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Original Research

Femoral Strength after Induced Lesions in Rats (*Rattus norvegicus*)

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Rats are a common model for the study of bone healing, with the cranium, femur, and tibia being the bones studied most frequently. This study examines noncritical-sized lesions that would allow rats to continue to bear weight without the need for fixation but that are sufficiently large to enable characterization of the healing process. We compared the femoral bone strength associated with 3 lesion sizes selected for use in future studies. Sprague–Dawley rats (age, 10 to 16 wk) were used to assess the ultimate breaking strength, stress, and break force of normal, unmanipulated femurs. We then created lesions of 3 different sizes in the mid- to distal diaphysis of the left and right femurs and characterized the associated decreases in bone strength. Femurs (*n* = 85) for this study were collected through tissue sharing from rats used in other acute surgical procedures and were tested by using a 3-point bending flexural materials-testing machine. Our hypothesis was that, as a model for bone healing, 3 induced lesions of different sizes would show incremental and proportional decreases in femoral strength, with the intermediate-sized (1.5-mm) lesion demonstrating a decrease of 20% to 40%. A lesion of 1.5 mm yielded a decrease in strength of 17% for both the left and right femurs. The strength of left femurs carrying intermediate lesions was significantly less than that of control, uninjured femur bones. In addition to providing validation for our own future bone-healing project, these data are a useful baseline for other investigators studying bone healing in a rat femur model.

Rodents, particularly rats, represent a reliable and affordable model for conducting basic research involving the skeleton.[2] Although biomechanical techniques for testing bone strength have been well documented, few studies define the theory, methods, and experimental procedures for evaluating the fracture toughness of bone (fracture resistance), especially whole-bone testing in small animals.[10] This said, femurs are still the ideal rat and mouse bones to use to evaluate the fracture toughness properties in small-animal model studies.[4,10] Bending tests are useful to assess the mechanical properties of bones from rodents and other small animals.[15] Even though this method of testing is referred to as a 'bending test,' the material (in this case, bone) is actually fractured to assess fracture toughness or breaking. For bending tests, long bones are loaded mainly in bending or compression during normal movement of the animals and are subject to both intrinsic and extrinsic large bending forces.[4,14] In rodents, locomotion results in alternating tension and compression on the cortex of weight-supporting bones during the gait cycle, with no limit on the magnitude or direction in which these forces can be exerted.[8] This makes testing of bending, compression, torsion or any combination of methods potentially applicable. Therefore we chose to conduct 3-point bending testing on rat femurs. Bones were stressed to the point of fracture and the values required were recorded for computer-assisted analysis.

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In the testing of bone, the fundamental structural properties of greatest importance are stiffness, strength, and toughness.[8,10] Measured and calculated values of importance are peak force (ultimate breaking strength), fatigue resistance, stress, strain, break force, and energy to break. We chose to collect and compare peak force (measured data) as well as stress and break force (both calculated data). We made these choices because the most important biomechanical property from a clinical point of view is the peak force, which corresponds to the ability of a patient's leg to resist high loading before a fracture or irreversible deformation occurs.

Strength can be tested as tension, compression, bending, or shear.[8,10] Strength as a material parameter is defined as the ultimate stress at which failure occurs, but strength is defined structurally as the ultimate load (or force) when failure of the system occurs.[8] In the current study, we tested the strength of rat femurs via 3-point bending. We hypothesized that the 1.5-mm lesion, which involved 39% of the bone circumference, would yield a 20% to 40% decrease in strength. In addition, the femurs with induced lesions showed a consistent decrease in strength, with larger lesions associated with lower peak force on both the right and left sides.

Materials and Methods

The biosamples used in this research were collected from rats maintained in our AAALAC-accredited facility and on an IACUC-approved protocol. The protocol followed the standards set forth in the *Guide for the Care and Use of Laboratory Animals*.[6] The tissue used in the current experiment came from tissue sharing from rats that were euthanized in accordance with their original protocol. Sprague–Dawley rats (age, 10 to 16 wk; weight, 350 to

375 g; male:female ratio, approximately 50:50; Taconic Farms, Hudson, NY) were selected to avoid the considerable bone modeling and skeletal growth associated with very young (age, less than 2 mo) rats.[3] Rats were healthy, previously untreated, and used acutely in a surgical training and tissue handling protocol. Within the original protocol from which these bones were obtained by tissue sharing, the rats were used only once, were anesthetized with ketamine and xylazine, and were euthanized by using an overdose of pentobarbital followed by cervical dislocation at the completion of procedures. Bones were harvested and frozen within 1 h after euthanasia. Femurs were harvested by disarticulating them at the coxofemoral and femoral tibial joints by using scalpel dissection. Soft tissue was removed through a combination of sharp dissection and gentle stripping with gauze pads. Every attempt was made to not leave any tendon, muscle, or cartilage, and most soft tissue was removed prior to storage of femurs in the freezer. Small amounts of soft tissue remained at the ends of the bones, but the femoral diaphyses where the lesions were created and where the testing was conducted were cleaned effectively.

Lesions were made by using either of 2 methods: (1) the lesion was made directly after collection of the bone, and then the bone was frozen or (2) the tissue was frozen, brought through a thaw cycle, injured, and then refrozen prior to testing. All tissue was placed in 0.9% NaCl-soaked gauze in double-lock zipper-closure bags to avoid freezer burn and stored frozen at −20 °C for as long as 3 mo. Tissue was prepared immediately and frozen within 1 h of collection because autolysis can begin within hours after the removal of bone from the body and thus adversely affect the mechanical properties of bone.[15] During the preparation time, tissue (primarily bone) was handled at room temperature. For lesion creation or strength testing, bones were thawed overnight (approximately 12 to 18 h) at refrigeration temperatures (5 °C) and brought to room temperature (18 to 21 °C) 1 h prior to testing or lesion preparation. Unless the tissue was being manipulated directly for breaking or lesion preparation, it was kept in saline-soaked gauze and at no time allowed to dry out.

Prepared tissues were separated according to right and left femurs for storage. Groups were established to representing all combinations of side (left or right) and lesion size (0, 1.0, 1.5, and 2.0 mm); all groups contained 10 femurs, except that for the right leg, no lesion (n = 15), for a total of 85 femurs. The lesion size as a percentage of the circumference of the average sized femur (an ellipse) was 26% at 1.0 mm, 39% at 1.5 mm, and 52% at 2.0 mm. Our hypothesis for lesion sizes was based on published information of segmental defects,[1,4,17] because very little information is available on this type of circular noncritical lesion in a rat model. The lesion size was based on the average rat femur having a diameter of 4 to 5 mm. A previous publication considered a 5-mm wedge-shaped segmental defect as a critical-sized model for the testing of biomaterials.[1] Even after healing for 42 d, the 5-mm defect was completely unstable and could not be used for biomechanical testing.[1] Another publication cited a 6-mm segmental defect as nonhealing and requiring stabilization with an external fixator.[17] A literature search regarding segmental defects showed that nonhealing lesions can range from 2 to 10 mm.[4] According to these sources, a 1.5-mm hole seemed to be appropriate for our study needs.

Lesions were created on the medial aspect of the mid- to distal femoral diaphysis, distal to the third trochanter, which extends quite distally in the rat. We chose to make our lesions in the diaphysis, which is composed primarily of cortical bone.[5] Cortical bone (also known as compact bone) is denser, harder, stronger, and stiffer than is the cancellous bone in the metaphyseal regions. In addition, we chose to avoid the metaphysis because the growth plates of rodents remain open throughout their lifespan. This characteristic accommodates ongoing bone growth and modeling, as seen in an immature skeleton.[2] A consistent circular surgical lesion was made in the harvested femurs to approximate an actual medial surgical approach. Using a surgical bone-drill system (Microtorque II, Ram Products, East Brunswick, NJ), we made a full-thickness defect through the medial side of the cortex and into the marrow cavity without disrupting the lateral cortex. The 1.0- and 2.0-mm lesions were made by using the no. 2 (0.039 in./ 0.9906 mm) and no. 7 (0.083 in./ 2.1082 mm), respectively, carbide tips included with the bone-drill system (Microtorque II, Ram Products**)**. The 1.5-mm lesions were made by using a 1.5-mm round carbide bit (Figures 1 and 2).

Strength testing by 3-point bending was conducted by using the bone-strength methodology of our collaborators at the University of Maryland, College Park, MD. The ultimate breaking strength was measured by using a load frame (model 5542, Instron, Canton, MA) and a 3-point bend fixture (model 2810-400, Instron) at a crosshead speed of 10 mm/min. The load cell for this testing (Instron 2530-416) was used with a maximum capacity of 500 N. Tissue was kept in 0.9% NaCl-soaked gauze until immediately before testing. Tissue was tested at room temperature (23 °C). Data (force in kg and extension in mm) were collected and analyzed with a vendor-provided commercial mathematical software package (Bluehill2, Instron). The external supporting pins were set at 19.26 mm for the appropriate fulcra distance for the rat femurs, which ranged in length from 32.58 to 37.46 mm. This spacing allowed both metaphyses to rest on the external supporting pins and for the central loading pin to apply force directly over the lesion (or distal 1/3 of the diaphysis for controls). The bones were very stable on the supporting pins and showed very little movement when the breaking force was applied. The load was placed on the bones in anterior–posterior bending.

The upper anvil had a diameter of 10 mm, and the lower external supporting pins each had a diameter of 4 mm, thus allowing for a minimal span of 4 mm and a maximal span of 194 mm (Figure 3). Dimensions of the femurs were measured by using digital calipers. The length was measured as the distance between the intercondylar fossa distally and the trochanteric fossa proximally and thus did not take into account the added length of the femoral head. Additional measurements from all bones were the outside diameter, height, and width at the level of the lesion (or standard mid- to distal diaphysis for controls). The inside diameter, height, and width were measured at all fracture points. The rat femurs we used fit the expected parameters of 30 to 40 mm long and 3 to 4 mm in diameter for testing by the 3-point bending method.[10]

The initial phase of this study measured the strength of intact left and right femurs. Then these values were compared with the decreased strength associated with 3 defined lesion sizes. These parameters will be used in support of a specific associated surgical study investigating bone-healing enhancement by using protein-based treatments. The lesion generated needs to be sufficient to enable differentiation between the various treatment groups but not so severe as to cause an actual fracture or require internal or external fixation in a live animal model. This feature is

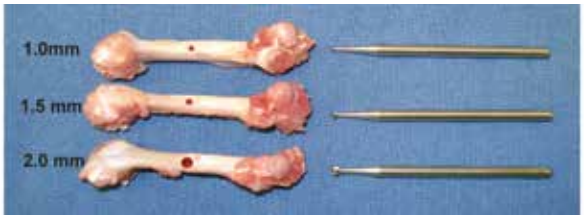

Figure 1. Examples of bit sizes and lesions in the right femur prior to breaking.

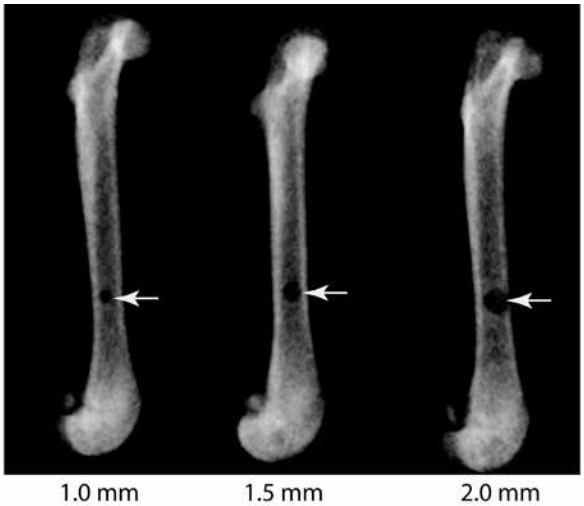

Figure 2. Radiographic representation of lesion sizes created by using a surgical bone drill prior to breaking of the femur. An arrow indicates each of the 3 lesions.

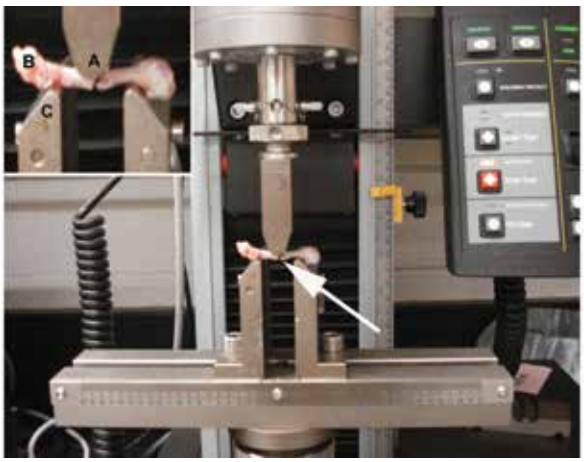

Figure 3. Image of the right femur after breaking, demonstrating the position of the external supporting pins, fracture through the lesion, and the break on the tension surface (convex side) first. The arrow in the main figure shows the lesion with the break; and the inset shows the (A) upper anvil, (B) right femur, and (C) lower supporting pins.

the main distinction between the circular noncritical defect model we are developing and the critical defect models previously reported in the literature.[1]

Peak force (or ultimate breaking strength; in kg) was measured directly by the software system in conjunction with the sevohydraulic materials testing machine. Ultimate strength is the maximal stress the bone can sustain, and breaking strength is the stress at which the bone actually breaks. In bone, ultimate stress and breaking strength are usually the same.[15] Other parameters compared were stress (in kg/cm^2) and break force (in kg). Stress is defined as force per unit area and is classified as compressive, tensile, or shear; these 3 types of stress almost always occur together even under the most simple loading schemes.[15]

The calculation for stress is:

$$\frac{F \times L \times C}{4 \times MI},$$

where F is the force to yield stress (in kg), L is the length between supporting fulcra points (in cm), C is half of the diameter of the bone parallel to the force applied (in cm), and MI is the moment of inertia (in cm^4, 0.0491 [BD^3 – bd^3, where B is the external bone diameter perpendicular to the force {in cm}, D is the external bone diameter parallel to the force {in cm}, b is the internal bone diameter perpendicular to the force {in cm}, and d is the internal bone diameter parallel to the force {in cm}]). The force required to break a bone is different from its intrinsic strength, because the ultimate load varies with the size of the bone.[14] Break force can be calculated as peak force minus baseline force but was normalized by the software as part of the flexure method.

Mean outcomes were compared among all lesion sizes by using one-way ANOVA. Tukey posthoc tests were used to conduct pairwise comparisons among all lesion sizes with to the no-lesion baseline. Models were run separately for left and right femurs. Linear contrasts were used to test whether there were increasing or decreasing trends with increasing lesion size. Data were analyzed by using SPSS version 20 (IBM, New York, NY); *P* values less than 0.05 were considered statistically significant.

Results

Strength data for intact rat femurs are presented in Table 1[ID] TBL1[/ID]. Our data showed that the 1.5-mm circular lesion was associated with a 17% decrease in the ultimate breaking strength, as calculated from the mean peak forces for each group. The ANOVA comparison for mean peak force, mean stress, and mean break force between groups (combined) was significant ($P < 0.05$) for the left side but not the right side for all 3 parameters (Table 1).

Data from all 3 lesion sizes for both left and right femurs were compared with baseline data (no lesion) by using Tukey Honestly Significant Difference posthoc analysis. These data were used to compare the mean peak force and the percentage decrease in strength associated with each lesion size (Figure 4). These reductions were significant ($P < 0.05$) for the 1.5- and 2.0-mm lesion sizes on the left femurs only. In addition, the magnitude of the decrease due to the 1.5-mm lesion (17%) was the same on both sides (Table 2). In addition, ANOVA for statistical trends found that larger lesion size was associated with lower peak force on both the right side ($P = 0.026$) and the left side ($P < 0.001$).

Discussion

The main objectives of this study were to establish peak force, stress, and break force for the left and right intact femurs of the rat and for femurs with 3 defined lesion sizes. These noncritical bone lesions are meant to be applicable to surgical healing models. Peak force or ultimate breaking strength is the classic measurement of bone strength. This measurement reflects the

Table 1. Mechanical properties of femoral bone strength (n = 85; mean ± 1 SD [range]) according to 3-point bending analysis

Side	Lesion size (mm)	Peak force (kg)	Stress (kg/cm^2)	Break force (kg)
Left				
	0 (n = 10)	13.280 (10.399–16.933)	85.572 (63.002–119.318)	14.049 (10.994–13.140)
	1.0 (n = 10)	11.414 (10.262–12.303)	53.497 (16.538–77.319)	12.033 (10.407–13.140)
	1.5 (n = 10)	10.799 (8.227–12.520)	40.355 (13.975–71.705)	11.511 (8.813–13.381)
	2.0 (n = 10)	8.330 (4.701–11.952)	21.799 (7.627–63.799)	8.891 (5.13–12.611)
	P value (ANOVA) between combined groups	0.001[a]	0.001[a]	0.001[a]
Right				
	0 (n = 15)	11.949 (6.200–15.762)	47.786 (15.478–107.518)	12.661 (6.746–16.602)
	1.0 (n = 10)	11.819 (8.510–15.541)	55.333 (25.504–108.569)	12.510 (9.194–16.481)
	1.5 (n = 10)	9.941 (8.209–11.581)	50.111 (16.361–101.254)	10.626 (8.758-12.403)
	2.0 (n = 10)	10.467 (7.511–13.500)	51.305 (18.502–95.700)	11.163 (8.158–14.368)
	P value (ANOVA) between combined groups	0.072	0.892	0.081

The same 3 measures of strength were used for both left and right femurs, but data were analyzed separately.
[a]P value is statistically significant.

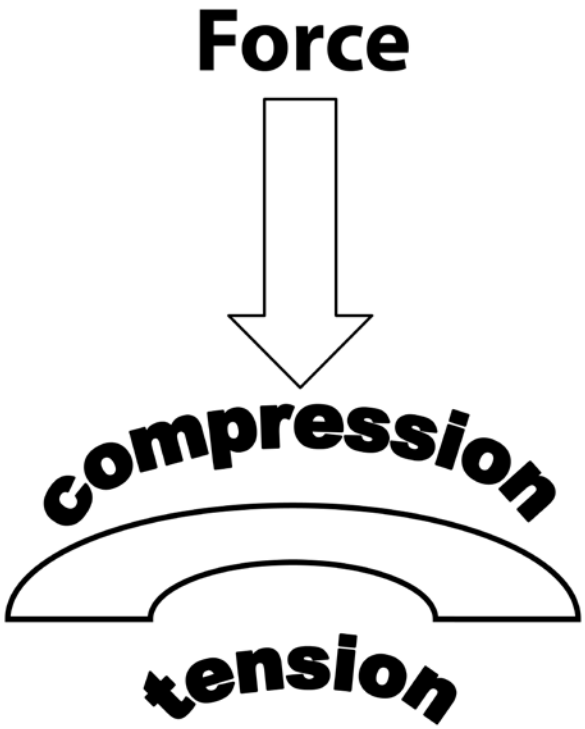

Figure 4. Peak force data for each group. Mean, horizontal line; 1 SD, vertical bar.

general integrity of the bone structure.[14] Measuring peak force allows comparison with data from previous publications and is recorded by the testing machine and computer software. Bone fracture occurs at the point when the energy-absorbing capacity of the bone is exceeded.[8] However that measurement does not account for the area over which the force is applied, the geometrical shape to which the force has been applied, differences in shape or length due to treatment, or potential differences in mineralization ('brittle' compared with 'rubbery' bones). These are the reasons why we included the additional data for stress and break force.

Bending results in maximal tensile force on the convex surface and maximal compressive forces on the concave side.[8] For our samples and the way they were oriented on the supporting pins, the tensile force was on the anterior side of the bone, and the compressive force on the posterior side of the bone, with the lesion in the middle. A cross section of this middle area should be a continuous gradient of stress from tension to compression.[8] Our samples were stronger in compression than in tension, with failure consistently noted on the tension surface (convex side; Figures 3 and 5).

As a tissue, bone is a 2-phase, porous, composite material composed mainly of collagen and minerals, and its mechanical properties are determined by the amounts, arrangements, and molecular structure of these materials.[14] The mineral component gives bone its strength and stiffness, and the collagen component contributes to the bone's work to failure or toughness, which is the material's ability to withstand both plastic and elastic deformations.[14] The mechanical properties of bone are determined by several physical attributes including microarchitecture (thickness and location of the bone trabecules), geometry, shape, size, and (above all) thickness of the cortical zone.[9] Our bones were oriented with the load directly over the lesion in the cortex and with force directed perpendicular to the bone. This orientation should have resulted in accurate measurement, because breaking begins on the external surface of the long bone shaft and because the structure that determines resistance to fracture is the cortical zone of the bone.[9] The actual decrease associated with the 1.5-mm circular lesion involving 39% of the rat femoral circumference was 17% in ultimate breaking strength for both the left and right sides. This value is similar but not as profound as a bone defect model created in the metaphysis of mice, which showed a 34% reduction in the bending moment compared with intact bone for a circular defect involving 20% of the bone circumference.[16]

The length of the bone in comparison to the span of the supporting pins is an important consideration for this type of testing. The span of the supporting pins must be sufficiently long to guarantee an accurate test; if the length is too short, most of the displacement induced by loading will be due to shear stress rather than bending.[15] In general, the length of the specimen should be about 16 times its thickness; however this requirement is not practical for bending tests of whole bones.[15] As a rule for the testing of adult rat femora, female samples should have a length of

Table 2. Decrease in peak force by lesion size relative to the peak force of the femur with no lesion on the same side

Side	Lesion size (mm)	Peak force (kg)	Difference in peak force (kg)	Decrease in strength (%)	*P*
Left					
	0 (*n* = 10)	13.280	—	—	—
	1.0 (*n* = 10)	11.414	1.866	14%	0.132
	1.5 (*n* = 10)	10.799	2.481	17%	0.025[a]
	2.0 (*n* = 10)	8.330	4.950	37%	0.000[a]
Right					
	0 (*n* = 15)	11.949	—	—	—
	1.0 (*n* = 10)	11.819	0.130	0.1%	0.999
	1.5 (*n* = 10)	9.941	2.007	17%	0.106
	2.0 (*n* = 10)	10.467	1.481	12%	0.325

Data are given as mean values.
[a]*P* value (Tukey HSD) is significant.

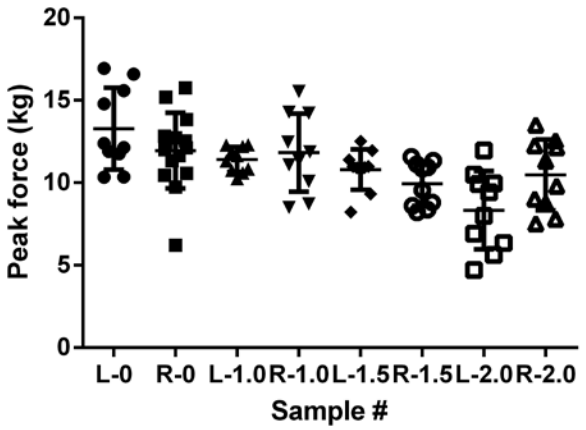

Figure 5. Representation of force in relation to the natural curvature of the rat femur (anterior–palmer) as oriented in this study. As expected, the break occurred first on the tension side.

15 mm and male samples should have a length of 20 mm to guarantee that 85% to 90% of the flexure is due to bending.[15] All of our specimens exceeded these limits for minimal length.

Physical properties that may have had an effect in the current study and that should be considered in future studies are the handing of specimens especially with regard to temperature during all phases of collection, storage, and testing. Our bones were harvested from euthanized animals, prepared, and transferred to the −20 °C freezer within 1 h and stored for a maximum of 3 mo before testing. Freezing human femurs at −20 °C for 20 d caused no change in the bending properties of the bone, and freezing at −20 °C for 30 d caused less than a 2% decrease in the Young's modulus of canine femurs.[7,15] Torsional strength and stiffness of femurs frozen at −20 °C for 14 d has been shown to remain consistent with those of fresh specimens.[7,15] Studies in the literature have used the use of storage periods from 3 h to 1 y.[7] After thawing, all of the bones we used were kept in saline-soaked gauze until immediately before testing. If bone is dried, its Young moduli and strength generally will increase, but its toughness will decrease.[15] For bones tested to breaking, drying can falsely increase strength measurements. For accurate measurement of mechanical properties, bone ideally should be tested at 37 °C; however, testing at room temperature does not yield a large error, except for fatigue testing.[15] No fatigue testing was conducted in the current study, and our bones were tested at a controlled room temperature of approximately 18 to 21 °C.

Possibilities for variation in bone strength even among the same treatment groups are differences in growth rates and laboratory conditions that might influence cortical and cancellous bone structures as well as the biomechanical composition of bone.[2] In addition, the mechanical properties of bone tissue can be influenced by genetics, physiologic factors, diet, and physical exercise.[9] Differences between male and female rats were not assessed in this study. Bones from male and female rats were assigned to testing groups by simple random sampling and not stratified. Another often-cited concern for the use of rodents in bone-healing studies is their lack of a Haversian system. This feature is why large animal species including dogs, nonhuman primates, pigs, and sheep are commonly considered for preclinical skeletal research, either in addition to rodents or instead of them.[2] However rats and mice have resorption cavities that are used for bone remodeling that are similar to the Haversian remodeling of larger animals.[1] This attribute supports the use of rodents for use in bone-healing studies.

For bone-healing and most other studies, outbred strains such as Sprague–Dawley (used here) or Lewis rats are the most commonly used due to their ready availability, low cost, and ease of handling. It has been suggested—and may be worth consideration for future studies—that the femoral bone of Wistar rats resembles the human femur more than does that of Sprague–Dawley rats.[4] In selecting a strain, variations in bone structure and mineral density among inbred strains of rats should be considered; this variability in skeletal phenotype tends to be both site- and strain-specific.[13]

The age of our rats was defined at 10 to 16 wk. However, any age-associated variation may be small, because the maximal breaking force required to fracture femurs at midshaft does not change with age because of architectural compensations, even though the tissue strength decreases with age.[3] Bone maturation progresses more slowly in rats than in many other mammals, and ossification is not complete in rats until after 1 y of age.[13] As rats grow, both body mass and regional muscle mass increase, so a weight-bearing bone like the femur actually will increase its mass and adapt its mechanical properties as the animal ages to satisfy the mechanical demands imposed by growth.[3] The development of the rat skeleton is similar to overall body growth and peaks at about 7 wk of age.[13] Our rats were all similar in age and were

considerably older than 7 wk, so age-associated factors likely did not create the differences noted.

Whole-bone fracture testing—especially in small animal models such as rats—are complicated due to the small physical size and large variation in the standard geometry of specimens. There may have been variation between the left and right femurs due to the natural curvature of the bones and the way they lay on the lower supporting pins with the lesion facing the tester for the right femurs and away from the tester for the left femurs. This uncertainty is further complicated by the fact that there is very little published information on the expected variation for these measurements in small animal models.[10] Although the technique proved challenging, these baseline data are what our study sought to provide. Another complicating aspect of testing bone strength is that bone is a brittle material that forms microcracks and displays some degree of inelasticity. These characteristics make the strength of bone a measurement of the stress required to deform and fracture it and, therefore, dependent on any flaws or defects present.[11] Although we tested bending strength, the dominant loading mode for natural weight bearing in the animal is assumed to be compression.[4] In future studies, this way of testing bone strength may be more applicable.

We chose to conduct 3-point rather than 4-point bending testing. The advantage of 3-point bending is its simplicity, but it has the disadvantage of creating high shear stress near the middle of the bone.[15] The mechanical properties of bone can vary with the kind of bone tested (cortical compared with cancellous), age and exact anatomic location, and variation in the testing conditions.[15] This type of biomechanical testing is commonly done on inorganic materials. Many of the unique properties of bone add to the difficulty of testing. Bone consists primarily of the inorganic compounds calcium phosphate and calcium carbonate, with small quantities of Mg, F, and Na.[12] These mineral crystals form hydroxyapatite, which precipitates in an orderly arrangement around the collagen fibers of the osteoid, giving bone its strength and rigidity.[12] In general, bone is a good composite material with a strength greater than that of either of its components.[8] We applied the basics of this type of materials testing to the very unique organic material of bone.

A last consideration for the future application of our data to actual surgical models is the expectation for healing of this type of lesion. In general after this type of drill hole defect, bone should heal through intramembranous ossification by mesenchymal cells, which differentiate directly into osteoblasts without cartilage formation.[5] In the actual surgical studies that this validation study will support, these drill holes may result in a reduction of bending stiffness during the healing process, causing periosteal activation of chrondogenesis and the deposition of callus-like tissue adjacent to the drill holes.[1,5]

Although we assumed that the circular lesion would reduce strength, we could only estimate percentages of decrease prior to conducting the study. We initially predicted that the 1.5-mm lesion, which involved 39% of the bone circumference, would yield a 20% to 40% decrease in strength. Baseline data were established for both the left and right femurs, yielding a mean peak force of 13.280 kg for the left femur and 11.949 kg for the right femur (Figure 4). In a pilot study in C57BL/6 mice, 20 pairs of left and right femurs were compared in 4-point bending and mechanical properties were observed to not differ.[7] Although the data for the 1.0- and 1.5-mm lesion sizes on the right side did not differ significantly from the control data, they were consistent with the data collected for the same lesion sizes on the left side and did show an incremental decrease in strength. In addition, ANOVA testing supported the trend of larger lesion size associated with lower peak force on both sides. We feel that the left femurs demonstrated the expected results, whereas the right femurs demonstrated possible complications and variability that can be encountered with this type of testing.

In conclusion, this type of destructive materials testing (3-point bending) is necessary because both clinical practice and experimental research still lack a noninvasive and precise method for evaluating fracture healing.[11] Baseline data have been established for the peak force of breaking of unmanipulated left and right femurs of rats and for those carrying induced lesions of 3 defined sizes.

Acknowledgments

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