A Non-Weight Bearing Model of Osteoporosis for Histomorphometric and Mechanical Evaluation of Bone Repair Using Bone Graft Substitutes

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Introduction: More than eighty percent of all fractures in people over 50 are caused by osteoporosis. A true animal model of human estrogen depletion osteoporosis is not known. However, a relevant local osteoporotic state can be obtained through prolonged, decreased weight bearing of a specific limb. The purpose of this study was to develop a canine non-weight bearing model to replicate the bone loss occurring in osteoporosis and to evaluate the ability of a synthetic bone graft substitute, compared to autogenous bone graft, to enhance bone volume and restore bone strength in this bone deficient environment. Understanding the differences in bone repair of osteoporotic sites versus normal bony sites when using bone graft substitutes could lead surgeons to modify their treatment choice.

Methods: Under an IACUC-approved protocol, 9 skeletally mature, male dogs (25-34 kgs) had their left front limb placed in a shoulder sling with the limb in flexion, to affect complete non-weight bearing, and were placed into one of three groups: no defects, autograft-treated defects, bone graft substitute-treated defects (Table 1). Cortical and medullary changes in the humerus and radius of the immobilized limb were followed by radiographs at 0, 16, and 26 weeks. After 26 weeks of immobilization, 6 of these animals had a critical-size, axial medullary defect (13mm dia. X 50mm) created in the proximal left humerus [1] and a large cylindrical defect (7 x 25 mm) was also created transversely in the distal radius. The humeral and radial defects were filled with the same graft material, either cancellous autogenous bone or a calcium sulfate/calcium phosphate composite bone graft substitute (PRO-DENSE®, Wright Medical Technology). Following the surgery, the animals were followed for an additional 13 weeks of non-weight bearing. Radiographs were obtained at 0, 2, 6, and 13 weeks post-operative.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Initial Non-Weight Bearing Period</th>
<th>Critical-Size Defects</th>
<th>Continued Non-Weight Bearing</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Defect</td>
<td>3</td>
<td>26-30 weeks</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Autograft</td>
<td>3</td>
<td>26 weeks</td>
<td>Yes</td>
<td>13 weeks</td>
</tr>
<tr>
<td>Bone Graft Substitute</td>
<td>3</td>
<td>26 weeks</td>
<td>Yes</td>
<td>13 weeks</td>
</tr>
</tbody>
</table>
Transverse, undecalcified stained sections of the bones were prepared. The area fractions of new bone and residual implanted materials in the defects were quantified using standard point-counting techniques. Total area of bone was quantified by digitizing high resolution contact x-rays of the specimen cross sections selecting for both cortical and trabecular bone. The yield strength and modulus of an 8mm dia. X 20mm test cylinder cored from the mid-level of each humeral defect was determined in unconfined, uniaxial compression tests at a crosshead speed of 0.5 mm/min. The area fraction, yield strength and modulus were also determined for the contralateral weight-bearing bones. The histomorphometric and biomechanical data were analyzed using the Friedman and Mann-Whitney tests. Data are presented as the mean and standard deviation.

**Results:** Global bone loss was achieved in the entire non-weight bearing front limb of the animals in all three groups. The mean area fraction of trabecular bone in the no-defect group was 10.4%±0.9 and was less than the weight bearing humeri (15.0%±2.1, p=0.083) (Figure 1). Notably, the area fraction of trabecular bone in these weight bearing humeri was comparable to that previously reported for normal humeri in healthy canines (p=0.584) [2], indicating that there was no hypertrophy of the bone in the contralateral weight bearing limb. The strength of bone (0.51 MPa±0.17) was less in the non-weight bearing no-defect group compared to the contralateral humerus (0.81MPa±0.26).

![Area Fraction of Bone in the Humerus](image)

Figure 1. Area fraction of new bone in non-weight bearing bone (blue bars) compared to the weight bearing contralateral bone (red bar).

Total bone area was also decreased in the non-weight bearing humerus and radius. Changes in total area were most notable in the radii and ranged from 21.3%±5.1 proximally to 36.3%±5.6 distally. For the humerus, the decrease ranged from 9.1%±2.7 proximally to 18.7%±7.5 distally. In the non-weight bearing humerus and radius, cortical bone loss was seen at both the periosteal and endosteal surfaces. Loss of bone in the medullary canal was evident as thinning of the individual trabeculae, as well as the apparent complete resorption of some trabeculae (Figure 2).
New bone formation was present in all of the defects in the radius and humerus. In the bone graft substitute group, the amount (14.1±7.6) and strength (0.81MPa±0.20) of new bone was equivalent to that of the weight bearing non-treated contralateral humeri (p=0.564). The newly formed trabeculae appeared thicker, had small residual particles of composite material incorporated within the trabeculae structure and were contiguous with surrounding native trabeculae. In addition, the surrounding native trabeculae also appeared thicker and more equivalent in size to those of the weight bearing humerus. In the autografted group, the area fraction (11.0±4.0) and strength (0.58MPa±0.40) of new bone was similar to that of non-weight bearing, no-defect humeri (p=0.827) (Figure 1). The trabeculae in the autografted humeri, both within and surrounding the created defects were decreased in thickness and interconnectivity compared to weight bearing humeri.

**Discussion:** The radiographic, quantitative histological findings and mechanical test results have demonstrated that, by placing the front limb of a canine in a non-weight bearing sling for at least 26 weeks, substantial loss of bone can be achieved in a gradient along the entire non-weight bearing limb. This was reflected most dramatically distally in the radius and was also seen in the humerus. In this bone deficient environment, the use of a calcium sulfate/calcium phosphate bone graft substitute in critical sized defects restored bone volume and strength to values similar to that of the weight bearing limb. Importantly, hypertrophy of the weight bearing contralateral bones was not noted.

**Significance:** This canine model can be utilized for studying bone graft substitute materials in an osteoporotic environment for potential clinical application. An impressive finding was the greater performance of the calcium sulfate/calcium phosphate bone graft substitute in contrast to autogenous cancellous bone graft in the restoration of bone volume and strength in osteoporotic bones.

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