

Bone mineral density changes during fracture healing: a densitometric study in rats

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Summary:

Dual-energy X-ray absorptiometry (DXA) is a quantitative technique for determination of bone mineral density (BMD). DXA was used to measure BMD of during fracture healing in rats. Stainless steel or titanium intramedullary nails were inserted into the right femora of male, two month old Sprague-Dawley rats. Closed mid-diaphyseal fractures were then created by three-point bending. Measurements of BMD were made in five regions of the femur in animals killed at 2, 8 and 12 weeks after surgery. Between weeks two and eight, there were significant increases in BMD in all five regions of interest for both treatment groups. There were no statistically significant differences in BMD between treatment groups. There are regional and temporal changes that occur in bones during fracture healing. We believe that that DXA is a useful technique for quantification of bone healing in this small animal model. The technique may have important clinical utility in the prediction of delayed or non-union.

Introduction:

Callus develops when a fracture undergoes secondary bone healing (3,16). Stability across a fracture is enhanced by the periosteal callus, which decreases mobility and strain on interfragmentary tissue by increasing the cross-sectional area and the moment of inertia. Experimentally, it is recognized that the mineral content of fracture callus increases within the first 3 months after injury (20). There is also a direct correlation between mineral content of callus and its mechanical strength (43). Thus, there is great interest in measuring serial changes in mineral content over time in order to predict ultimate bone strength.

Intramedullary fixation is used commonly to provide stability to long bone fractures in humans (6, 49). This type of fixation restores bony alignment and permits early weight bearing. The intramedullary nail serves as an internal splint with both bone and rod contributing to interfragmentary stability. Because this type of fixation is not rigid, repair occurs by secondary healing with formation of external callus. Currently, however, complications such as delayed union or non-union may arise from excessive flexibility, rod deformation, fatigue fractures, or migration. Basic research into fracture healing is therefore essential to determine the optimum

materials, geometry, and structural properties for fixation devices.

Dual-energy X-ray absorptiometry (DXA) is a non-invasive imaging technique that permits the quantitative measurement of bone mineral density (BMD) (7, 9, 11, 13, 14, 15, 18, 24, 29, 34, 35, 36, 37, 46). Recently the introduction of ultra-high resolution software has made it possible to analyze BMD changes in small regions of interest, and hence in small animals such as rats (22, 30, 31, 33, 38, 39, 42,). Small animal models have been used previously to study the response of the skeletal system to trauma, enhancing our understanding of the biology of fracture repair (12, 28, 30). The application of DXA to study fracture healing in experimental models is therefore a logical progression. The objectives of the current study were: (1) to investigate the use of DXA in a small animal fracture model that relies on intramedullary fixation, (2) to quantify the temporal and regional changes in BMD that occur during bone healing, and (3) to examine the effects of implant stiffness on fracture repair.

Materials and Methods:

Surgical Implants. Intramedullary nails (30 mm long) were fabricated from either surgical grade stainless steel or titanium. The stainless steel nails were 1.13 mm in diameter with a mean structural stiffness of 24.0 N/mm. The titanium nails had a diameter of 1.00 mm with mean stiffness of 10.5 N/mm. Stiffness was measured on an Instron 1195 tensile testing machine at our institution.

Animal Model. Forty-eight, two month old, male Sprague-Dawley rats (200-250 gm) were included in this study. The study was undertaken with the approval of an institutional review board, and all animals used in the study were treated in accordance with the laws and regulations of the home country.

The small animal model used in this study was based on that described by Jackson (28). A standard experimental femoral fracture was produced in such a way that alignment of the femur was maintained without interfering with formation of external fracture callus. Each animal was anesthetized with an intraperitoneal injection of a 1:1:2 mixture of fentanyl-fluanisone ("Hypnorm" Janssen Animal Health), midazolam ("Hypnovel" Roche), and sterile water. The right hindlimb was clipped and prepared aseptically for surgery. The distal femur was exposed through a medial parapatellar arthrotomy. After exposing the intercondylar notch, a starting hole was made with an 18gauge needle, and an unreamed, unlocked intramedullary nail was inserted retrograde into the medullary canal through the intercondylar fossa. Care was taken to insure that the distal end of the intramedullary nail was flush with the surface of the femur. The joint capsule and skin were closed with simple interrupted sutures of 6/0 polyglactin 910 ("Vicryl," Ethicon).

Following intramedullary nailing, a closed mid-diaphyseal fracture was established using a pneumatic three-point bending guillotine applied to the lateral side of the right femur (28). Before recovery from anesthesia, a mediolateral radiograph was taken. If the fracture was not located in the mid-shaft of the femur or if the fracture was comminuted, the animal was excluded from the study. 54 animals had surgery, 6 were excluded to yield a study group of 48 animals. Analgesics ("Temgesic," Reckitt and Coleman) and antibiotics ("Synulox," Beecham Animal Health) were administered routinely after surgery. Unrestricted weight-bearing was allowed post-operatively.

Groups of eight animals from each treatment group were sacrificed at three time points (2 weeks, 8 weeks, and 12 weeks). At harvest, hindlimbs were disarticulated at the hip, with removal of soft tissues and intramedullary nails. The femora were then placed in lactated Ringers solution at 4°C until densitometric analysis. Contralateral, unoperated (left) femora were also harvested as controls.

Densitometry. BMD was measured by DXA on a Hologic-QDR 1000 scanner (Waltham, MA), using an ultra-high resolution small animal software package (scan resolution 0.1 mm). All densitometry measurements were made under a standard 2.5 cm depth of water (21). Ultra-high resolution software was used, all soft tissues were dissected free, and the distance between bone and imaging table was standardized (36). Post-mortem specimens were used for better edge-detection (21).

The Hologic-QDR 1000 produces a photon beam at two different energies (43 and 110 keV) that passes through a collimator and an internal calibration wheel and then through the bone (8). This beam is detected by a cadmium tungstate scintillator, coupled to a photomultiplier tube. The bone mineral content (BMC) is determined by the attenuation of the photon beam. An edge detection algorithm defines the bone outline, and the osseous area (cm²) is quantified. The mean BMD (g/cm²) of each region is then calculated automatically as the ratio of BMC per unit area.

For each scan, five regions of interest were defined and analyzed semi-automatically ([Figure 1a](#)). Region 1 represented the entire proximal epiphyseal and metaphyseal regions of the femur. Regions 2 and 3 were mid-shaft, extending ten pixels proximal and ten pixels distal to the line of the fracture, respectively, and included both periosteal and endosteal fracture callus. They were defined semi-automatically after the fracture line, which divided these two regions, was defined manually. Ten pixels was chosen because all callus was included within this measurement. The medial and lateral borders were determined automatically by the software edge-detection. Region 4 represented the entire distal metaphyseal and epiphyseal regions of the femur. The fifth region (Total) was defined as the entire femur. Similar regions were defined in the contralateral control femora ([Figure 1b](#)).

Statistical analysis. For each of the five regions of interest, comparisons were made both within groups, comparing operated versus control limbs, and between groups, comparing stainless steel versus titanium, at the three timepoints (2, 8, and 12 weeks). Statistical analyses within groups were by two-way ANOVA with post-hoc matched-group Student's *t* tests. For comparisons of mean BMD between the two groups, two sample independent-groups Student's *t* tests were utilized. A $p < 0.05$ was considered statistically significant in all instances (10).

Results:

Gross Healing. All of the animals tolerated the surgery well. As mentioned previously six animals were excluded at the time of surgery for poorly positioned fractures. Grossly there was no evidence of infection, and new bone formation was evident in all forty-eight animals at the time of euthanasia. There were no non-unions or malunions. The intramedullary nails were well-fixed in all cases.

Densitometry:

The data for BMD at each region, time point, and treatment group is summarized in Tables [1](#), [2](#), and [3](#).

Comparison within groups:

Control group. In the control group (contralateral, unfractured femur), there were steady and statistically significant increases in BMD at both the 8 and 12 week time points (Tables [1](#), [2](#), and [3](#)). At 2 weeks, BMD values in the control femora were greater in the more proximal and distal regions (1 and 4), however, by 8 weeks and again at 12 weeks, this pattern had changed such that the greatest BMD values were found in the diaphyseal regions (regions 2 and 3). There were statistically significant increases in rates of change between weeks 2 and 8 in all regions, except total, with greatest rates of change in the midshaft, regions 2 (16.8%) and 3 (18.6%). Between weeks 8 and 12, there was further increase with rates of change varying from 1.5% in region 4 to 7% in region 2. These net positive rates of change reached statistical significance ($p < 0.05$) in the midshaft regions (2 and 3) and in total BMD.

Stainless steel. Between weeks 2 and 8, BMD increased significantly ($p < 0.01$) in all five regions of the femora (Table 2). Between weeks 8 and 12, there were no statistically significant increases in BMD in the stainless steel test group (Table 3). The rates of change varied from a net -2.6% in region 2 to 3.6% in region 4.

Titanium. Between weeks 2 and 8, BMD increased significantly ($p < 0.01$) in all five regions of the femora (Table 2). The range varied from 7.2% (region 4-titanium) to 20.7% (region 3-titanium). BMD values at 12 weeks

were lower than at 8 weeks (Table 3), and the loss of BMD was reflected in the net negative rates of change, which ranged from -0.3% in region 4 to -16.8% in region 2. This reached a level of statistical significance ($p < 0.05$) in regions 1, 2, and total.

Comparison between groups:

While the patterns of BMD change within groups did reach levels of statistical significance, comparisons between regional and temporal changes in BMD across treatment groups (stainless steel versus titanium), did not reveal any statistically significant differences at any time point in any region.

Discussion:

Fracture repair is a complex process that requires the ordered synthesis of matrix proteins and deposition of mineral. The quantitative measurement of fracture healing will allow a better understanding of the mechanisms that underlie this process. DXA is an important research technique for assessing BMD changes in the skeletal system. This study illustrates the use of DXA as a tool for the quantification of densitometric changes that occur during fracture healing and supports previous studies in which DXA has been used to measure BMD in small regions of interest (22, 35, 37, 41). DXA a simple technique that quantifies BMD; precision ranges from 1 to 2% (22, 35). DXA offers distinct advantages in quantifying fracture callus. Fracture callus is three-dimensional; it is asymmetric and heterogeneous, making it difficult to quantify radiographically (17). Because both the mineral content within the callus and the actual volume of callus tissue are incorporated in the calculation of BMD (g/cm^2), DXA provides a single measure of several important variables (1, 2, 35, 46).

The regional and temporal changes in BMD that occur after fracture have potential clinical ramifications both for the progression of weight-bearing and the timing of hardware removal. How the BMD of healthy callus changes as a function of time and in relation to other regions of the bone might be useful clinically. For example, it might be possible to predict nonunions, by observing a slower progression or by noting a contrast in the relative density of the callus as compared with other regions.

In this study, statistically significant changes in femoral BMD were seen between weeks 2 and 8 in both treatment groups and the control group. These findings are consistent with those of Grundes and Reikeras who showed that the rate of fracture healing in rats is maximal in the first 8 weeks (19). Similar results have been reported by Markel and Bogdanske in a canine model (35). They followed densitometric changes in fracture gap tissue and concluded that time from injury was a significant predictor of both BMD and mechanical characteristics.

Maximum BMD was found proximal and distal to the fracture line in the nascent periosteal and endosteal callus. BMD also increased significantly in the distal and proximal regions of the femora, together with dramatic increases in BMD in the contralateral control femora. An increase in the rate of bone remodeling has been reported after skeletal trauma (5), and it has been postulated that this regional acceleratory phenomenon may be caused by release of cytokines from cells at the fracture site (16). These humoral factors may enhance bone turnover at distant sites throughout the skeleton (12, 23), and may have contributed to the higher BMD values found in the proximal and distal regions of the femora. While it is also possible that the increases in BMD, seen in the contralateral control femora, were due to increased loading as rats favored the fractured sides, the increases were found at the later endpoints (8 and 12 weeks) when weight-bearing would be expected to have been resumed.

In the control femora, BMD values were greatest in the distal and proximal metaphyseal regions (1 and 4) at the initial 2 week endpoint, while in the fractured side the greatest values were found mid-shaft in the callus. By 8 weeks and again at 12 weeks, the diaphyseal regions (2 and 3) in the contralateral control side had undergone significant increases in BMD, and the regional pattern (2 and 3 > 1 and 4) became similar to that of the fractured side. These regional and temporal differences may be related to the relative amounts of trabecular, cortical, and/or woven bone present in the particular region of interest at the specific endpoint. It is well known that

trabecular bone is more metabolically active than dense cortical bone (7, 45). Therefore changes in BMD may have been reflected more quickly in those regions with a greater proportion of trabecular bone, such as the proximal and distal metaphysis. Furthermore, it is known that the mechanical strength of rat bone continues to increase to one year of age (4), and it is possible that the relatively young age (2 months) of the rats is reflected in the changes seen in the control limbs.

The results are also interesting because they may reveal how much denser a healthy callus is than the rest of the bone as a function of time. For example, it is conceivable that, by contrasting the relative densities of the distal end of the femur to the callus region, poor healing might be detected before mechanical failure occurs.

Blood supply can also influence BMD and bone healing (25, 40). While this model utilized an unreamed intramedullary nail, which theoretically protects endosteal blood supply, the intramedullary canal was certainly violated upon nail insertion, and it is also conceivable that the nutrient artery was disrupted during creation of the fracture. Proximal blood supply could be diminished by both of these mechanisms, resulting in lower BMD in the distal metaphysis (region 4) as compared with the proximal metaphysis (region 1).

This experiment also compared the effects of two different types of intramedullary nails on fracture healing. The model produces a controlled, transverse fracture with the nail serving as an internal splint. This allows early, unrestricted activity and interfragmentary compression, while preventing angulation and displacement (28). There is, however, no rotational stability, and furthermore, the nails, initially at least, obtained a relatively loose fit in the medullary canal. The stainless steel nails were approximately twice as stiff as the titanium nails, and although we found no statistical differences between groups, there were different trends after 8 weeks. In the stiff stainless steel group, the BMD was maintained, while in the titanium group BMD began to decline. The stainless nails were also approximately 13% larger than the titanium nails. Perhaps with the stainless nails occupying larger portions of the marrow cavity, BMD in the stainless-pinned femurs was less.

From clinical and basic research, it is known that materials with poor biomechanical properties may adversely affect healing (19, 26, 27). Pritchard has shown that mechanical factors play a fundamental role in fracture healing and influence osseous versus cartilaginous union (44). Fixation with overly rigid nails may result in decreased movement between fracture ends diminishing callus formation (19, 25), while nails that are overly flexible may fail to maintain alignment. Wang *et al* have demonstrated that intramedullary nails with approximately 20 to 50% of normal femoral stiffness stimulated abundant callus and hastened return of structural properties when compared with stiffer nails (48). Aro and coworkers have shown that flexible nails result in more fibrous tissue while rigid nails result in more cartilaginous tissue (1). Husby *et al* recently studied the effects of intramedullary reaming and nailing on the porosity of rat femurs and concluded that the stiffness of the intramedullary nail influenced cortical porosity and that stiff nails (stainless steel) had the most prominent strain-shielding effect (25). There seems to be preferential stress-shielding in the mid-diaphyseal region, with subsequent transfer of stresses through the implant to the proximal and distal femur. Similar stress transfer mechanisms have been reported with rigid plate fixation devices (47, 50) and arthroplasty prostheses (32).

[Literature:](#)

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