of bone differ between individuals with OI types I (mild OI) and III (severe OI)? The secondary question was: how do properties vary between regions of interstitial and osteonal bone? These questions were investigated using small bone specimens from children with OI, collected during routine surgical procedures.

# 2. Methods

This study investigated the effects of disease severity (phenotype) on the local elastic modulus and hardness of bone tissue from children with OI types I and III, using nanoindentation. The effect of the choice of indentation site on the measured properties was also investigated.

## 2.1. Specimens

Bone specimens were collected from lower extremity long bones of young individuals with OI. Inclusion criteria were: pediatric patients between 5 and 18 years of age with OI types I and III, and who are undergoing routine surgery at Shriners Hospitals for Children – Chicago for fracture repair and/or deformity correction of lower extremity long bones. Bone specimens selected for this study had a lamellar microstructure, without the appearance of callous tissue. A total of twelve bone specimens were collected for this study, of which one specimen was excluded due to the presence of callous tissue and a lack of lamellar microstructure, indicating remodeling activity at a fracture site. Eleven bone specimens, obtained from ten donors, were therefore included in this study. Five of the donors (ages 7–16) had the mildest form, OI type I, while the other five (ages 7–14) had a more severe form, type III ([Table 1](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "t0005)). Donor ages did not differ significantly between the two groups (Student's *t*-test, *P* = 0.5). The specimens were obtained under appropriate informed consent and IRB approval (#10101309 from Rush University Medical Center, and #HR-2167 from Marquette University). The unfixed specimens were stored at − 85°C until testing. A previous study found that storage at a similar temperature (− 80°C) for up to 5 years did not have significant effects on histological features or mechanical properties of bones ([Salai et al., 2000](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0220)).

Table 1. Specimen descriptions: Donor age, gender, OI type, history of bisphosphonate treatment, and anatomic site.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Specimen** | **OI type** | **Age** | **Gender** | **Anatomic site** | **Bisphosphonate treatment** | **# Indents osteonal** | **# Indents interstitial** |
| 1 | I | 16 | M | Tibia (p.d.) | Yes | 10 | 10 |
| 2 | I | 7 | F | Tibia (m.d.) | Yes | 11 | 0 |
| 3[⁎](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "tf0005) | I | 14 | F | Tibia (m.d.) | Yes | 9 | 3 |
| 4 | I | 13 | M | Femur (m.d.) | Yes | 7 | 9 |
| 5[⁎](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#tf0005) | I | 11 | F | Femur (p.d.) | Yes | 8 | 10 |
| 6 | I | 15 | M | Femur (d.d.) | Yes | 0 | 8 |
| 7 | III | 12 | M | Tibia (m.d.) | No | 5 | 9 |
| 8 | III | 12 | M | Tibia (m.d.) | No | 2 | 14 |
| 9 | III | 14 | M | Tibia (m.d.) | Yes | 14 | 5 |
| 10 | III | 7 | F | Tibia (m.d.) | No | 5 | 6 |
| 11 | III | 12 | F | Femur (p.d.) | Yes | 13 | 10 |

(p.d.) Proximal diaphysis; (m.d.) middle diaphysis; and (d.d.) distal diaphysis.

⁎Specimens 3 and 5 were obtained from the same donor during surgical procedures that were performed three years apart.

## 2.2. Specimen preparation

Prior to nanoindentation, the specimens were thawed and prepared for embedding. The specimens were cross-sectioned under constant water irrigation using a diamond saw (IsometTM Low Speed Saw; Buehler, Lake Bluff, IL, USA). The cross-sections were oriented such that the exposed surface was approximately perpendicular to the longitudinal axis of the long bone, using the periosteal surface of the specimen as a reference. The specimens were fixed and dehydrated in graded ethanol solutions: 70%, 24 h; 80%, 2 h; 95%, 2 h; 95%, 3 h; 100%, 2 h; 100%, 2 h; and 100%, 3 h. After dehydration, the specimens were air-dried for approximately 5 min and embedded under vacuum in a low viscosity epoxy resin (Epo-Thin®; Buehler, Lake Bluff, IL, USA). After polymerization, the specimen surfaces were ground on a grinder–polisher (Metaserv® 3000; Buehler, Lake Bluff, IL, USA) using progressively fine grit sizes: 400, 600, 800, and 1200. The surfaces were polished using a 3 μm aluminum oxide coated disc (Fibrmet®; Buehler, Lake Bluff, IL, USA). Final polishing was done with a polishing cloth (Microcloth®; Buehler, Lake Bluff, IL, USA) and a 0.05 μm alumina suspension (Micropolish® B; Buehler, Lake Bluff, IL, USA).

## 2.3. Nanoindentation testing

Testing was performed on a nanoindenter (Nano Indenter XP; MTS, Eden Prairie, MN, USA), using a Berkovich diamond indenter tip. Measurements of modulus and hardness were obtained using the Continuous Stiffness Measurement (CSM) algorithm that was associated with the nanoindenter. The CSM algorithm applies a low magnitude oscillating force superimposed onto the nominally increasing load ([Li and Bhushan, 2002](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0150)). This method has been used by others in the characterization of bone material properties ([Wang et al., 2008a](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0270), [Wang et al., 2008b](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0300)). With this test method, the indenter penetrates the specimen surface with a defined frequency, amplitude, strain rate and maximum depth. Modulus and hardness are measured continuously as a function of penetration depth. Frequency and amplitude were set at 45 Hz and 2 nm, respectively, as was done in previous studies ([Wang et al., 2008a](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0270), [Wang et al., 2008b](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0300)). A strain rate of 0.05 s− 1, and a depth limit of 2000 nm were used. As in previous studies, Poisson's ratio was assumed to be 0.3 for the bone specimens ([Fan et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0060), [Fan et al., 2007a](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0065), [Wang et al., 2008a](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0270), [Wang et al., 2008b](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0300)). Modulus and hardness were calculated automatically as a function of indentation depth using the CSM algorithm. For each indent, average modulus and hardness values were obtained between indentation depths of 800–1600 nm, a range over which these measurements were approximately constant.

A total of 20 indents per specimen were performed. For each specimen, indentation was performed in four separate, pre-defined clusters, each located in a different region of lamellar bone. Bone microstructure was described at each indent site using an optical microscope. The indent sites were divided in two microstructural groups: osteonal bone and interstitial lamellar bone. Typical indent sites are shown in [Fig. 1](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "f0005). Microstructure at each indentation site was identified by two independent observers (JJ and CA). Data points for which the two observers did not agree on the type of lamellar microstructure were excluded from the data set. Indents that were found to be in contact with void spaces such as osteocyte lacunae, resorption spaces and Haversian canals were also excluded from the dataset. Finally, indents that were located in non-lamellar bone (e.g., woven bone), and those for which the site could not be distinguished clearly as being located in osteonal or interstitial bone regions were also excluded.

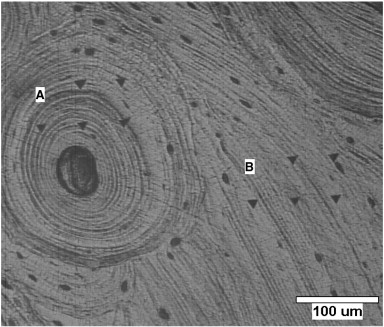


Fig. 1. Location of typical indentation sites. (A) Osteonal bone region. (B) Interstitial lamellar bone region. The bone cross section shown was obtained from the femoral diaphysis of a 16-year-old male with OI type I.

A total of 168 indents were included in this study. Approximately half (85) of the indents were in the specimens of OI type I, while the others (83) were in those of type III. Half of the indents (84) were located in interstitial lamellar bone. The other half of the indents were in osteonal regions ([Table 1](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#t0005)).

## 2.4. Statistical analysis

The effects of disease severity (OI types I vs. III) and lamellar microstructure (osteonal vs. interstitial) on local elastic modulus and hardness were analyzed statistically using linear mixed effects models. The statistical analysis was performed by a statistician using an open source software package (The R Project for Statistical Computing, [www.r-project.org](http://www.r-project.org/)). OI severity and microstructure were defined as fixed factors in the model. Specimen number (1–11) was treated as a random factor. The effects of four potential covariates were also explored: patient age, patient gender, anatomic site (femur or tibia), and patient history of bisphosphonate treatment (yes or no). Covariates that were found to be significant (*P* < 0.05) were included in the final statistical models.

# 3. Results

Modulus and hardness results for each OI type (I and III) are shown in [Fig. 2](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "f0010). Within each OI severity group, the results are presented for each microstructure (interstitial and osteonal) in [Fig. 2](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#f0010).

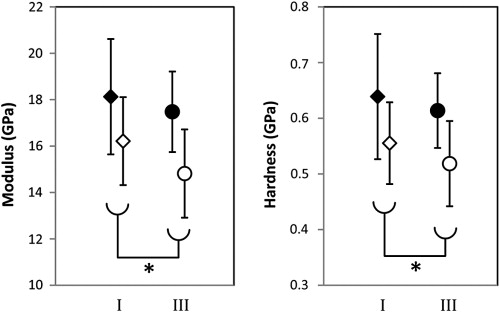


Fig. 2. Elastic modulus (left) and hardness (right) results for all indents. Results for OI type I are shown as diamonds: interstitial (♦) and osteonal bone regions (♢). Results for OI type III are shown as circles: interstitial (●) and osteonal regions (○). Shown are means and standard deviations. \**P* < 0.05 between OI types I and III, based on linear mixed effects models (see [Table 2](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "t0010), [Table 3](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "t0015)).

## 3.1. Building of statistical model for modulus

Of the possible covariates investigated, only anatomic site (tibia vs. femur) had a significant effect on modulus (*P* = 0.014) and was retained for the final statistical model. The other possible covariates, i.e., donor age, gender and history of bisphosphonate treatment, did not have a significant effect on modulus (*P* > 0.05). Therefore, these factors were not included in the final model.

## 3.2. Results of statistical model for modulus

The results of the final linear mixed model for modulus are presented in [Table 2](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#t0010). Based on the model results, OI disease severity had a significant effect on modulus (*P* = 0.024). Individuals with OI type III had lower moduli than did those with type I by approximately 7% (16.3 GPa vs. 17.5 GPa). Bone microstructure also had a significant effect on modulus (*P* < 0.001), with osteonal bone having lower modulus than interstitial lamellar bone by approximately 13%. Finally, the effect of anatomic site was also significant (*P* = 0.014) with the modulus being higher in the tibia than the femur by approximately 8%.

Table 2. Results of the linear mixed model for modulus.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Coefficient (GPa)** | **SE (GPa)** | ***P* value** |
| Intercept (OI type I, interstitial bone, femur) | 17.53 | 0.47 | < 0.001 |
| Severity = OI type III | − 1.23 | 0.55 | 0.024 |
| Microstructure = Osteonal | − 2.21 | 0.28 | < 0.001 |
| Anatomic site = Tibia | 1.40 | 0.57 | 0.014 |

## 3.3. Building of statistical model for hardness

With respect to the hardness model, two covariates were found to have statistically significant effects: patient gender and history of bisphosphonate treatment (*P* < 0.05). Therefore, these two factors were included as covariates in the final model for hardness.

## 3.4. Results of statistical model for hardness

Results of the linear mixed model for hardness are presented in [Table 3](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#t0015). As seen in [Table 3](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#t0015), OI severity had a significant effect on bone tissue hardness (*P* = 0.003), OI type III having lower hardness than OI type I by 8%. Bone microstructure also had a significant effect (*P* < 0.001), with osteonal bone having lower hardness than interstitial bone by 11%. Finally, hardness was higher for males than females by 6%, and individuals with a known history of bisphosphonate treatment prior to tissue donation had a lower hardness by 6% (*P* < 0.04).

Table 3. Results of the linear mixed model for hardness.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Coefficient (GPa)** | **SE (GPa)** | ***P* value** |
| Intercept (OI type I, interstitial bone, female) | 0.656 | 0.026 | < 0.001 |
| Severity = OI type III | − 0.054 | 0.018 | 0.003 |
| Microstructure = osteonal | − 0.735 | 0.013 | < 0.001 |
| Gender = male | 0.041 | 0.015 | 0.006 |
| Bisphosphonates = yes | − 0.044 | 0.021 | 0.039 |

# 4. Discussion

Little data is currently available to describe the material properties of bones in individuals with OI, and bone material properties have not yet been characterized for the most common form, OI type I. The primary goal of this study was to determine if bone material properties differ between individuals with OI types I and III. This question was investigated using nanoindentation. To determine the role (if any) of microstructure, these properties were also compared between interstitial and osteonal bone regions.

Osteotomy specimens are often sent for routine histopathology or discarded following orthopedic surgeries in children with OI. These specimens can provide a valuable resource for characterizing bone material behavior. Because of its small scale, nanoindentation lends itself well to the testing of these osteotomy specimens. Nevertheless, use of this technique to characterize bone tissue has some limitations. Calculation of elastic modulus assumes local isotropy of the mechanical properties of the bone specimens, which may not be true. A previous nanoindentation study of OI bone, however, did not find significant modulus differences between longitudinal and transverse directions, suggesting that OI bone tissue at the sub-microstructural scale may be more isotropic than healthy bone tissue ([Fan et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0060)). Modulus calculation in nanoindentation also requires knowledge of the specimen's Poisson's ratio. In the present study, a Poisson's ratio of 0.3 was assumed. Varying Poisson's ratio of bone specimens as input from 0.2 to 0.4 was reported to affected modulus results by 8–9% ([Hoffler et al., 2005](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0130), [Turner et al., 1999](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0255)). Moreover, bone tissue behavior is viscoelastic in nature, and experimental factors such as specimen dehydration ([Hengsberger et al., 2002](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0110), [Hoffler et al., 2005](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0130), [Rho and Pharr, 1999](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0200)), loading rate ([Hoffler et al., 2005](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0130), [Mittra et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0175)), specimen mounting media ([Mittra et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0175)) and time of storage before testing ([Mittra et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0175)) have been demonstrated to affect modulus measured by nanoindentation. For example, it is a common practice to fix and dehydrate the bone specimens prior to nanoindentation ([Fan et al., 2007a](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0065), [Isaksson et al., 2010](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0135), [Mittra et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0175), [Mulder et al., 2007](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0180), [Turner et al., 1999](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0255)) in order to facilitate handling, storage, and testing. Modulus of bone measured by nanoindentation, however, was found to be higher under dry vs. wet conditions ([Bushby et al., 2004](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0025), [Guidoni et al., 2010](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0100), [Hoffler et al., 2005](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0130)), and this phenomenon has been attributed to the contribution of the non-mineralized phase ([Guidoni et al., 2010](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0100)). To minimize undue experimental variability between the specimens, dehydration protocol, embedding media, time of storage (2 weeks prior to testing), and loading rate were kept constant in this study. Nonetheless, in light of the abovementioned limitations that are inherent to the use of nanoindentation to characterize bone tissue, emphasis of the current results should be placed on the relative effects of the factors rather than on the absolute values.

Another limitation in this study is the small number of specimens in each OI type. As in any small sample study, the reliability of the statistical conclusions is hindered by the small number of specimens, and a heavy reliance on the chosen statistical models is required.

The results of the present study were within the range of values reported in previous nanoindentation studies of OI bone ([Fan et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0060), [Fan et al., 2007a](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0065), [Fan et al., 2007b](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0070), [Weber et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0280)). Current results for osteonal regions of OI type III were similar to those of another study in which the indentation sites were also located within osteons ([Fan et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0060)). For interstitial regions, previously published average results for modulus (19–22 GPa) and hardness (0.7–0.8 GPa) for severe OI were somewhat higher than the current results ([Fan et al., 2007a](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0065), [Weber et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0280)). These differences may be attributed to the abovementioned experimental factors, differences between anatomic site (iliac crest vs. long bones), and/or differences in indentation sites within interstitial bone.

The main finding of this study was that OI severity had a small but significant effect on the material properties measured. Elastic modulus and hardness were higher in individuals with OI type I than in those with type III. The reason behind this observation is not fully understood. It has been postulated that the higher modulus previously reported in severe OI compared to normal bone ([Weber et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0280)) could be attributed to a more highly mineralized bone matrix ([Roschger et al., 2008a](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0210)). A relationship between local modulus and local mineralization has indeed been observed in previous studies of animal ([Burket et al., 2011](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0015), [Hoc et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0120)) and human bones ([Smith et al., 2010](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0230)). A scanning electron microscopy study found that mean bone mineralization density of iliac crest biopsies was higher in children with OI than in normal controls and it was further noted that mineralization density tended to be lower in OI type I than in OI types III–V ([Boyde et al., 1999](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0010)). The current finding of higher elastic modulus and hardness in OI type I than in type III is therefore surprising in that it does not seem to fit the assumption that a higher modulus is attributed to higher mineralization. Nonetheless, in a recent nanoindentation study in a mouse model for OI (*oim*), modulus was not found to correlate with local measurements of bone matrix mineralization ([Vanleene et al., 2012](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0260)). Therefore, the effect of OI severity on modulus and hardness may be related to factors other than mineralization density, such as: size, shape and composition of the mineral crystals, collagen structure, and/or mechanical interaction between the collagen fibrils and the mineral crystals.

On the collagen side, OI type I is typically associated with a null COL1A1 mutation that results in a reduced production of type I collagen ([Barsh et al., 1982](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0005), [Willing et al., 1992](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0290), [Willing et al., 1996](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0285)). In OI type I, collagen is normal in composition and structure but an insufficient quantity is produced ([Barsh et al., 1982](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0005), [Smith, 1994](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0235)). OI type III, on the other hand, is associated with amino substitution defects within type I collagen molecules, i.e., collagen quality is affected rather than its quantity ([Forlino et al., 2011](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0080), [Smith, 1994](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0235)). These qualitative collagen mutations could affect the mechanical behavior of the collagen fibril differently than quantitative ones (OI type I). For example, a computational study has demonstrated that structural mutations within type I collagen molecules could compromise fibril mechanical properties by altering stress distribution within the fibrils ([Gautieri et al., 2009](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0090)). At the ultrastructural level, abnormal type I collagen fibril diameters have been reported in OI, although there is disagreement as to whether their diameters are higher ([Cassella and Ali, 1992](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0030), [Cassella et al., 1994](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0035)) or lower ([Stoss and Freisinger, 1993](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0240)) than normal. Conflicting information also exists when comparing the fibril diameters between phenotypes. One study noted that fibril diameter tended to be larger in OI type I and smaller in OI type IV ([Cassella and Ali, 1992](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0030)), while another reported the opposite, i.e., that fibril diameter was smaller in OI type I than in types III and IV ([Stoss and Freisinger, 1993](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0240)), although no statistical analysis supported this observation.

On the mineral side, compositional and structural abnormalities have also been reported in OI bone. Ca/P ratio was found to be lower than normal in OI, although phenotypes were not compared ([Cassella and Ali, 1992](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0030), [Cassella et al., 1995](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0040)). Apatite crystals were also found to be smaller than normal in children with OI and their size was noted to decrease with disease severity ([Traub et al., 1994](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0250), [Vetter et al., 1991](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0265)). Similar observations were also reported in animal models of OI ([Fisher et al., 1987](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0075), [Fratzl et al., 1996](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0085), [Grabner et al., 2001](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0095), [Vanleene et al., 2012](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0260)). Further work is warranted to investigate the roles of local bone tissue composition and ultrastructure on the mechanical properties of bone in humans with OI.

The current study presents the first mechanical characterization of bone tissue in humans with OI type I. Although the study did not include a normal control group, the results can be compared against those of other previously published studies to get a sense of how modulus compares between OI phenotypes and normal bone. A few studies have looked at tensile and flexural mechanical properties of pediatric bone at the mesoscale ([Currey and Butler, 1975](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0050), [Hirsch and Evans, 1965](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0115)). Modulus results from the current study lie within the range of moduli previously for pediatric bone specimens ([Hirsch and Evans, 1965](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0115)), however, it could be misleading to compare results obtained by nanoindentation directly to control values obtained at the mesoscale. Moduli of normal as well as OI pediatric iliac crest biopsies have been measured by nanoindentation ([Weber et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0280)). However, modulus values for severe OI in that study were higher than those of the current study. This discrepancy is likely due to differences in indentation site or other experimental factors, as discussed earlier. For this reason, cross-study comparisons of these nanoindentation results should be based on relative observations rather than absolute values. While no significant difference in elastic modulus has been found between individuals with OI types III and IV ([Fan et al., 2007a](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0065), [Fan et al., 2007b](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0070)), modulus was 13% higher in iliac crest biopsies from children with severe OI than in age-matched normal controls ([Weber et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0280)). One might expect the elastic modulus of bone in milder OI (type I) to fall between those of severe OI (type III) and normal tissue, however, results of the current study indicate that this might not be the case ([Fig. 3](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "f0015)). In the current study, elastic modulus was slightly higher in OI type I than in type III. As discussed previously, further research is required to explain this observation.

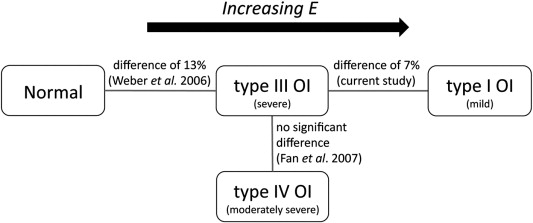


Fig. 3. Synthesis of the effects of OI type on the elastic modulus of human bone tissue by nanoindentation: comparison of the relative results of the current study to those of previous studies of pediatric OI bone.

Studies in mice have also produced uncertainties regarding the effect of OI on the elastic modulus of bones. Some mechanical studies of whole bones reported higher modulus in *oim/oim* mice, representative of severe OI, than in wild-type control littermates ([Miller et al., 2007](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0165), [Misof et al., 2005](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0170), [Rao et al., 2008](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0185)), while no significant difference was seen in another study ([McCarthy et al., 2002](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0160)). On the other hand, *oim* mice were found to have lower modulus by nanoindentation than their wild-type littermates ([Vanleene et al., 2012](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0260)). For the *Mov13* mice, which produce bone pathology analogous to mild OI, elastic modulus was reported to be higher than that of control littermates in the anterior femoral region, but not in the posterior region ([Jepsen et al., 1997](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0140)). The present study indicates that, at the sub-microstructural level, average elastic modulus is slightly greater in individuals with mild OI than in those with a severe form of the disorder.

Bone microstructure had a significant effect on both modulus and hardness. These properties were higher in interstitial lamellar regions than in osteonal regions. Similar results were reported in other nanoindentation studies of bovine ([Rho and Pharr, 1999](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0200)) and adult human bones ([Hoffler et al., 2005](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0130), [Rho et al., 2002](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0205)). These differences are likely attributed to differences in degrees of mineralization between these regions. Osteonal regions tend to be less mineralized than interstitial bone ([Eschberger and Eschberger, 1986](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0295)), and a relationship has been observed between local bone modulus and degree of mineralization ([Gupta et al., 2005](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0105), [Mulder et al., 2007](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0180), [Tai et al., 2005](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0245)).

Elastic modulus and hardness measure resistance to elastic (recoverable) and localized plastic (irrecoverable) deformation, respectively. Although they represent distinct properties, a relationship exists between them in bone tissue. In the current study, hardness results correlated positively with modulus (Pearson's correlation, slope = 0.038, R2 = 0.80, *P* < 0.001). This relationship is consistent with previous studies, and it has been attributed to these two properties having similar relationships with local bone mineral content ([Currey and Brear, 1990](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0045), [Hodgskinson et al., 1989](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0125), [Hoffler et al., 2005](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0130)).

Bisphosphonates have become commonly used in the treatment of children with OI, as a means to reduce fracture risk by increasing bone mass ([Rauch and Glorieux, 2004](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0190)). Most but not all of the individuals who donated the bone specimens used in this study had a known history of bisphosphonate treatment. This factor was not controlled in our study design; nonetheless the significance of its association with the measured properties was explored. In a previous study of OI bone, hardness and modulus were not found to be affected after two to three years of pamidronate treatment ([Weber et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0280)). In the current study, history of bisphosphonate treatments had no significant effect on modulus, but a small significant effect on hardness was observed. Therefore, some material properties may indeed be affected by the use of bisphosphonates. It should also be emphasized that bone material strength or toughness were not measured in these nanoindentation studies, and that the effect of bisphosphonates on those properties has not yet been determined. Bisphosphonates inhibit osteoclasts, which play an important role in the cortical bone remodeling process. Consequently, this class of drugs may adversely affect the material strength and toughness of cortical bone tissue. Nevertheless, as previously stated by Weber et al., a reduction in fracture incidence after pamidronate use in OI is likely attributed to an increase in bone mass rather than to changes in bone material properties ([Weber et al., 2006](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0280)).

In the present study, modulus was slightly higher in the tibia than the femur. A similar observation was also reported in a macroscopic-scale tensile study of human bone specimens ([Burstein et al., 1976](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0020)). Males also had slightly higher bone tissue hardness than did females, although the meaning behind this observation is not clear. No difference in modulus was seen between the genders, and this observation is in agreement with the results of a previous study ([Lindahl and Lindgren, 1967](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub" \l "bb0155)).

The structural behavior of whole bones is dependent not only on material properties but on geometry as well. Histological studies have shown that individuals with severe OI tend to have less bone tissue than those with mild OI, as evidenced by thinner cortices and decreased trabecular bone volume ([Rauch et al., 2000](https://www.sciencedirect.com/science/article/pii/S0268003312002306?via%3Dihub#bb0195)). Therefore, due to a combination of decreased bone volume and lower tissue modulus, the overall structural stiffness of whole bones could be appreciably lower in individuals with severe OI than in those with mild OI.

# 5. Conclusions

Results of the present study indicate that bone material properties are affected by OI severity, and that modulus and hardness are higher in individuals with OI type I than type III. Based on the current findings, it would be ideal if future studies aimed at characterizing the mechanical properties of bones in OI considered each phenotype separately. Further research is recommended to determine how other material properties, such as bone material strength and toughness, are affected by OI severity and by bisphosphonate treatments. Knowledge of these bone material properties in OI will contribute to the ability to develop models to assist in predicting fracture risk.

# Conflict of Interest

The authors declare that there are no financial or personal relationships with other people or organizations that could inappropriately influence this study.

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# References

Barsh et al., 1982. G.S. Barsh, K.E. David, P.H. Byers. **Type I *osteogenesis imperfecta*: a nonfunctional allele for pro alpha 1 (I) chains of type I procollagen.** *Proc. Natl. Acad. Sci. U. S. A.,* 79 (1982), pp. 3838-3842

Boyde et al., 1999. A. Boyde, R. Travers, F.H. Glorieux, S.J. Jones. **The mineralization density of iliac crest bone from children with *osteogenesis imperfecta*.** *Calcif. Tissue Int.,* 64 (1999), pp. 185-190

Burket et al., 2011. J. Burket, S. Gourion-Arsiquaud, L.M. Havill, S.P. Baker, A.L. Boskey, M.C. van der Meulen. **Microstructure and nanomechanical properties in osteons relate to tissue and animal age.** *J. Biomech*., 44 (2011), pp. 277-284

Burstein et al., 1976. A.H. Burstein, D.T. Reilly, M. Martens. **Aging of bone tissue: mechanical properties.** *J. Bone Joint Surg. Am*., 57 (1976), pp. 956-966

Bushby et al., 2004. A.J. Bushby, V.L. Ferguson, A. Boyde. **Nanoindentation of bone: comparison of specimens tested in liquid and embedded in polymethylmethacrylate.** *J. Mater. Res*., 19 (2004), pp. 249-259

Cassella and Ali, 1992. J.P. Cassella, S.Y. Ali. **Abnormal collagen and mineral formation in *osteogenesis imperfecta*.** *Bone Miner*., 17 (1992), pp. 123-128

Cassella et al., 1994. J.P. Cassella, P. Barber, A.C. Catterall, S.Y. Ali. **A morphometric analysis of osteoid collagen fibril diameter in *osteogenesis imperfecta*.** *Bone*, 15 (1994), pp. 329-334

Cassella et al., 1995. J.P. Cassella, N. Garrington, T.C. Stamp, S.Y. Ali. **An electron probe X-ray microanalytical study of bone mineral in *osteogenesis imperfecta*.** *Calcif. Tissue Int*., 56 (1995), pp. 118-122

Currey and Brear, 1990. J. Currey, K. Brear. **Hardness, Young's modulus and yield stress in mammalian mineralized tissues.** *J. Mater. Sci. Mater. Med*., 1 (1990), pp. 14-20

Currey and Butler, 1975. J.D. Currey, G. Butler. **The mechanical properties of bone tissue in children.** *J. Bone Joint Surg. Am*., 57 (1975), pp. 810-814

Eschberger and Eschberger, 1986. J. Eschberger, J. Eschberger. **Microradiography.** A.F. Von Recum (Ed.), *Handbook of Biomaterials Evaluation: Scientific, Technical, and Clinical Testing of Implant Materials*, Macmillan Publishing Company, New York, NY (1986)

Fan et al., 2006. Z. Fan, P.A. Smith, E.C. Eckstein, G.F. Harris. **Mechanical properties of OI type III bone tissue measured by nanoindentation.** *J. Biomed. Mater. Res. A*, 79 (2006), pp. 71-77

Fan et al., 2007a. Z. Fan, P.A. Smith, G.F. Harris, F. Rauch, R. Bajorunaite. **Comparison of nanoindentation measurements between *osteogenesis imperfecta* Type III and Type IV and between different anatomic locations (femur/tibia versus iliac crest).** *Connect. Tissue Res*., 48 (2007), pp. 70-75

Fan et al., 2007b. Z. Fan, P.A. Smith, F. Rauch, G.F. Harris. **Nanoindentation as a means for distinguishing clinical type of *osteogenesis imperfecta*.** *Composites Part B*, 38 (2007), pp. 411-415

Fisher et al., 1987. L.W. Fisher, E.D. Eanes, L.J. Denholm, B.R. Heywood, J.D. Termine. **Two bovine models of *osteogenesis imperfecta* exhibit decreased apatite crystal size.** *Calcif. Tissue Int*., 40 (1987), pp. 282-285

Forlino et al., 2011. A. Forlino, W.A. Cabral, A.M. Barnes, J.C. Marini. **New perspectives on *osteogenesis imperfecta*.** *Nat. Rev. Endocrinol*., 7 (2011), pp. 540-557

Fratzl et al., 1996. P. Fratzl, O. Paris, K. Klaushofer, W.J. Landis. **Bone mineralization in an *osteogenesis imperfecta* mouse model studied by small-angle x-ray scattering.** *J. Clin. Invest*., 97 (1996), pp. 396-402

Gautieri et al., 2009. A. Gautieri, S. Uzel, S. Vesentini, A. Redaelli, M.J. Buehler. **Molecular and mesoscale mechanisms of *osteogenesis imperfecta* disease in collagen fibrils.** *Biophys. J.,* 97 (2009), pp. 857-865

Grabner et al., 2001. B. Grabner, W.J. Landis, P. Roschger, S. Rinnerthaler, H. Peterlik, K. Klaushofer, *et al.* **Age- and genotype-dependence of bone material properties in the *osteogenesis imperfecta* murine model (oim).** *Bone*, 29 (2001), pp. 453-457

Guidoni et al., 2010. G. Guidoni, M. Swain, I. Jager. **Nanoindentation of wet and dry compact bone: influence of environment and indenter tip geometry on the indentation modulus.** *Philos. Mag.,* 90 (2010), pp. 553-565

Gupta et al., 2005. H.S. Gupta, S. Schratter, W. Tesch, P. Roschger, A. Berlanovich, T. Schoeberl, *et al.* **Two different correlations between nanoindentation modulus and mineral content in the bone-cartilage interface.** *J. Struct. Biol.,* 149 (2005), pp. 138-148

Hengsberger et al., 2002. S. Hengsberger, A. Kulik, P. Zysset. **Nanoindentation discriminates the elastic properties of individual human bone lamellae under dry and physiological conditions.** *Bone*, 30 (2002), pp. 178-184

Hirsch and Evans, 1965. C. Hirsch, F.G. Evans. **Studies on some physical properties of infant compact bone.** *Acta Orthop. Scand*., 35 (1965), pp. 300-303

Hoc et al., 2006. T. Hoc, L. Henry, M. Verdier, D. Aubry, L. Sedel, A. Meunier. **Effect of microstructure on the mechanical properties of Haversian cortical bone.** *Bone*, 38 (2006), pp. 466-474

Hodgskinson et al., 1989. R. Hodgskinson, J.D. Currey, G.P. Evans. **Hardness, an indicator of the mechanical competence of cancellous bone.** *J. Orthop. Res.,* 7 (1989), pp. 754-758

Hoffler et al., 2005. C.E. Hoffler, X.E. Guo, P.K. Zysset, S.A. Goldstein. **An application of nanoindentation technique to measure bone tissue lamellae properties.** *J. Biomech. Eng.,* 127 (2005), pp. 1046-1053

Isaksson et al., 2010. H. Isaksson, S. Nagao, M. Malkiewicz, P. Julkunen, R. Nowak, J. Jurvelin. **Precision of nanoindentation protocols for measurement of viscoelasticity in cortical and trabecular bone.** *J. Biomech*., 43 (2010), pp. 2410-2417

Jepsen et al., 1997. K.J. Jepsen, M.B. Schaffler, J.L. Kuhn, R.W. Goulet, J. Bonadio, S.A. Goldstein. **Type I collagen mutation alters the strength and fatigue behavior of Mov13 cortical tissue.** *J. Biomech*., 30 (1997), pp. 1141-1147

Lewis et al., 2006. G. Lewis, J. Xu, N. Dunne, C. Daly, J. Orr. **Critical comparison of two methods for the determination of nanomechanical properties of a material: application to synthetic and natural biomaterials.** *J. Biomed. Mater. Res. B Appl. Biomater*., 78 (2006), pp. 312-317

Li and Bhushan, 2002. X. Li, B. Bhushan. **A review of nanoindentation continuous stiffness measurement technique and its applications.** *Mater. Charact*., 48 (2002), pp. 11-36

Lindahl and Lindgren, 1967. O. Lindahl, G.H. Lindgren. **Cortical bone in man: II. Variation in tensile strength with age and sex.** *Acta Orthop. Scand*., 38 (1967), pp. 141-147

McCarthy et al., 2002. E.A. McCarthy, C.L. Raggio, M.D. Hossack, E.A. Miller, S. Jain, A.L. Boskey, *et al.* **Alendronate treatment for infants with *osteogenesis imperfecta*: demonstration of efficacy in a mouse model.** *Pediatr. Res.,* 52 (2002), pp. 660-670

Miller et al., 2007. E. Miller, D. Delos, T. Baldini, T.M. Wright, N. Pleshko Camacho. **Abnormal mineral–matrix interactions are a significant contributor to fragility in oim/oim bone.** *Calcif. Tissue Int.,* 81 (2007), pp. 206-214

Misof et al., 2005. B.M. Misof, P. Roschger, T. Baldini, C.L. Raggio, V. Zraick, L. Root, *et al.* **Differential effects of alendronate treatment on bone from growing *osteogenesis imperfecta* and wild-type mouse.** *Bone*, 36 (2005), pp. 150-158

Mittra et al., 2006. E. Mittra, S. Akella, Y.X. Qin. **The effects of embedding material, loading rate and magnitude, and penetration depth in nanoindentation of trabecular bone.** *J. Biomed. Mater. Res. A,* 79 (2006), pp. 86-93

Mulder et al., 2007. L. Mulder, J.H. Koolstra, J.M. den Toonder, T.M. van Eijden. **Intratrabecular distribution of tissue stiffness and mineralization in developing trabecular bone.** *Bone*, 41 (2007), pp. 256-265

Rao et al., 2008. S.H. Rao, K.D. Evans, A.M. Oberbauer, R.B. Martin. **Bisphosphonate treatment in the oim mouse model alters bone modeling during growth.** *J. Biomech*., 41 (2008), pp. 3371-3376

Rauch and Glorieux, 2004. F. Rauch, F.H. Glorieux. ***Osteogenesis imperfecta*.** *Lancet*, 363 (2004), pp. 1377-1385

Rauch et al., 2000. F. Rauch, R. Travers, A.M. Parfitt, F.H. Glorieux. **Static and dynamic bone histomorphometry in children with *osteogenesis imperfecta*.** *Bone*, 26 (2000), pp. 581-589

Rho and Pharr, 1999. J.Y. Rho, G.M. Pharr. **Effects of drying on the mechanical properties of bovine femur measured by nanoindentation.** *J. Mater. Sci. Mater. Med*., 10 (1999), pp. 485-488

Rho et al., 2002. J.Y. Rho, P. Zioupos, J.D. Currey, G.M. Pharr. **Microstructural elasticity and regional heterogeneity in human femoral bone of various ages examined by nano-indentation.** *J. Biomech*., 35 (2002), pp. 189-198

Roschger et al., 2008a. P. Roschger, N. Fratzl-Zelman, B.M. Misof, F.H. Glorieux, K. Klaushofer, F. Rauch. **Evidence that abnormal high bone mineralization in growing children with *osteogenesis imperfecta* is not associated with specific collagen mutations.** *Calcif. Tissue Int*., 82 (2008), pp. 263-270

Roschger et al., 2008b. P. Roschger, E.P. Paschalis, P. Fratzl, K. Klaushofer. **Bone mineralization density distribution in health and disease.** *Bone*, 42 (2008), pp. 456-466

Salai et al., 2000. M. Salai, T. Brosh, N. Keller, M. Perelman, I. Dudkiewitz. **The effects of prolonged cryopreservation on the biomechanical properties of bone allografts: a microbiological, histological and mechanical study.** *Cell Tissue Bank*., 1 (2000), pp. 69-73

Sillence et al., 1979. D.O. Sillence, A. Senn, D.M. Danks. **Genetic heterogeneity in *osteogenesis imperfecta*.** *J. Med. Genet*., 16 (1979), pp. 101-116

Smith, 1994. R. Smith. ***Osteogenesis imperfecta*: from phenotype to genotype and back again.** *Int. J. Exp. Pathol*., 75 (1994), pp. 233-241

Smith et al., 2010. L.J. Smith, J.P. Schirer, N.L. Fazzalari. **The role of mineral content in determining the micromechanical properties of discrete trabecular bone remodeling packets.** *J. Biomech*., 43 (2010), pp. 3144-3149

Stoss and Freisinger, 1993. H. Stoss, P. Freisinger. **Collagen fibrils of osteoid in *osteogenesis imperfecta*: morphometrical analysis of the fibril diameter.** *Am. J. Med. Genet*., 45 (1993), p. 257

Tai et al., 2005. K. Tai, H.J. Qi, C. Ortiz. **Effect of mineral content on the nanoindentation properties and nanoscale deformation mechanisms of bovine tibial cortical bone.** *J. Mater. Sci. Mater. Med*., 16 (2005), pp. 947-959

Traub et al., 1994. W. Traub, T. Arad, U. Vetter, S. Weiner. **Ultrastructural studies of bones from patients with *osteogenesis imperfecta*.** *Matrix Biol.,* 14 (1994), pp. 337-345

Turner et al., 1999. C.H. Turner, J. Rho, Y. Takano, T.Y. Tsui, G.M. Pharr. **The elastic properties of trabecular and cortical bone tissues are similar: results from two microscopic measurement techniques.** *J. Biomech*., 32 (1999), pp. 437-441

Vanleene et al., 2012. M. Vanleene, A. Porter, P.V. Guillot, A. Boyde, M. Oyen, S. Shefelbine. **Ultra-structural defects cause low bone matrix stiffness despite high mineralization in *osteogenesis imperfecta* mice.** *Bone*, 50 (2012), pp. 1317-1323

Vetter et al., 1991. U. Vetter, E.D. Eanes, J.B. Kopp, J.D. Termine, P.G. Robey. **Changes in apatite crystal size in bones of patients with *osteogenesis imperfecta*.** *Calcif. Tissue Int*., 49 (1991), pp. 248-250

Wang et al., 2008a. X. Wang, M.R. Allen, D.B. Burr, E.J. Lavernia, B. Jeremic, D.P. Fyhrie. **Identification of material parameters based on Mohr–Coulomb failure criterion for bisphosphonate treated canine vertebral cancellous bone.** *Bone*, 43 (2008), pp. 775-780

Wang et al., 2008b. X. Wang, D. Sudhaker Rao, L. Ajdelsztajn, T.E. Ciarelli, E.J. Lavernia, D.P. Fyhrie. **Human iliac crest cancellous bone elastic modulus and hardness differ with bone formation rate per bone surface but not by existence of prevalent vertebral fracture.** *J. Biomed. Mater. Res. B Appl. Biomater.,* 85 (2008), pp. 68-77

Weber et al., 2006. M. Weber, P. Roschger, N. Fratzl-Zelman, T. Schoberl, F. Rauch, F.H. Glorieux, *et al.* **Pamidronate does not adversely affect bone intrinsic material properties in children with *osteogenesis imperfecta*.** *Bone*, 39 (2006), pp. 616-622

Willing et al., 1992. M.C. Willing, C.J. Pruchno, M. Atkinson, P.H. Byers. ***Osteogenesis imperfecta* type I is commonly due to a COL1A1 null allele of type I collagen.** *Am. J. Hum. Genet.,* 51 (1992), pp. 508-515

Willing et al., 1996. M.C. Willing, S.P. Deschenes, R.L. Slayton, E.J. Roberts. **Premature chain termination is a unifying mechanism for COL1A1 null alleles in *osteogenesis imperfecta* type I cell strains.** *Am. J. Hum. Genet.,* 59 (1996), pp. 799-809