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VALIDATION OF A MICRO-CT APPROACH FOR CHARACTERIZATION OF MURINE AND HUMAN BONE IN OSTEOGENESIS IMPERFECTA

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INTRODUCTION

Osteogenesis imperfecta (OI) is a rare genetic, musculoskeletal disease characterized by mutations in type I collagen, ultimately leading to abnormal collagen synthesis and assembly [1]. Although it is highly heterogeneous, common symptoms include frequent bone fractures, decreased stature, and progressive spinal and limb deformities. Currently patients are classified into eight clinical groups, where Type III is the most severe form compatible with life. To date, there is little biomechanical data of OI bone. Because of the limited availability and small size of human OI specimens, most research has focused on animal models. One commonly studied murine model of Type III OI is the homozygous *oim* B6C3Fe *a/a-Coll1a2^{oim}/J* strain (*oim/oim*). The goal of this study was to validate a system for the evaluation of *oim/oim* and human OI bone samples using micro-computed tomography (μ CT).

METHODS

Animals. All animals were obtained from Jackson Laboratory (Bar Harbor, ME) and studies were performed under approval of an Institutional Animal Care and Use Committee (IACUC) protocol. Five *oim/oim* and five wild type (+/+) femora were harvested from male mice and fresh frozen at -70°C until μ CT evaluation. Average ages for the *oim/oim* and +/+ strains at tissue dissection were 9 and 10 weeks, respectively.

Imaging. All scans were performed using a μ CT system developed at the Zablocki VA Medical Center (Milwaukee, WI) [2]. The system is composed of a 100.50 X-ray source (3-mm focal

spot; Comet North America), an AI-5830-HP image intensifier (North American Imaging) coupled to a Silicon Mountain Design SMD1M-15 CCD camera (DALSA), and a specimen micromanipulator stage, all mounted on a precision rail. Femora were thawed, placed in 1.5-mL Eppendorf tubes filled with saline, mounted on the specimen stage, and scanned in continuous mode (33 kVp, 242 μA , 7-frame average) at two magnification levels. Reconstructions were performed using a standard Feldkamp algorithm, with resulting voxel sizes of 17 and 34 μm , corresponding to high and low magnification, respectively.

Geometrical analysis. Cortical and trabecular regions of interest (ROIs) were isolated and analyzed for several structural morphometric parameters using ImageJ (v1.42; NIH) and MicroView (v2.1.2; GE Healthcare). Femoral length was measured using MicroView's line tool. Longitudinal cortical regions were selected from the low magnification scans, where each ROI extended distally 2.5 mm from the femoral midpoint. These regions were analyzed for cross-sectional area (CSA) and cortical thickness (Ct.Th). Cylindrical trabecular regions were located in the high magnification scans just proximal to the distal femoral growth plate, extending proximally 1.5 mm with a diameter of 1 mm. After applying a local threshold (determined using MicroView's auto-threshold option), trabecular ROIs were evaluated for bone volume fraction (BV/TV) and trabecular number (Tb.N), thickness (Tb.Th), and spacing (Tb.Sp).

Statistical analysis. Data from each genotype was pooled and compared for statistical significance using an unpaired student's t-test.

RESULTS AND DISCUSSION

Structural data. Femoral length was not significantly different between the two groups. However, *oim/oim* cortical and trabecular indices were generally inferior to controls (Table 1) and agreed well with OI mouse literature [3,4]. *Oim/oim* cortical geometry was more flattened and ellipsoidal in appearance (Figure 1B). Mid-shaft *oim/oim* cortices showed a 16% reduction in CSA, suggesting decreased resistance to bending loads, as found in a prior study [3]. *Oim/oim* trabecular regions appeared more open in structure (Figure 1B). This was confirmed by our findings of significantly reduced BV/TV and Tb.N, with a corresponding increase in Tb.Sp (Table 1). These results agree with prior findings that OI mice have decreased trabecular bony tissue that is also of reduced mechanical viability [3,4].

Extension to human OI bone. After establishing agreement with murine literature, we applied our methods to a human OI bone specimen. Pediatric OI patients suffer frequent fractures that require routine surgical intervention. Small bone fragments are often removed during these procedures. One such specimen was collected (under written consent and IRB approval) and fresh frozen until μ CT imaging was conducted. The bone was scanned at 24- μ m voxel resolution and analyzed using similar methods to the animal study. The wedge-shaped sample was composed almost exclusively of trabecular bone (Figure 2); thus, only applicable parameters were calculated. Surprisingly, the specimen showed increased BV/TV and Tb.N, but decreased Tb.Sp (Table 1) compared to healthy femoral head tissue [5]. One possible explanation is that this patient was treated with bisphosphonates, which have been shown to have similar effects in murine studies [3,4]. To our knowledge, this is the first application of μ CT to determine morphometric parameters in human OI bone.

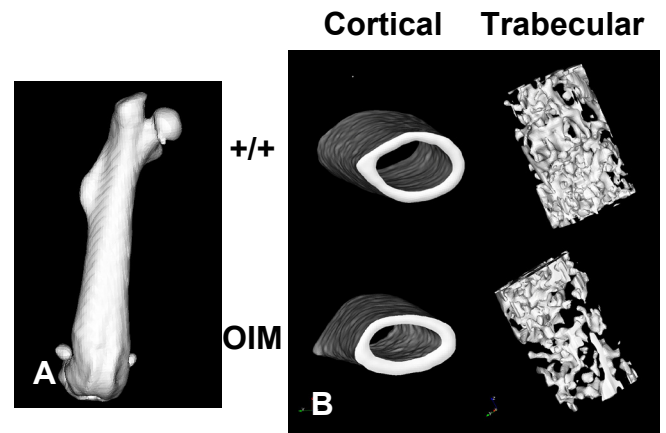


Figure 1: Representative surface-shaded renderings of mouse femora from μ CT data. (A) Whole femur. (B) Cortical and trabecular ROIs.

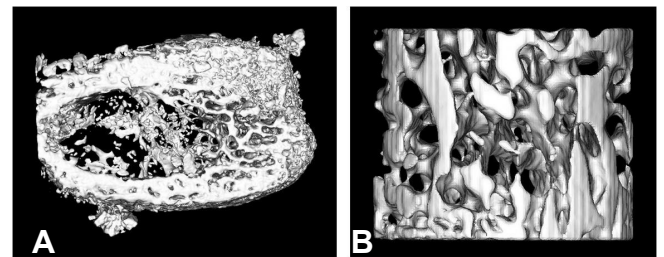


Figure 2: Human OI bone sample. (A) Wedge-shaped specimen with dimensions 10.5 x 6.25 x 5.56 mm. (B) Cylindrical trabecular ROI with 3-mm diameter and 2.5-mm height.

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Table 1: Summary of calculated bone parameters.

Genotype	Morphometric Parameters					
	CSA (mm ²)	Ct.Th (mm)	BV/TV	Tb.N (mm ⁻¹)	Tb.Th (mm)	Tb.Sp (mm)
Control (+/+)	0.87 ± 0.15	0.20 ± 0.03	0.25 ± 0.05	6.89 ± 1.71	0.036 ± 0.006	0.12 ± 0.04
Oim/oim	0.73 ± 0.10*	0.19 ± 0.01	0.14 ± 0.03[†]	3.67 ± 1.03[†]	0.038 ± 0.005	0.28 ± 0.09[†]
Healthy Human [5]	-	-	0.26 ± 0.08	1.60 ± 0.29	0.194 ± 0.033	0.64 ± 0.11
Human OI	-	-	0.45	2.90	0.154	0.19

* $p < 0.06$ between +/+ and *oim/oim*.

[†] $p < 0.05$ between +/+ and *oim/oim*.