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Reduced diaphyseal strength associated with high intracortical vascular porosity within long bones of children with osteogenesis imperfecta

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ABSTRACT

Osteogenesis imperfecta is a genetic disorder resulting in bone fragility. The mechanisms behind this fragility are not well understood. In addition to characteristic bone mass deficiencies, research suggests that bone material properties are compromised in individuals with this disorder. However, little data exists regarding bone properties beyond the microstructural scale in individuals with this disorder.

Specimens were obtained from long bone diaphyses of nine children with osteogenesis imperfecta during routine osteotomy procedures. Small rectangular beams, oriented longitudinally and transversely to the diaphyseal axis, were machined from these specimens and elastic modulus, yield strength, and maximum strength were measured in three-point bending. Intracortical vascular porosity, bone volume fraction, osteocyte lacuna density, and volumetric tissue mineral density were determined by synchrotron micro-computed tomography, and relationships among these mechanical properties and structural parameters were explored.

Modulus and strength were on average 64–68% lower in the transverse vs. longitudinal beams ($P < 0.001$, linear mixed model). Vascular porosity ranged between 3 and 42% of total bone volume. Longitudinal properties were associated negatively with porosity ($P \leq 0.006$, linear regressions). Mechanical properties, however, were not associated with osteocyte lacuna density or volumetric tissue mineral density ($P \geq 0.167$). Bone properties and structural parameters were not associated significantly with donor age ($P \geq 0.225$, linear mixed models).

This study presents novel data regarding bone material strength in children with osteogenesis imperfecta. Results confirm that these properties are anisotropic. Elevated vascular porosity was observed in most specimens, and this parameter was associated with reduced bone material strength. These results offer insight toward understanding bone fragility and the role of intracortical porosity on the strength of bone tissue in children with osteogenesis imperfecta.

1. Introduction

Osteogenesis imperfecta (OI), also known as brittle bone disease, is a genetic disorder related to type I collagen and resulting in a high susceptibility to bone fractures [1]. The true prevalence of OI is unknown; however, it has been estimated to affect between 1:30,000 and 1:5000 births [1–4]. There is no cure for OI. Severity varies widely, from mild to lethal in the perinatal period, and several genetic mutations have been associated with various types of OI [5–8]. The mechanisms responsible for bone fragility in OI, however, remain poorly understood, and better understanding of these mechanisms is of high value toward identifying new treatment and rehabilitative strategies for individuals with this disorder.

Bone fragility in OI likely stems in part from a characteristic low bone mass. Individuals with OI tend to have very low areal bone mineral density (aBMD), which can be the result of decreased bone size and/or decreased volumetric bone mineral density (vBMD) [9,10]. In a backscattered electron imaging study of iliac crest biopsies, cortical and trabecular bone were described as "markedly sparse" in children with severe and moderately severe forms of OI, and a "dearth of bone" was noted in some children with mild OI [11]. Decreased trabecular and cortical thickness, as well as reduced bone volume fraction were also observed in histomorphometric studies of iliac crest biopsies from children with OI [12,13].

In addition to bone mass deficiencies, several abnormalities in bone tissue composition have been described, and these irregularities may also contribute to the characteristic bone fragility. The causative genetic

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defects in OI are related to type I collagen, the main organic component of bone. A mild form of the disorder, OI type I, has been attributed to an insufficient production of type I collagen [14–17]. More severe forms, OI types III and IV, have been linked to amino substitution defects within the collagen molecules [6,17,18], while recently identified recessive forms of the disorder have been associated with deficiencies in other proteins that interact with collagen [5,19]. Irregularities in collagen fibril diameters have also been observed in the OI population [20–22]. Within the inorganic matrix, alterations in the size, shape, and composition of the bone mineral crystals [20,23,24], and increased matrix mineralization density have been noted in children with OI [13,25,26]. Murine models have provided support to the hypothesis that the brittleness of bone in OI can be attributed in part to compromised material properties of the bone tissue. For example, in mouse models of mild and severe forms of OI (*mov13* and *oim* models, respectively) bone material strength was 11–43% lower than that of control mice [27–29]. It has not been confirmed, however, whether these observations are also true in humans, as little data is yet available to describe bone material strength in individuals with this condition [30].

A few previous studies have used nanoindentation to measure elastic modulus, a property denoting material-level stiffness, for pediatric OI bone [25,31–34]. Within that microstructural scale, the elastic modulus of bone tissue was found to be higher in children with severe or moderately severe OI (types III and IV) vs. age-matched controls [25], and this property was slightly higher in children with mild (type I) vs. severe (type III) OI [31]. Interestingly, contrary to observations in normal bone tissue [35–37], no significant difference in modulus was observed between indents taken parallel vs. perpendicular to the long bone axis [32], which led to the speculation that OI bone may exhibit more isotropic properties than does typical bone. These results confirm that bone material properties are affected in OI; however, important limitations with these studies should be acknowledged. First, the small size of the indents excluded the effects of pores such as vascular spaces, which can largely influence the "effective" material properties at the mesoscale. Therefore, due to the complex hierarchical structure of bone, it is not clear whether these observations made within the microstructural scale hold true in OI bone at larger scales. Finally, these nanoindentation studies do not provide any information regarding bone material strength, an important property in determining fracture risk.

In a recent pilot study by our group, two osteotomy specimens from long bone diaphyses of children with OI were tested in bending [30], and their bone material strength was lower than values reported for typical pediatric bone [38]. Visual inspection of these bone specimens revealed the appearance of considerable porosity within a tissue region that is typically occupied by dense cortical bone: throughout the mid-diaphyseal cortex, near to the periosteal surface. Based upon this observation, it was speculated that the decreased strength observed in those bone specimens could be the result of abnormally high intracortical bone porosity. Further investigation by high-resolution computed tomography confirmed the presence of unusually high vascular porosity, on average 21%, within long bone diaphyseal cortex of children with OI, when compared with control specimens from children with no known musculoskeletal disease, for which the average porosity was 3% [39]. A similar finding was since then reported by another group in a scanning electron microscopy study of mid-diaphyseal osteotomy specimens from children with OI type III, which described the presence of "flattened and elongated lacunar spaces" within regions of bone "normally taken up by compact cortex" [40]. The current study builds upon our group's recent work, and offers an in-depth analysis of relationships between cortical tissue architecture, including vascular porosity, and the mechanical properties of diaphyseal bone in children with OI.

The specific objectives of this study were as follows: (1) to measure the flexural properties, i.e., elastic modulus, flexural yield strength, and maximum flexural strength, of cortical bone tissue from the long bone diaphyses of children with OI; (2) to assess anisotropy within OI bone tissue by comparing the material properties of specimens oriented transversely vs. longitudinally to the long bone axis; and (3) to explore relationships between the material properties and intracortical vascular porosity in this population. Relationships between the mechanical properties and other microstructural parameters, namely osteocyte lacuna density and volumetric tissue mineral density, were also explored.

2. Materials and methods

2.1. Bone specimens

Twelve cortical bone specimens were collected from long bone diaphyses of nine children with OI (Tables 1 and 2). These specimens, varying in shapes and sizes, were obtained during routine corrective orthopaedic surgeries (Table 2) at Shriners Hospitals–Chicago, with informed consent/assent from the donors and under an IRB-approved protocol (Rush University Medical Center #10101309, Marquette University #HR-2167).

2.2. Specimen preparation

The bone specimens were machined into a total of 59 rectangular beams with a low speed diamond saw (IsoMet Low Speed Saw, Buehler, Lake Bluff, IL) and a 0.3 mm-thick wafering blade (Series 15HC Diamond, Buehler, Lake Bluff, IL), using methods described and validated in earlier work [30]. Beam dimensions of approximately 5–6 mm in length, 0.7 mm in depth, and 1 mm in width were chosen based on the dimensions of the smallest of these donated osteotomy specimens. Each specimen was kept fresh-frozen at −85 °C prior to machining and testing. Machining of the beams was achieved with the following steps. Under constant irrigation, each specimen was first machined into a slice having a thickness equal to the desired beam depth. These specimens were small and varied in size and shape; however, care was taken to obtain the beams from a relatively consistent location. The beams were cut from a slice of bone that was obtained as close as possible to the periosteal surface. This was achieved by gluing the periosteal surface onto a wood mandrel and making two consecutive cuts at distances of 1.2 and 0.2 mm, respectively, from the referenced periosteum–mandrel interface. These cut distances were selected taking into account the thickness of the wafering blade. The slice was subsequently cut into beams of the desired width. While cutting each slice into beams, the slice was gripped onto a ¼-inch thick acrylic backing to prevent undue bending deformation of the slice. Each beam was machined such that its long axis was oriented either longitudinally (i.e., proximal–distal orientation, 40 beams) or transversely (i.e., circumferential direction, 19 beams) relative to the long bone axis of the donated specimen (Table 2). Beam depth and width were measured with a digital micrometer (Model 293–340, Mitutoyo Corporation, Japan), and the average depth and width were 668 μm (standard deviation 60 μm) and 1005 μm (46 μm), respectively.

2.3. Flexural testing

Each beam was loaded to failure using a three-point bending test assembly designed for the specific purpose of characterizing small bone specimens [30]. The loading nose and supports consisted of 1/16-in. (1.59 mm) diameter stainless steel dowel pins, which were fixed into grooves machined in an upper and a lower aluminum platen using cyanoacrylate. A span length (L) of 4 mm (actual measurement 3.973 mm) was chosen to accommodate the size of the osteotomy specimens that were collected for this study. The bending jig was assembled onto an electromechanical testing system (Model 3345, Instron®, Norwood, MA, USA) with a 50 N capacity load cell (Model 2519–102, Instron®, Norwood, MA, USA). An external linear variable differential transformer, LVDT (Model 2601, Instron®, Norwood, MA, USA), was used to determine beam deflection at the mid-span.