Fragility of Vertebral Trabecular Bone under Various Loading Orientations in Ovariectomized (OVX) Rats*

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Abstract
We developed a new estimation system using rapid prototyping technology that focuses on the relationship between the architecture and mechanical properties of trabecular bone. The system uses three-dimensional acrylic resin models of trabecular bone constructed from micro-CT data to predict the mechanical properties of trabecular bone. We used this method to clarify the relationship between loading orientation and bone fragility in the vertebral trabecular bone of ovariectomized (OVX) rats. Twenty 6-week-old female Wistar rats were randomly divided into two groups (OVX and normal groups). Five rats from each group were killed at 3 and 6 weeks following operations and the L4 vertebra was removed. Bone specimens from both groups were scanned using a micro-CT system. Subsequently, resin models of the bone were fabricated at 60× magnification from the micro-CT data sets using laser stereolithography. The resin models were subjected to compressive testing in three orthogonal orientations corresponding to the craniocaudal, mediolateral, and anteroposterior anatomic axes. The results showed that the elastic modulus and ultimate stress were lower in the models of OVX rats than in the normal rats, the mechanical properties of trabecular bone structure from OVX rats deteriorated with increasing time postoperatively, and the elastic moduli in the mediolateral and anteroposterior axes were especially reduced relative to the decrease in the craniocaudal axis in the OVX rats.

Key words: Bone, Osteoporosis, Rat, Mechanical Property, Rapid Prototyping Technology

1. Introduction
Osteoporosis is a progressive bone disease that increases the risk of fracture (1). Currently, medication is widely used to treat patients with osteoporosis and its efficacy is evaluated clinically with the bone mineral density (BMD) (2) using dual X-ray absorptiometry (DXA) (1)(3). Bone strength depends on the integration of bone density and bone quality, and osteoporosis is characterized not only by a decrease in bone density, but also by deterioration in trabecular bone quality, including the architecture, damage, and mineralization. Research on trabecular bone has advanced exponentially with remarkable developments in measurement apparatus, such as high-resolution micro-computed
tomography (CT), micro-magnetic resonance imaging, nano-indentation systems, three-dimensional (3-D) morphometric analysis, and finite element model analysis. Many studies have examined how the microstructure contributes to mechanical strength by deriving the microstructural parameters of trabecular bone (4), such as the connectivity density, volume fraction, and other 3-D bone morphometric parameters, and have clarified the relationships between those parameters and the mechanical properties of trabecular bone (5)-(9). Recently, several studies have reported new methods of analyzing trabecular bone (10)-(13), but it is extremely difficult to quantify the risk of bone fracture due to the various complex factors involved in the biomechanics of bone.

Therefore, we developed a new estimation system using rapid prototyping technology that focuses on the effects of trabecular architecture on the mechanical properties of trabecular bone (14). This system uses 3-D acrylic resin models of trabecular bone constructed from micro-CT data sets and allows prediction of the strength of trabecular bone in vivo. In addition, multiple mechanical tests can be carried out under various loading conditions using the same model because rapid prototyping technology allows us to produce several high-precision copies of the same model (Fig. 1). Previous studies have shown this method is a valid technique for predicting the mechanical properties of trabecular bone structure (14)-(16).

In this study, we used our rapid prototyping technology estimation system to elucidate the relationships among the compressive load orientation (craniocaudal, mediolateral, and anteroposterior anatomic axes), osteoporotic changes in the trabecular architecture of OVX rats, and the mechanical properties of trabecular bone structure. These data provide a basis with which to evaluate new products for treating osteoporosis.

![Fig. 1 Illustration of the bone estimation system. Trabecular architecture is scanned using micro-CT. A three-dimensional image of the bone specimen is reconstructed, and the image is converted into CAD data (STL data). The resin model is fabricated from the trabecular bone image using a laser stereolithography apparatus. Mechanical testing is performed on resin model specimen.](image-url)
2. Materials and Methods

2.1 Animal specimen preparation

Twenty, 6-week-old, female Wistar rats were divided randomly into two groups. Rats in the control group were subjected to a sham operation (control group), while those in the second group underwent a bilateral ovariectomy (OVX group). Five rats from each group were killed at 3 and 6 weeks to extract their L4 vertebrae. The average value, standard deviation, and range of the weight at pre-operation, 3 and 6 weeks in the control and OVX rats are shown in Table 1. The bones were stored at –20°C until scanning. All animal experiments were conducted according to the Guidelines for Animal Experimentation of Niigata University of Medical and Dental Sciences.

<table>
<thead>
<tr>
<th>Weight (g)</th>
<th>Control (n=10)</th>
<th>OVX (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Pre-operation</td>
<td>272.9</td>
<td>11.9</td>
</tr>
<tr>
<td>3 week</td>
<td>309</td>
<td>11.7</td>
</tr>
<tr>
<td>6 week</td>
<td>331.9</td>
<td>11</td>
</tr>
</tbody>
</table>

2.2 Digital imaging

After preparing the specimens, to obtain 3-D data sets of the trabecular bone structure, the bone specimens were scanned using a micro-CT system (SMX-130CT; Shimadzu, Japan) with a spatial resolution of 20 µm, a 30 µm slice thickness, 50 kV tube voltage, 36 µA tube current, and a 512×512 image matrix. Subsequently, a 3-D image of the bone specimens was reconstructed with a 20-µm voxel size and converted into computer-assisted design (CAD) data in an STL file using image reconstruction software (Mimics 7.0; Materialise, Chiba, Japan). Cylinder images, 0.83 mm in diameter and 0.83 mm high, were then extracted from the STL data for one vertebra along the cranio-caudal (z-axis), mediolateral (x-axis), and anteroposterior (y-axis) anatomical axes (Fig. 2).

To assess the morphometric indices, morphological analyses of the binarized volume of interest (VOI) were performed directly using Pafitt’s definitions according to the plate model \(^5\), \(^17\). The morphometric indices were the bone volume fraction (BVF,%), which represents the percentage of trabecular bone volume (BV) relative to the total tissue volume (TV) in the VOI, trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp).

![Fig. 2](image)

Fig. 2  Bone model specimen preparation protocol. Cylinder images (φ0.83 mm×0.83 mm) were extracted from the one vertebral STL data along anatomical axes in the cranio-caudal (z-axis), mediolateral (x-axis), and anteroposterior (y-axis) axes. Bone model specimens (φ50 mm×50 mm) were fabricated at 60 × magnification from the cylinder images.
2.3 Bone model preparation

Rapid prototyping technology was applied to make bone models using the vertebral STL data and acrylic resin. This technology allows us to construct a high-precision, 3-D acrylic resin model directly from the STL data. Table 2 gives the mechanical properties of the acrylic resin. All of the resin specimens were fabricated at 60× magnification from the cylindrical images using laser stereolithography (Solidjet2000; Denken, Japan).

2.4 Mechanical testing

The resin models were subjected to destructive compression testing using a material-testing machine (Autograph-10kN; Shimadzu, Japan) to impart compression loads at a constant strain rate of 0.001 s⁻¹. The elastic modulus was calculated for each model from the respective stress–strain curves. The ultimate stress was defined based on the point of maximum stress and the apparent sectional area in that region of the model. During testing, we recorded the fracture behavior of the resin model specimens using a video camera.

2.5 Statistics

Differences in the elastic modulus and ultimate stress between the control and OVX groups were assessed using an unpaired t-test using STATCEL version 2 (Add-in on MS Excel; Japan). The results were considered statistically significant at p<0.05.

3. Results

The average value, standard deviation, and range of the 3-D morphometric indices at 3 and 6 weeks are summarized in Table 3.

Table 2. Mechanical properties of acrylic resin.

<table>
<thead>
<tr>
<th></th>
<th>Elastic modulus (bending), MPa</th>
<th>3100</th>
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<tr>
<td></td>
<td>Strength (bending), MPa</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>Shore hardness (D)</td>
<td>75</td>
</tr>
</tbody>
</table>

Table 3. Bone morphological characteristics of control and OVX group.

<table>
<thead>
<tr>
<th></th>
<th>(3 week)</th>
<th>(6 week)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n=5)</td>
<td>OVX (n=5)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>BVF, %</td>
<td>55.9</td>
<td>3.3</td>
</tr>
<tr>
<td>Tb.N, 1/mm</td>
<td>2.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Tb.Th, µm</td>
<td>112.3</td>
<td>22.1</td>
</tr>
<tr>
<td>Tb.Sp, µm</td>
<td>110.3</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>54.6</td>
<td>4</td>
</tr>
<tr>
<td>Tb.N, 1/mm</td>
<td>2.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Tb.Th, µm</td>
<td>110</td>
<td>15.6</td>
</tr>
<tr>
<td>Tb.Sp, µm</td>
<td>113.3</td>
<td>9.2</td>
</tr>
</tbody>
</table>

Figure 3 shows typical stress–strain curves in the craniocaudal (z-axis) orientation for control and OVX rats at 6 weeks. The mechanical elastic modulus and ultimate stress in the three orthogonal orientations (x, y, and z) at 3 weeks are shown for both groups in Figures 4 and 5, respectively. As expected, the elastic modulus and ultimate stress in models of the OVX rats were lower than in those of the control rats. The decrease in the elastic modulus and ultimate stress of the OVX rats relative to the control animals was calculated as follows:

\[
\text{Decrease, } \% = \frac{\text{OVX}}{\text{Control}} \times 100.
\]  

The decrease in the mechanical properties of the OVX rat models compared to the controls at 3 and 6 weeks are shown in Fig. 6. Comparing the models of the OVX and control rats, the elastic modulus decreased 58.8, 77.2, and 73.9\% in the x-, y-, and z-axes, respectively, and the ultimate stress decreased 62.9, 74.3, and 80.1\% at 3 weeks. At 6 weeks, the elastic modulus in models of OVX rats was 22.5, 23.8, and 51.2\% of the control models in the x-, y-, and z-axes, respectively, and the ultimate stress decreased 49.8, 43.3, and 70.2\%. The mechanical properties of trabecular bone structure from OVX rats deteriorated with increasing time postoperatively. The elastic modulus was reduced especially in the mediolateral and anteroposterior axes relative to the decrease in the craniocaudal axis.

4. Discussion

In general, the mechanical properties of trabecular bone depend on both bone mass and bone quality. Several aspects of bone quality, which range from the macroscopic to the microscopic, should be considered. Microscopically, bone tissue properties such as mineralized matrix and microdamage are important parameters of bone quality. The accumulation of microdamage in trabecular bone leads to bone fragility\(^{(18)}\). Follet reported that a greater mineralization of the cancellous tissue led to its higher stiffness and compressive strength\(^{(19)}\). Conversely, Edward showed that osteoporosis in rat manifests as a loss in bone mass, whereas the elastic and hardness properties of the surviving bone tissue remain relatively unchanged\(^{(20)}\). The macroscopic architecture is also an important parameter of bone quality. Therefore, various studies have investigated the relationship between bone architecture and its mechanical properties\(^{(5)-(9)}\), and several new techniques for estimating the mechanical properties of trabecular bone have been reported\(^{(10)-(13)}\).
The elastic modulus in the three orthogonal orientations (x, y and z) at 3 weeks in both control and OVX groups. Decrease in the elastic modulus of the OVX rats relative to the control rats in x, y and z were 58.8, 77.2 and 73.9%, respectively.

The ultimate stress in the three orthogonal orientations (x, y and z) at 3 weeks in both control and OVX groups. Decrease in the ultimate stress of the OVX rats relative to the control rats in x, y and z were 62.9, 74.3 and 80.1%, respectively.

The decrease in mechanical properties of the OVX rat models compared to the controls at 3 and 6 weeks. x-, y- and z-axis are mediolateral, anteroposterior and craniocaudal orientations, respectively.
In many analyses, the mechanical properties of trabecular bone are tested using human or animal bones. The mechanical properties of trabecular bone are intricately influenced by various parameters of bone quality. Therefore, it is difficult to evaluate the contribution of each of the aforementioned parameters of bone quality to the mechanical properties of trabecular bone separately. In this study, we could ignore the parameters related to bone mass and tissue properties and focus on the contribution of architecture to the mechanical properties of trabecular bone.

The temporal changes in the mechanical properties of trabecular bone in OVX rats were clarified in a 3-D analysis of trabecular architecture using rapid prototyping technology. The mechanical properties of trabecular bone structure strongly depended on the loading orientation in both the control and OVX rats. Our results were very similar to those of studies examining the relationship between the structural and orthogonal compressive properties of trabecular bone using the mechanical testing of human trabecular bone (5)–(7). The mechanical properties of trabecular bone structure from OVX rats deteriorated with increasing time postoperatively. Moreover, in the OVX rats, the elastic modulus was especially reduced in the mediolateral and anteroposterior axes relative to the decrease in the craniocaudal axis. We postulate that the marked decrease in the elastic modulus in OVX rats with time is one of the causes of bone fragility. In our experiments with models of OVX rat specimens, we observed a general tendency toward increased bone fragility with time. Our system was sufficient for evaluating the mechanical properties of trabecular bone structure, employing models of a minuscule region of vertebral bone fabricated from 3-D images using acrylic resin.

Several studies have examined how microstructure contributes to mechanical strength, and in various studies, the mechanical testing was performed under a uniaxial load only. The mechanical properties of trabecular bone vary with the loading orientation because trabecular bone is mechanically anisotropic. Therefore, it is best to estimate the mechanical strength under various loading conditions. Our method, which uses 3-D resin models of trabecular bone constructed from micro-CT data sets, allows the prediction of the strength of trabecular bone under various loading conditions.

Animal experiments are required for studying the effects of medicine on the mechanical properties of osteoporotic bone. Recently, Waarsing et al. developed an in vivo micro-CT system (21) that allows laboratories to perform longitudinal studies. Others have reported the temporal changes in architecture in a rat model of osteoporosis using the in vivo micro-CT system (22). A unique advantage of our approach is that studying osteoporotic changes in the trabecular architecture can provide a powerful means of predicting the in vivo consequences of such changes in fragility and strength. Moreover, this method may lead to new techniques for the in vivo analysis of laboratory animals.

In conclusion, the mechanical properties of trabecular bone structure varied with the loading orientation because trabecular bone is an anisotropic material. The mechanical properties of trabecular bone structure in the OVX rats were especially affected by the loading orientation over time. These findings indicate that the mechanical strength of trabecular bone is best estimated under various loading conditions, which can be accomplished using our method.

Acknowledgment

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References

(21)J.H. Waarsing, et al., Detecting and tracking local changes in the tibiae of individual