INTRODUCTION

There is currently no cure for the skeletal dysplasia osteogenesis imperfecta (OI), a debilitating disease that occurs in approximately 1 in every 20,000 human births. OI is caused by a genetic mutation that results in brittle bones and many fractures.

In a mouse model of the disease, oim, we have characterized bone structure using X-ray microtomography, and found that oim bone has decreased bone volume, cortical area, cortical thickness, and moment of area compared to wild type (WT) bone [1]. In three-point bending tests, oim bone exhibited classic brittle behavior with fracture occurring just after the yield point of the tissue. In three-point bending, oim bones fractured obliquely at the distal end of the diaphysis, whereas WT bones fractured transversely under the midspan loading nose.

To date, the role of porosity in determining OI bone fracture toughness is unknown. Because material structure at all hierarchical levels is critical in fracture mechanics, differences between oim bone and WT bone ultrastructure may explain the dramatic increase in fracture rates. The aim of this study was to quantitatively investigate the cortical tissue porosity in C57BL/6 (B6) WT mice and B6C3Fe-α/-α-coll1a2<sup>−/−</sup> brittle bone oim mice using synchrotron radiation-based computed tomography (SR CT).

METHODS

Three tibiae and four humeri from B6 WT mice and three tibiae and three humeri from oim mice, all 8 weeks-old, were dissected free after sacrifice, aligned and embedded at both ends in low viscosity bone cement (PMMA; Cemex®). During preparation and before imaging, the samples were wrapped in gauze moistened with physiologic solution (PBS) and stored at -20°C.

Bones were oriented vertically and immersed in PBS during scanning in the beamline for tomographic microscopy and coherent radiology (TOMCAT) of the Swiss Light Source. High-resolution 3D representations of the posterior tibial and the lateral humeral mid-diaphysis of the bones were acquired for both mouse strains using SR CT at nominal resolution of 700 nm. For each 3D data set, a total of 1001 projections were acquired over a range of 180 degrees and at a photon energy of 17.5 keV. The data were reconstructed using filtered back-projection. Histogram-based global thresholding (isodata algorithm) was applied to segment bone matrix from air and soft tissue [2].

The Analyse Particles routine in BoneJ was used to segment individual osteocyte lacunae and cortical canals by sequential region labelling and perform subsequent quantitative morphometric analysis [3]. Cortical bone volume (CLTV) was calculated on the whole bone level, and the canal network was characterised on the tissue level by measures such as number of canals (N.Ca), canal number density (N.Ca/Ct.TV), canal volume (Ca.V), canal volume density (Ca.V/Ct.TV), mean canal volume (Ca.V/N.Ca), mean canal thickness (Ca.Th).

On the cellular level, osteocyte lacunae were quantified. We derived number of lacunae (N.Lc), lacuna number density (N.Lc/Ct.TV), lacuna volume (Lc.V), lacuna volume density (Lc.V/Ct.TV) and mean lacuna volume (Lc.V/N.Lc).

Morphometric measures in oim and B6 WT bone were compared using the Student's independent t-test for variables with normal distributions and the Mann-Whitney rank test for variables with non-normal distributions. P-values smaller than 0.05 were considered to be significant.

RESULTS

Morphometric evaluation (Table 1) of the canal network in the B6 WT and oim groups indicated a statistically significant increase in the number of canals within the oim bones (p<0.005). Results also showed comparable canal volume density between B6 WT and oim bones, suggesting canals are more numerous but smaller in oim bone (Figure 1; differences in canal volume and thickness were not statistically significant).

Morphometric analysis at the cellular level showed a statistically significant increase in lacuna number and volume density in oim bone (p<0.0001), while mean lacuna volume was similar for both mouse strains.

DISCUSSION

We collected and analyzed synchrotron CT data that indicate greater porosity in oim bone than in B6 WT, which is in agreement with the high bone turnover quantified in oim bone [4]. We believe that the greater number of lacunae and canals may contribute to differences in fracture mechanics between ductile WT bone and brittle oim bone. Previous studies have found that cracks originate at canals and propagate through osteocyte lacunae [5, 6]. Because fracture in brittle bone occurs with little load and under small strain, high rates of fracture are common in OI. Understanding how the fractures in brittle bone propagate and how this process differs in normal bone could inform treatments to strengthen the bone in order to avoid fracture initiation and propagation.

Though our sample size was limited, we have detected significant changes in porosity in oim bone, warranting a more thorough investigation of porosity in oim bone and the relationship to fracture properties. Previous studies have demonstrated that morphological changes are dependent on mouse strain, gender, anatomical site (i.e. posterior vs. anterior, superior vs. inferior) and bone (i.e. tibia vs. humerus) [7]. Larger samples sizes will allow us to investigate these differences in oim bone in more detail. We used the standard B6 mouse as WT, however a more appropriate control in future studies will be B6C3Fe-α/-α-<sup>−/−</sup>. The small statistical significance found for the canal morphometric indices is probably due to the high variance in the dataset, especially from B6 WT bone.

In conclusion, we have demonstrated how osteocyte lacunae and canal networks contribute to 3D cortical porosity in oim bone, which may have implications in the mechanical integrity of the bone. These findings might help to better understand human OI bone disease, where bone fracture is the major concern.

REFERENCES

1. Vanleeuwe, M., et al 55th ORS 2009, Las Vegas, USA

Figure 1. SR CT slide of B6 WT (left) and oim (right) tibial cortical bone.

<table>
<thead>
<tr>
<th>Morphometric index</th>
<th>B6 WT</th>
<th>oim</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>N.Ca/Ct.TV (mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>370 ± 85</td>
<td>778 ± 467</td>
<td>0.005</td>
</tr>
<tr>
<td>Ca.V/Ct.TV (%)</td>
<td>3.4 ± 4.4</td>
<td>3.6 ± 2.4</td>
<td>n.s</td>
</tr>
<tr>
<td>Ca.V/N.Ca (10&lt;sup&gt;³&lt;/sup&gt;m³)</td>
<td>105 ± 168</td>
<td>48 ± 32</td>
<td>n.s</td>
</tr>
<tr>
<td>Ca.Th (µm)</td>
<td>7.6 ± 1.4</td>
<td>7.4 ± 0.6</td>
<td>n.s</td>
</tr>
<tr>
<td>N.Lc/Ct.TV (mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>72981 ± 9033</td>
<td>127472 ± 13668</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lc.V/Ct.TV (%)</td>
<td>2.73 ± 0.29</td>
<td>4.23 ± 0.24</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lc.V/N.Lc (µm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>376 ± 35</td>
<td>350 ± 59</td>
<td>n.s</td>
</tr>
</tbody>
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Table 1. Morphometric indices characterizing cortical porosity (mean ± standard deviation) of B6 WT and oim mice.