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In Vivo Whole Body and Appendicular Bone Mineral Density in Rats: A Dual Energy X-ray Absorptiometry Study

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Bone mineral density (BMD) of the whole body and hind limb of young adult rats, with and without a sham-operated stifle joint was studied, using dual energy x-ray absorptiometry (DEXA) at three time points. Data from the whole body scan were used for analyses of BMD, bone mineral content (BMC), fat, lean, body weight (BW), percentage of BMC (%BMC), percentage of fat (%fat), and percentage of lean (%lean), none of which were significantly different between the groups at any time point. Significant ($P < 0.05$) differences in BMD, BMC, %BMC, BW, fat, %fat, and %lean were apparent at the second and third scans, compared with the initial scan, within both groups. Changes in whole body BMD, BMC, and %BMC as well as BW were highly correlated with time in both groups. In the hind limb scans, regions of interest (ROIs) were created to obtain values of BMD and BMC from the whole femur, whole tibia including the fibula, distal portion of the femur, and proximal portion of the tibia. Significant differences were not found between the groups for any ROIs. However, significant BMD and BMC increases were evident in all ROIs at the second and third scans, compared with the initial scan. Similar to those in the whole body scan, BMD and BMC obtained from ROIs were highly correlated with time. The positioning technique for the whole body and appendicular scans was analyzed by calculating percentage of the coefficient of variation (%CV) at the beginning of the study. The %CV was low and acceptable in ROIs for the hind limb and for all parameters of the whole body scan, except fat. The results suggest that in vivo DEXA scanning of the rat whole body and appendicular skeleton is highly reproducible and useful to study the whole skeleton, as well as a region of a long bone of the rat. Values for the sham-operated rats were not significantly different from those for the untreated controls, which suggests that soft tissue damage around the stifle joint did not alter BMD in the subchondral bone of the distal portion of the femur and proximal portion of the tibia.

Bone density or bone mineral density (BMD) is a common parameter used to diagnose and monitor treatments of skeletal diseases such as osteoporosis and osteopetrosis (1). Although conventional radiography and more advanced quantitative computer tomography (QCT) have great value for clinicians, bone densitometry, using non-invasive methods, has become the standard tool to evaluate BMD (2, 3). Bone densitometers operate on a principle in which photon energy generated from a radioactive or x-ray source passes through a test subject. Bone and soft tissue can be differentiated by the ability to attenuate photons (3). In general, bone densitometers, using an x-ray source, are preferable since exposure to radiation is minimized. A breakthrough in bone densitometer technology was the dual energy X-ray absorptiometer (DEXA), which was designed to overcome limitations of single x-ray and single photon absorptiometers, such as problems related to thickness between and within subjects (3). Dual energy x-ray absorptiometers involve use of two x-rays that have low and high peak levels of energy for soft tissue and bone (2). Thus, DEXA can analyze subjects of different composition and thickness with greater accuracy and precision and can separate tissue components of the test subject. Although DEXA has been developed to measure bone mineral, the development of new software allows DEXA to produce body composition data that are highly correlated with those generated by chemical analysis for body composition (4-6). Importantly, recent advances enable analysis of focal regions of the skeleton in small animals, such as the rat, with great accuracy in a short period.

The laboratory rat is particularly suited to study the etiopathogenesis of complex skeletal diseases and to evaluate the therapeutic efficacy of treatments (7). Although BMD data have been used to study osteoporosis in female rats following ovariohysterectomy (8-11), localized changes in BMD that accompany osteoarthritis, osteonecrosis, bone repair with or without fixation, bone fracture, joint replacement, immobilization, and remobilization also could be studied. Thus, development of in vivo techniques to accurately analyze BMD at focal sites in the skeleton could increase our understanding of disease processes and provide a method to evaluate changes in bone structure during the pathogenesis of diseases or chronologic responses to treatments.

In vivo BMD scanning of the whole body of rats has been conducted, especially in studies of osteoporosis and nutrition (4, 5,10-12). Also, a specific focal region of the skeleton can be de-

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fined and analyzed from the whole body scan. To facilitate accurate analysis of limbs, bone densitometers are equipped with separate programs to scan the appendicular skeleton. However, terminal postmortem measurements (13-16) and only a few in vivo studies of rat limbs have been reported (10, 11, 17).

To better understand changes in BMD during disease, repeated measurements are necessary to follow the pathogenesis of destruction of the skeleton and its potential recovery in response to therapeutic intervention. However, information and techniques concerning in vivo bone densitometry of rodent limbs, as well as that of larger species, such as dogs, and chronologic studies are limited. The objective of the study reported here was to evaluate an in vivo technique to determine BMD of hind limbs, as well as the whole body, of normal young adult rats at three time points. To evaluate the usefulness of the technique in experimentally manipulated animals, a second group of rats, in which a stifle joint was surgically opened, was added for comparison. This group of animals was equivalent to sham-operated rats in destabilized stifle joint models of osteoarthritis.

Materials and Methods

Animals. The study was conducted under a protocol approved by the Auburn University Institutional Animal Care and Use Committee for 12 male Wistar rats (*Rattus norvegicus*). Rats weighing 350 to 399 g and between the ages of 120 and 140 days old were purchased by the Department of Laboratory Animal Health, College of Veterinary Medicine, from a United States Department of Agriculture-licensed vendor (Harlan, Indianapolis, Ind.). The health surveillance reports provided by the vendor were negative for all agents tested (comprehensive screening including serologic, bacteriologic, parasitologic, histologic, and polymerase chain reaction analyses for *Helicobacter* sp.). Health surveillance, using the dirty bedding technique, also was performed by personnel of Auburn University. Sentinel animals that had resided in the room containing the experimental rats were sent to the Missouri University Research Diagnostic and Investigative Laboratory. All results (serologic, bacteriologic, parasitologic, and histologic) were negative.

On receipt, the rats were given a physical examination and housed individually in standard shoe box-type cages (48 cm long, 25 cm wide, and 20 cm deep, with 920 cm^2 in floor area). A commercially formulated diet (5P00 Prolab RMH 3000, PMI International, Inc., Brentwood, Mo.) and water were provided ad libitum. Husbandry and treatment of the rats were in accordance with the standard operating procedures of the Department of Laboratory Animal Health, College of Veterinary Medicine, Auburn University, and "The Guide for the Use and Care of Laboratory Animals." Rats were allowed two weeks to adapt to the diet and housing environment and were assigned randomly to one of two groups of six rats each: group 1, control and group 2, sham-operated stifle joint. Sham operation of the stifle joint is commonly used to control the effects of soft tissue injury in iatrogenically induced arthritis.

Study design. Sham operations were performed on rats under general anesthesia that was induced with halothane (Fort Dodge Animal Health, Fort Dodge, Iowa) and maintained by intraperitoneal administration of sodium pentobarbital (50 mg/kg of body weight; The Butler Co., Columbus, Ohio). The joint capsule of the right stifle joint of the rats of group 2 was exposed by use of aseptic surgical approach to the medial site of the joint, as described by Williams and co-workers (18). When reached, the joint capsule was opened by an incision and was closed, using 5-0 Dexon absorbable sutures. After opposing the musculature, the skin was sutured, using a simple interrupted pattern.

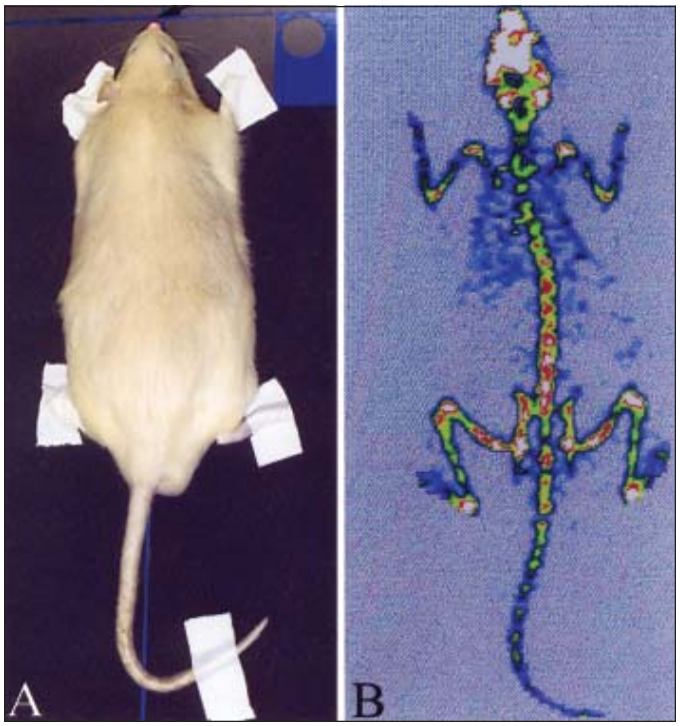

Figure 1. Positioning of a rat during a whole body scan by use of dual x-ray absorptiometry (DEXA). (A) The rat's body was positioned in ventral recumbency on the bare table. Forelimbs were abducted craniolaterally while the manus was positioned in palmar recumbency, and hind limbs were abducted laterally with slightly flexed stifle and hock joints while the pes was positioned in plantar recumbency. Positioning was stabilized by use of one-inch-wide Zonas porous tape. The rat was scanned from the nose (arrow) to the tail. (B) Image of the total body scan of a rat.

Densitometry. Bone densitometry was conducted, using the general anesthesia protocol described previously. All scans were performed, using a DPX-L model dual energy X-ray absorptiometer (Lunar Corp, Madison, Wis.). Quality assurance procedures were performed daily in accordance with procedures recommended by the manufacturer. The Lunar software package for total body scans of small animals included four programs that were designed to handle animals weighing < 400 g up to 5 kg. Rats were expected to weigh more than 550 g at the completion of the study, and the "detail < 5 kg slow" software, version 4.6, was selected as suggested by Lunar. The anesthetized rat was positioned on the bare scan table in a ventral recumbency position. Forelimbs were abducted craniolaterally while the manus was in the palmar recumbency, and hind limbs were abducted laterally, with slightly flexed stifle and hock joints while the pes was in plantar recumbency. Positioning was maintained with one-inch-wide Zonas porous tape (Johnson & Johnson). The tail was curled to reduce scan time. The body was then scanned, starting from the nose and extending to the end of the tail (Fig. 1).

Data acquisition. Data from the whole body scan included quantitative measurement of BMD, bone mineral content (BMC), soft tissue, lean, fat, percentage of tissue fat, and percentage of regional fat. Body weight, percentage of BMC (%BMC), and

percentage of lean (%lean) were calculated from the total body scan data.

To obtain quantitative absorptiometry scan data of individual limbs, rats were scanned using "small animal appendicular" software version 4.6f. The appendicular scans were conducted during the same anesthetic event, following the total body scan. Five scanning modes with various levels of resolution were available. Hind limbs were scanned individually, using "slow" mode software. With the rat positioned in ventral recumbency, the hind limb was placed on a 3.0-cm-thick slab of plexiglass that served as a tissue equivalent in accordance with the instructions of the manufacturer. Each limb was abducted caudolaterally, with the extended hip, stifle, and hock joints and the pes in a slight dorsal recumbency position. The position was stabilized by use of one-inch-wide Zonas porous tape (Johnson & Johnson). Care was taken to avoid excess pressure on the stifle and hip joints. The positioning indicator beam, which designated the starting position, was placed approximately five millimeters distal to the tibiotarsal joint. Scanning was considered complete when the femoral head in the acetabulum was completely visible on the computer screen. A scan width of 40 mm was used to ensure that the entire hind limb was included in the scan (Fig. 2). The first of three scans was conducted on all rats of groups 1 and 2 after the normalization period but before surgery. Additional scans were conducted at six and 10 weeks.

To validate the positioning technique as well as operator and machine precision, one rat from group 1 was scanned five consecutive times, repositioned each time, using the total body software, and again using appendicular software to determine a coefficient of variation (CV). The percentage coefficient of variation (%CV) is commonly used to evaluate the reproducibility of absorptiometry (1-6).

All appendicular scans were examined for misplaced bone edges. Recalculations of edges and corrections were made if misplaced edges were identified. Limb bones in the appendicular scan were analyzed by creation of rectangular boxes designating regions of interest (ROIs). Quantitative data for BMD and BMC from each ROI were obtained, using auto analysis software. Regions of interest were the whole femur, whole tibia, distal portion of the femur, and proximal portion of the tibia. The whole femur was outlined by an ROI that included the entire femur, except a small part of the femoral head located in the acetabulum. A second ROI that included the whole tibia and the fibula was created. The distal end of the ROI was placed immediately below the articulating end of the distal portion of the tibia, which was defined by the widening of the distal portion of the tibia at the level of the malleus. The distal portion of the femur was defined by the third ROI, which was 4.5 mm deep and contained the entire femoral condylar area. A long edge of the ROI touched the patellar region, and a short edge was on the caudal end of the condyle. The proximal portion of the tibia was defined by the fourth ROI that paralleled the articular surface and extended 3.5 mm into its epiphysis. Determination of ROIs was conducted by the same investigator for all rats at all time points.

Statistical analysis. All parameters of the whole body scan, as well as BMD and BMC values of each ROI were analyzed, using analysis of variance (ANOVA) for repeated measures, and the least significant (LSD) test for multiple comparisons, using statistical analysis system (SAS) software (release version 6.12, SAS Institute, Inc., Cary, N.C.). For the multiple comparison tests, $P \leq 0.05$ was considered significant. Changes in BMC and BMD in the whole body and ROIs also were analyzed by use of regression analysis with Excel software (1997, Microsoft, Redmond, Wash.).

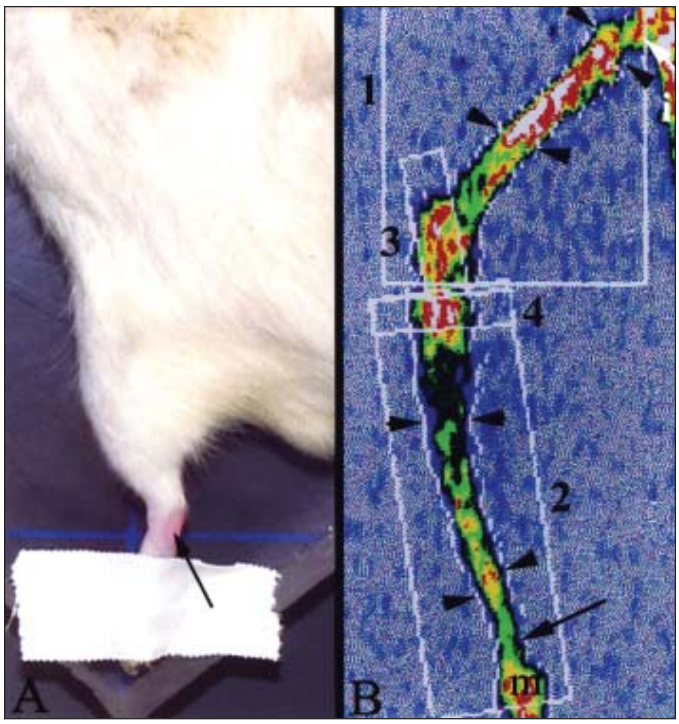

Figure 2. Rat hind limb scan obtained by use of DEXA. (A) While the rat was positioned in ventral recumbency, the hind limb was placed on a 3.0 cm thick slab of plexiglass that served as a tissue equivalent. The limb was abducted caudolaterally with the extended hip, stifle, and hock joints and the pes positioned in slight dorsal recumbency. Care was taken to avoid putting excess pressure on the stifle and hip joints. The positioning indicator beam (arrow), which designated the starting position, was placed approximately 5 mm distal to the tibiotarsal joint. (B) The scanning procedure for the hind limb started from just distal to the tibiotarsal joint defined by the malleus (m) and stopped when the femoral head (white arrow) was completely visible on the computer screen. Bone edges (arrowheads) were identified as dots by the auto analysis of the software. The ischium (I), which caused a minor scanning problem was also visible when the proximal portion of the femur was scanned. A part of the distal portion of the tibia (black arrow) just above the malleus was thin at the first time point. Bone mineral density (BMD) and bone mineral content (BMC) were obtained from regions of interest (ROIs). The ROIs were the whole femur (1), whole tibia (2), distal portion of the femur (3), and proximal portion of the tibia (4). The ROI of the whole tibia had a lower BMD than did that of other ROIs, which was visible as a color difference on the scan.

Results

Sham-operated rats began bearing weight on the right hind limb a day after surgery and healing was uneventful. The %CVs for the whole body and ROIs of the hindlimb were acceptable (Tables 1 and 2). Values for BMD, BMC, %BMC, body weight, lean, fat, %lean, and %fat for young adult rats are recorded in Table 3. Parameters of the whole body scan did not indicate significant differences ($P > 0.05$) between groups at any time point (Table 3). There were significant differences ($P < 0.05$) within groups over time for all parameters except %lean. The changes in BMD ($R^2 = 0.825$ and $R^2 = 0.7849$), BMC ($R^2 = 0.9213$ and $R^2 =$

Table 1. Percentage coefficient of variation (%CV) and mean ± SD of the rat whole body scan

Variable	BMD (g/cm²)	BMC (g)	Tissue (g)	Fat (g)	Lean (g)	BW (g)
%CV	1.4	0	0.8	15.5	0.656	0.624
Mean ± SD	0.325 ± 0.004	4 ± 0	409 ± 3.3	26.4 ± 4	382.4 ± 2.5	413 ± 3.3

BMD = bone mineral density; BMC = bone mineral content; BW = body weight.

Table 2. The %CV and mean ± SD for BMD (g/cm²) and BMC (g) in the regions of interest (ROIs) of the rat hind limb

Variable	Whole femur		Whole tibia		Distal portion femur		Proximal portion tibia	
	BMD	BMC	BMD	BMC	BMD	BMC	BMD	BMC
%CV	0.08	2.5	2.2	0.6	2.3	6.1	5.9	3.2
Mean ± SD	0.315 ± 0.002	0.542 ± 0.013	0.219 ± 0.004	0.437 ± 0.002	0.305 ± 0.007	0.127 ± 0.007	0.278 ± 0.016	0.059 ± 0.001

Table 3. Whole body scan results (mean ± SD)

Variable	First Scan		Second Scan		Third Scan	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
BMD (g/cm²)	0.333 ± 0.006	0.327 ± 0.008	0.363 ± 0.01	0.353 ± 0.01	0.377 ± 0.004	0.366 ± 0.01
BMC (g)	4.6 ± 0.55	4 ± 0.63	8 ± 0.89	7.83 ± 0.75	10.17 ± 0.75	9.5 ± 0.55
Weight (g)	423.60 ± 12.5	399.3 ± 26.5	550.83 ± 30.7	513.67 ± 16.6	582.50 ± 41.1	552 ± 26.83
Lean (g)	392.8 ± 10.1	370 ± 20.3	493.17 ± 19.7	473.1 ± 17.09	517.67 ± 24.4	494.83 ± 19.8
Fat (g)	26 ± 4.84	25 ± 10.1	49.17 ± 11.51	32.33 ± 10.05	54.83 ± 18.6	547.5 ± 23.47
%BMC	1.09 ± 0.13	1 ± 0.12	1.45 ± 0.11	1.53 ± 0.17	1.75 ± 0.11	1.72 ± 0.13
%Lean	92.74 ± 0.9	92.73 ± 2.16	89.6 ± 1.64	92.12 ± 1.94	89.01 ± 2.66	89.74 ± 3.82
%Fat	6.12 ± 1.01	6.18 ± 2.23	8.86 ± 1.63	6.28 ± 1.93	9.28 ± 2.6	28.5 ± 3.96

Significant differences between the groups were not observed.

0.9267), %BMC (R^2 = 0.8336 and R^2 = 0.8702), BW (R^2 = 0.821 and R^2 = 0.8747), lean (R^2 = 0.8634 and R^2 = 0.8534), but not fat (R^2 = 0.5114 and R^2 = 0.2677), %fat (R^2 = 0.3394 and R^2 = 0.0987), and %lean (R^2 = 0.4557 and R^2 = 0.0144) were highly correlated with time ($P < 0.05$) for groups 1 and 2, respectively (Fig. 3).

There were no significant BMD and BMC differences ($P > 0.05$) in ROIs between the groups at any time point. At the initial scan, there were some significant difference between the right and left ROIs (Tables 4 and 5). Within the groups, significant differences ($P < 0.05$) in BMD and BMC for all ROIs were found at the second and third scans, compared with the initial scan (Tables 4 and 5). Increases in BMC and BMD for ROIs of the hind limb also were correlated with time ($P < 0.05$) for the right whole femur (R^2 = 0.751 and R^2 = 0.8021; R^2 = 0.8482 and R^2 = 0.8961), left whole femur (R^2 = 0.713 and R^2 = 0.8874; R^2 = 0.8664 and R^2 = 0.9487), right whole tibia (R^2 = 0.7639 and R^2 = 0.942; R^2 = 0.644 and R^2 = 0.8787), left whole tibia (R^2 = 0.815 and R^2 = 0.865; R^2 = 0.8204 and R^2 = 0.894), right distal portion of the femur (R^2 = 0.8687 and R^2 = 0.8037; R^2 = 0.6277 and R^2 = 0.6994), left distal portion of the femur (R^2 = 0.8687 and R^2 = 0.8037; R^2 = 0.5846 and R^2 = 0.5747), right proximal portion of the femur (R^2 = 0.7274 and R^2 = 0.7282; R^2 = 0.7731 and R^2= 0.7603), and left proximal femur (R^2= 0.7221and R^2= 0.8991; R^2= 0.7114 and R^2 = 0.8387) in both groups (group-1 BMD and group-2 BMD; group-1 BMC and group-2 BMC), respectively (Fig. 4). The whole tibia had a significantly ($P < 0.0001$) lower BMD value, compared with that for other ROIs at all time points for both groups.

Discussion

On the basis of results of in vivo and postmortem studies on man and animals, including rats, dual energy x-ray absorptiometry has been validated to be an accurate and precise method to measure BMD (2, 10, 11, 19, 20). Quantitative data obtained by use of DEXA is reproducible, with a low CV (6, 10, 19, 20). Except for the value for fat (15%), %CV was low for all parameters of the whole body scan. This value was similar to the 11% CV reported by Rose and co-workers (5). The high fat CV most likely was due to limitations of DEXA in differentiating fat from water (4, 5). The high fat %CV might have been a contributing factor to the high standard deviation in fat and %fat data at various time points. Thus, DEXA may not be useful to measure fat content of the rat, and values for fat content obtained by DEXA should be substantiated by use of other techniques. Regardless, the low %CVs, especially for BMC (0%) and BMD (1.4%), indicate that our positioning technique for whole body scans was highly reproducible. The %CVs for BMC and BMD were comparable to those (1.3 and 1.5%, respectively) reported by Jacez and co-workers (12). The low %CVs for BMC and BMD of the femur and the tibia in this in vivo study also were comparable to those obtained from the excised hind limb (16), with and without muscle trimming (13-15), and those of the intact hind limb (10, 11, 17) of other studies (Table 6).

Although CVs were slightly higher for BMD of the proximal portion of the tibia (5.9%) and for BMC of the distal portion of the femur (6.1%), they were acceptable. The higher CVs may have resulted from slight differences in placement of the ROIs. Also, both anatomic sites are located at a movable region of the body; thus, their orientation may have been changed slightly at each repositioning. The DEXA scanning interprets three-dimensional subjects in two dimensions; consequently, small changes in positioning can alter the regional thickness of sites for analysis. This is supported by the various locations of higher CVs for BMD and BMC, which would be expected as the topographically irregular prominences of epiphyses are realigned. For studies of BMD and BMC of the appendicular skeleton, as well as the whole body, reproducibility in repositioning is important in DEXA scanning; therefore, positioning of subjects should be performed by one person, and precision testing should be performed on all scanning protocols.

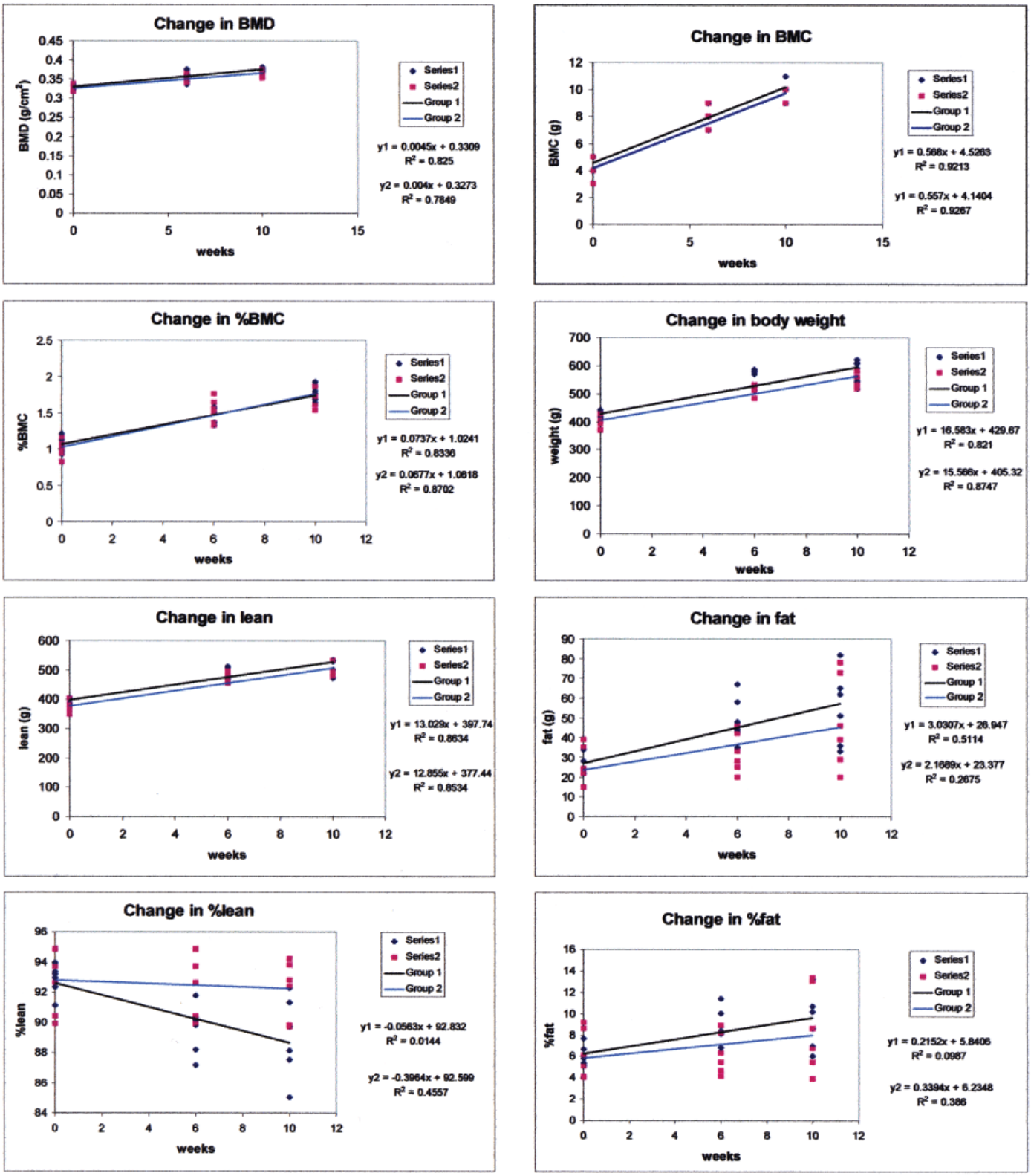

Figure 3. Values for BMD, BMC, percentage of BMC (%BMC), body weight, lean, fat, percentage of lean (%lean), and percentage of fat (%fat) were determined by use of the whole body DEXA scan over time.

Dual x-ray absorptiometry has been developed to measure BMD and BMC; however, it also provides reliable values for soft tissue, lean, fat, %tissue fat, and %regional fat. Body weight, %BMC, and %lean are calculated from the total body scan data (4-6, 20). The quantitive data obtained in this study using the whole body scan are comparable to those of other studies con-

Table 4. Bone mineral density (g/cm^2; mean ± SD) of ROIs of hind limbs of rats

Variable	First Scan		Second Scan		Third Scan	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Whole femur (L)	0.271 ± 0.011*	0.263 ± 0.019*	0.329 ± 0.028	0.318 ± 0.011	0.353 ± 0.026	0.344 ± 0.009
Whole femur (R)	0.247 ± 0.024	0.246 ± 0.019	0.323 ± 0.02	0.316 ± 0.016	0.348 ± 0.026	0.328 ± 0.009
Whole tibia (L)	0.201 ± 0.05*	0.19 ± 0.014	0.232 ± 0.015	0.235 ± 0.004	0.252 ± 0.009	0.249 ± 0.004
Whole tibia (R)	0.188 ± 0.023	0.185 ± 0.009	0.228 ± 0.01	0.227 ± 0.003	10.253 ± 0.008	0.247 ± 0.004
Femur						
Distal portion (L)	0.256 ± 0.01	0.266 ± 0.012*	0.308 ± 0.002	0.306 ± 0.004	0.344 ± 0.02	0.334 ± 0.01
Distal portion (R)	0.244 ± 0.028	0.234 ± 0.021	0.315 ± 0.018	0.304 ± 0.002	0.331 ± 0.031	0.329 ± 0.01
Tibia						
Proximal portion (L)	0.257 ± 0.015*	0.248 ± 0.017*	0.309 ± 0.016	0.315 ± 0.013	0.343 ± 0.035	0.342 ± 0.005
Proximal portion (R)	0.229 ± 0.026	0.228 ± 0.031	0.304 ± 0.036	0.318 ± 0.027	0.335 ± 0.022	0.337 ± 0.025

Significant differences between groups were not observed at any time point. Whole tibia had significantly ($P < 0.05$) lower BMD values, compared with those for other ROIs, at all time points in both groups. Asterisk (*) indicates the difference between the left and right side for the same ROI.
L = left; R = right.

Table 5. Bone mineral content (BMC) (g; mean ± SD) for ROIs of hind limbs of rats

Variable	First scan		Second scan		Third scan	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Whole femur (L)	0.371 ± 0.018*	0.38 ± 0.024*	0.533 ± 0.045	0.547 ± 0.024	0.645 ± 0.067	0.616 ± 0.033
Whole femur (R)	0.336 ± 0.049	0.346 ± 0.026	0.528 ± 0.052	0.517 ± 0.048	0.616 ± 0.055	0.598 ± 0.035
Whole tibia (L)	0.368 ± 0.015*	0.337 ± 0.021	0.466 ± 0.047	0.485 ± 0.039	0.541 ± 0.037	0.542 ± 0.025
Whole tibia (R)	0.315 ± 0.062	0.322 ± 0.026	0.462 ± 0.025	0.446 ± 0.034	0.54 ± 0.034	0.544 ± 0.046
Femur						
Distal portion (L)	0.102 ± 0.009*	0.104 ± 0.012*	0.123 ± 0.019	0.132 ± 0.01	0.154 ± 0.023	0.15 ± 0.022
Distal portion (R)	0.083 ± 0.016	0.091 ± 0.011	0.128 ± 0.021	0.128 ± 0.01	0.142 ± 0.022	0.133 ± 0.01
Tibia						
Proximal portion (L)	0.06 ± 0.002*	0.058 ± 0.005	0.073 ± 0.006	0.077 ± 0.006	0.084 ± 0.009	0.084 ± 0.002
Proximal portion (R)	0.048 ± 0.01	0.05 ± 0.008	0.069 ± 0.006	0.07 ± 0.008	0.081 ± 0.006	0.081 ± 0.005

Significant differences in ROIs between the groups at any time point.
See Table 4 for key.

ducted on the rat. Particularly, increased values for BMD, BMC, BW, lean, fat, %BMC, %fat, but not %lean, at six and 10 weeks are comparable to results of studies of other animals and the rat obtained by use of DEXA or chemical analysis (4, 5, 21).

Bone mineral density and BMC also increased in ROIs of the hind limb at six and 10 weeks. In ROIs of the hind limb, BMD was low at the first scan; however, it gradually approached that of the whole body suggesting that BMD of the appendicular skeleton is lower than that of the axial skeleton of young and young adult rats. This may be due to appendicular growth of the rat. Unlike higher mammals, physes in long bones of adult rats remain open and growth of the appendicular skeleton continues into adulthood (22). However, physes lose their efficiency with increasing age and appendicular skeletal growth drops markedly (22).

Interestingly, the ROI that contained the whole tibia and fibula had a lower BMD value than that for the other ROIs at all time points. The reason for this difference is unclear. However, the biomechanics of the hind limb of the rat in its typical crouched posture might influence the physical forces placed on the tibia and, in turn, its BMD.

Appendicular bone density measurements in rats have been reported. Postmortem studies involved use of excised limbs with intact musculature and skin (13-15) or individual bones without musculature, which allowed only terminal BMD measurements (16). Such studies did not capitalize on the non-invasive value of DEXA to evaluate interim changes in the skeleton of the living animal. Although in vivo analysis of rat appendicular BMD has been conducted (10, 11, 17, 23), published reports are scarce and limited to only a small segment of the hind limb. In vivo studies were conducted on the tibia without scanning the whole limb to obtain BMD data from the whole tibia with the fibula (17) and tibial metaphysis (23). In this study, the rat hind limb, excluding the portion distal to the tibiotarsal joint, was scanned, which allowed evaluation of the whole femur, whole tibia, distal portion of the femur, and proximal portion of the tibia. However, additional parameters of metaphyses and diaphyses of the femur and tibia could be analyzed separately. Exclusion of digits and metatarsal bones decreased time to complete the scanning procedure.

Two regions of the hind limb presented minor analytical problems in this study. At the first scan, the diameter of the distal end of the tibia close to the malleus (Fig. 2) was thin, and the software had difficulty identifying locations of two separate edges. The second region was the proximal portion of the femur close to the femoral head. As illustrated in Figure 2, scans of the proximal portion of the femur included part of the ischium. If BMD was higher in the ischium than the femur, the software placed the edges of the femur on the ischium. Both problems were corrected by recalculation of the bone edges, using the DEXA software. However, researchers and clinicians must be aware of such problems and carefully examine the bone edges in each appendicular scan before proceeding with mineral analysis.

Mechanical destabilization of the stifle joint in laboratory animals, including the rat, is a preferred in vivo model to study the etiopathogenesis and therapy of osteoarthritis (18, 24, 25). Subchondral bone is subject to pathologic changes in people with osteoarthritis (26, 27). In fact, increased bone density has been suggested as an etiopathogenetic factor in osteoarthritis (28). Thus, in the study reported here, ROI for the distal portion of the femur and the proximal portion of the tibia contained subchondral bone as well as articular cartilage.

New therapeutic strategies for osteoarthritis include modification of the density and pathologic changes of subchondral bone (29). Monitoring of in vivo changes in subchondral bone, using quantitative data, necessitates use of techniques such as

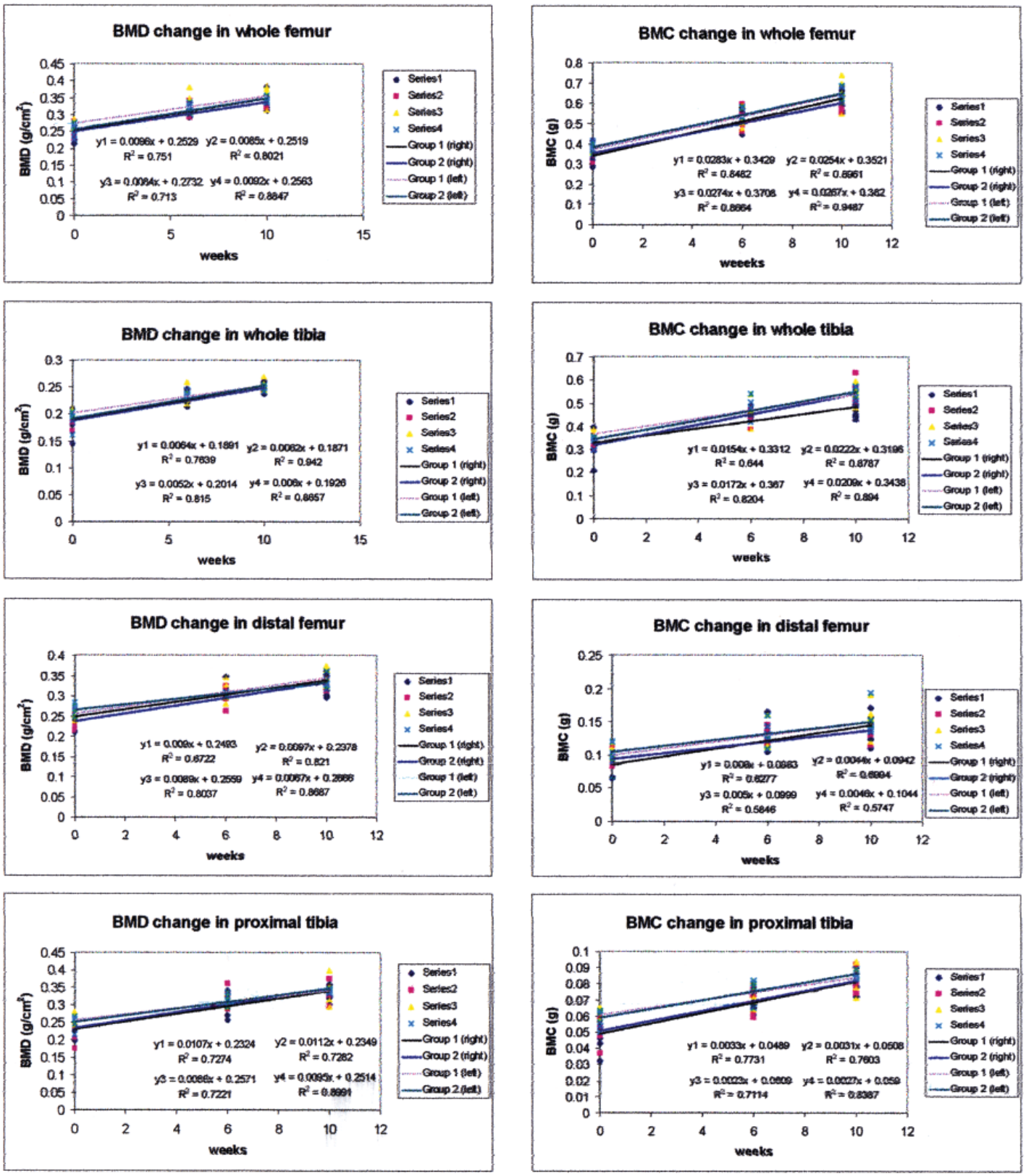

Figure 4. Changes in BMD and BMC in regions of interest (ROIs) for the hind limb over time.

the one used in this study. Development of techniques to monitor BMD of the human proximal portion of the tibia also has been a recent focus (30). In an osteoporosis model, QCT was used to obtain BMD in vivo and ex vivo in the distal portion of the femur and proximal portion of the tibia of the rat (31). Bone mineral density data obtained by use of QCT are expressed as

Table 6. Comparison of percentage co-efficient of variation (%CV) of BMD and BMC obtained from the ROIs for the hind limb of a repositioned rat with those of published reports

ROI								
W Femur		W Tibia		D Femur		P Tibia		Author (reference No.)
BMD	BMC	BMD	BMC	BMD	BMC	BMD	BMC	
0.08	2.5	2.2	0.6	2.3	6.1	5.9	3.2	Present study[a]
0.52	–	–	–	0.96[d]	–	1.6[d]	–	Griffin and co-workers (10)[a]
–	–	1.2	–	–	–	–	–	Ammann and co-workers (11)[a]
0.9	1.2	–	–	1.8[d]	5.6[d]	–	–	Kannus and co-workers (13)[b]
0.9	1.2	1.3	2.8	–	–	–	–	Kannus and co-workers (14)[b]
0.15	0.24	–	–	–	–	–	–	Jiang and co-workers (15)[b]
0.8	–	–	–	–	–	–	–	Seco and co-workers. (16)[b,c]
–	–	1.89	–	–	–	–	–	Iwamoto and co-workers (17)[a]

[a]In vivo analysis.
[b]Postmortem analysis.
[c]Muscle trimmed.
[d]Reported, but not directly comparable with analysis of this study because, in the published report, greater length of bone than that of the ROI of this study was analyzed.
W = whole; D = distal portion; P= proximal portion; – = not reported.

"cm^3", which is a better measurement than data expressed as cm^2 in DEXA, but analysis by use of QCT is not appreciably better than analysis by use of DEXA (1-3, 9). Quantitative CT is also more expensive than is DEXA and is not accessible to most researchers. Pastoureau and co-workers (32) used DEXA in a postmortem analysis of subchondral bone of the distal portion of the femur in partially meniscectomized guinea pigs. Unlike in that study, in our study, we analyzed bone changes at three time points, which included the initial status and progression of changes in multiple ROIs such as the distal portion of the femur. Studies of osteoporosis involving use of DEXA to evaluate the distal portion of the femur (10, 13) and proximal portion of the tibia (10) of rats were reported; however, the length of ROIs used in the studies were greater and contained large portions of the diaphysis than that of our study. Therefore, subchondral bone of the distal portion of the femur or the proximal portion of the tibia was the focus of those studies and was not comparable to results of our study.

Absorptiometers provide two separate values, BMD and BMC, to express bone mineral contents of ROIs quantitatively. Although BMD is a calculated value (g/cm^2), most researchers prefer to use and report only BMD data (17, 32). However, BMC is an absolute value (g) of total bone minerals in ROIs or whole body and may be more useful, especially in studies where repeated measurements of the growing skeleton are conducted. Bone densitometry, including DEXA, interprets BMD in a two-dimensional perspective. However, bone growth is circumferential, and addition of minerals due to new bone growth would be recognized as increased BMD except at the edges of the bone. Therefore, reporting of BMD and BMC provides a more comprehensive analysis of changes in bone minerals and may compensate for minor repositioning problems.

Dual energy x-ray absorptiometry is a standard procedure used in human medicine to monitor BMD (1-3). Reference populations for people of various ages, either sex, and even ethnic backgrounds, are compiled in the sophisticated DEXA software and are used as a standard to evaluate human subjects for BMD and fracture risk. Although results of studies conducted on dogs, cats, and sheep have been reported, the published data are insufficient to form reference populations (6, 16). This has been the major limiting factor for use of bone densitometry in clinical veterinary medicine. However, bone densitometric studies on rodents, such as rats, increased after commercial companies added small animal software to DEXA machines. The software applicable to analyze small animals such as the rat is designed solely for research purposes, and reference populations are not available. Also, there are insufficient reports on whole body as well as the appendicular skeleton of animals, including rats, that include different development stages. Since the existing published data have not been combined to form reference populations, researchers must use control animals for baseline reference values. In vivo or postmortem data for regions of the rat appendicular or axial skeleton have not included whole body BMD or BMC values (10, 11, 17, 23, 32). Focal changes in the skeleton following treatment may misrepresent its generalized condition. Thus, results of ROIs in the appendicular or axial skeleton should be accompanied by analysis of the total body as well as the bone where ROIs are located. Importantly, availability of reference populations formed by collection of new and summarization of published whole body and appendicular bone density data from rats at various ages and strains and of either sex would enrich the field of comparative medicine and would be invaluable information for skeletal studies conducted on rats.

Sham operation is a control used to define the potential influence of surgical manipulation on results of iatrogenically induced experiments. In the study reported here, differences were not observed in whole body or appendicular scans between sham-operated and untreated control rats. These results indicate that BMD changes due to injuries to the synovium and musculature around the rat stifle synovium are limited, which provides more confidence in BMD data collected from surgically treated rat stifle joints.

In conclusion, we found that in vivo DEXA scanning was useful in rats to monitor BMD of a specific region of the appendicular skeleton as well as the whole body, and provides valuable information to our understanding of many diseases associated with the skeleton. A generalized increase in BMD and BMC, which was uniform in rats of same age and sex and was highly correlated with time, was observed in the whole body and hind limb. Differences between sham- operated and untreated control rats were not observed, which suggested that soft tissue damage around the stifle joint due to the sham operation did not cause BMD changes in the subchondral bone of the distal portion of the femur, proximal portion of the tibia, as well as the whole tibia, femur, and the rest of the skeleton.

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