Relation to Musculoskeletal Conditions: Weight-bearing alters the early healing response in distraction osteogenesis. This study was performed to determine if these changes affect late consolidation of the regenerate zone.

Introduction: In a clinical study, weight-bearing has been correlated with increased density of formed callus during distraction osteogenesis. In addition, recent laboratory studies have demonstrated that callus volume, mineral content, and fracture strength are increased when micromotion is applied during the consolidation phase of limb lengthening. Using a rat model, we recently demonstrated that weight-bearing during limb lengthening leads to early increases in osteocalcin and type I collagen mRNA expression, increases in bone morphogenic protein (2 and/or 4) expression, and a decrease in type II collagen formation. Our model was designed to reduce the risk of osteopenia associated with immobilization, which may confound the results of mechanical testing. This study was performed to determine how these biological changes affect late consolidation of the regenerate zone.

Methods: All procedures involving animals were reviewed and approved by our institution’s animal welfare committee. Unilateral right-sided femoral lengthenings were performed on 37 male Sprague Dawley rats (400-500 gm, age < 6 mos.). Under Nembutal anesthesia, a unilateral four-pin external fixator was applied to the femur via a muscle splitting approach. After application of the external fixator, a mid-diaphyseal osteotomy was made with a micro-oscillating saw, and the wound was closed in layers. The rats were divided into two experimental groups. Rats in the first group (twenty) were allowed unrestricted weight-bearing, while the rats in the second group (seventeen) were made non-weight-bearing by a through-knee amputation performed at the time of the initial operation. After a two-day latency period, all rats were lengthened 0.5 mm per day for four days, to a final lengthening (six weeks post-op).

Routine and Quantitative Histology: The femurs from these animals were cleaned, potted, and tested to failure in axial tension at a rate of 25 mm/min. Using a servohydraulic materials testing machine (MTS 810, MTS Systems Inc., Eden Prairie, MN). During testing, load and displacement data were recorded with a digital data acquisition system.

Routine Histology: The femurs from the remaining animals of each group were harvested in bloc. Three specimens from each group were embedded in paraffin, serially sectioned and stained with H&E, Safranin-OFast Green, and Masson-Trichrome. The remaining femurs were embedded in polymethylmethacrylate. Serial sagittal sections were cut, ground, and stained with Toluidine Blue. Digital images of the distraction zone were captured at low power magnification (IP Lab, Signal Analytics, Vienna, VA). Cortical bone (CB), periosteal bone (PB), interzone bone (IZB), and interzone cartilage (IZC) were manually segmented (Fig. 1). The respective areas were calculated with NIH Image 1.61 and summed to yield a total area (TA) for each bone.

Statistical Analysis: The results of mechanical testing, mineral content determination and quantitative histology were compared using unpaired Student’s t-tests. Results: Thirty-three animals survived the protocol and were available for evaluation. Three rats in the weight-bearing group died during the protocol, possibly due to anesthetic stress. The remaining 30 rats were classified as either weight-bearing or non-weight-bearing.

Mechanica Testis aMineral Content Thirteen specimens were available.

\( \text{Stiffness (N/mm)} \)

\[
\begin{array}{ccc}
\text{Weight-Bearing} & \text{Non-Weight-Bearing} & p-value \\
(n=5) & (n=5) & \\
314.1±164.4 & 256.6±130.1 & 0.58 \\
97.5±63.6 & 94.2±61.0 & 0.94 \\
41.3±66.9 & 15.1±13.0 & 0.47 \\
57.7±17.4 & 58.9±31.3 & 0.36 \\
\end{array}
\]

Discussion: At five weeks of consolidation, there was significantly more new bone formation in the distraction zone of the weight-bearing rats. The mechanical properties of the regenerate zone in the weight-bearing rats, although not significantly greater than the corresponding values in the non-weight-bearing rats, did show increased energy to failure and stiffness. Taken together, these results suggest that weight-bearing produces a significant effect on bone formation. When viewed in the context of our previous findings of early weight-bearing-related increases in osteocalcin and type I collagen mRNA expression, and increases in bone morphogenic protein (2 and/or 4) expression, these findings suggest that the early biological changes we previously observed may lead to increased bone formation in the regenerate zone, which ultimately translates to a functional difference in the healed distraction gap.

References:

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Figure 2. Quantitative Histology Results.

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