BIOMECHANICAL PROPERTIES, OSTEOPOROSIS, AND FRACTURE POTENTIAL OF METATARSAL TRABECULAR BONE FROM MOOSE IN ISLE ROYALE NATIONAL PARK

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ABSTRACT: We investigated relationships between trabecular bone mechanical properties and bone mineral density of metatarsal bones from moose (*Alces alces*) of known age, sex, and cause of death in Isle Royale National Park, Michigan. Bone density mineral equivalent values were determined using quantitative computed tomography scans (QCT) of 10 mm cross-sections of cancellous bone from the distal metatarsus. Cylindrical specimens were taken from the same anatomic position as the region of interest in the scan. Each specimen was weighed, measured, and photographed for trabecular microstructure under transmitted light microscopy. Using compression tests to failure, we found that modulus of elasticity of trabecular bone was significantly correlated with bone mineral density ($r^2 = 0.91$, $P = 0.0005$). There were also significant relationships between both apparent density and volume fraction of bone ($r^2 = 0.75$, $P = 0.0001$) and bone mineral density on QCT. Loss of bone mineral occurs as the result of environmental, nutritional, hormonal, and/or genetic causes and increases fracture risk. This study has implications for the understanding of moose bone fragility related to mechanical and geometric properties such as elasticity and trabecular microstructure in routine activities of daily living (foraging, ambulating, and bedding-down) as well as when subjected to stressors such as kicking predators, running, and falling.

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Metabolic bone disease and its musculoskeletal morbidity have been more intensively studied in recent years. Rarified bone tissue, loss of bone strength, and ultimate fracture are the major clinical sequelae of osteoporosis, the most common metabolic bone disorder in the world (Marcus 1991). Most osteoporotic fractures are due to low bone mineral density as there is a close relationship between bone mineral density and strength (Carter and Hayes 1977). But bone fragility is also related to bone material and geometric properties such as elasticity and trabecular microstructure (Parfitt *et al.* 1983).

One of the most effective techniques for discriminating bone density changes is quantitative computed tomography (QCT), which provides precise 3-dimensional imaging and direct density measurements (Cann *et al.* 1985). Measurement of mineral content by QCT can be used to predict density of trabecular bone and is correlated with mechanical properties (McBroom *et al.* 1985). Significant positive correlations exist between mechanical properties and equivalent mineral density measured by QCT (Lang *et al.* 1988) and correlations are much higher for cancellous bone than cortical bone (Rho *et al.* 1995). Therefore, QCT is a useful non-invasive diagnostic method for predicting bone stresses under high-risk loading conditions especially in metabolically active trabecular bone where changes in bone mass due to remodeling are more likely to occur (Marcus 1991).

Mechanical properties are extremely variable between species and between bones (Currey 1985, 1987). However, across species, bone elasticity has a strong relationship to density (Carter and Hayes 1977). While osteoporosis in humans is a health problem of growing public concern, fragile bones have dramatic implications for other species as well. In most animal studies of skeletal changes, the examination of bone is limited to histological studies and/or ash content, but measurement of mechanical properties can reveal important relationships, such as decreases in stiffness and strength following pregnancy and lactation (Peng *et al.* 1987) and antler growth (Banks *et al.* 1968). Metabolic bone disease in moose (*Alces alces*), may compromise their ability to maneuver in deep snow, reduce their capacity to run from or fight-off attacks, and leave them vulnerable to predation.

The purpose of this study was to test for relationships between bone mineral density and biomechanical properties of cancellous bone from the distal hind leg of moose under loading conditions at 2 strain rates. This information will enhance our understanding of bone performance in a variety of loading and stressor situations encountered in the daily routines of moose.

METHODS

Moose skeletal remains have been collected in Isle Royale National Park, Michigan (48° N, 89° W) from 1958 to 1997 as part of a long-term study of wolf (*Canis lupus*)/moose population dynamics (Mech 1966, Peterson 1977, Hindelang 1996). Sex was determined by the presence or absence of antlers or antler pedicles, and age was estimated from counts of cementum annuli (Wolfe 1969). For this study, bones from moose of known sex and age, and only bones of moose greater than 7 years old were used because we have determined that osteoporosis is rare in moose that die before age 7 (Hindelang and Peterson 1996). Bone specimens were obtained from the distal metatarsus and were in a defatted and dried state. The bones were packed in ultrasonic gel and scanned on a Phillips Tomoscan LXC (Phillips Medical Systems, 1300 Norwood, Itasca, Illinois, USA) quantitative computed tomography (QCT) bone mineral densitometry unit (Image Enhancements, 6 Gerke, San Francisco, California, USA) at Portage Health System in Hancock, Michigan. Scans were taken transversely in the proximal-to-distal direction and at 10 mm cross-sections operating at 130-kilovolt potential, 150 milliampere-seconds, with a calibration phantom of known concentrations of dipotassium hydrogen phosphate (K_2HPO_4) for reference (Cann *et al.* 1985).

Cross-sections for study were chosen from the cancellous bone in the distal portion just proximal to the epiphyseal fusion line, as this area demonstrated the greatest changes in density in a pilot study (Hindelang *et al.* 1992), and had the largest concentration of metabolically active cancellous bone (Genant *et al.* 1987). Bone density mineral equivalent values in milligrams per cubic centimeter were obtained for each region of interest from the same anatomic position as specimens cut for the measurement of mechanical properties. Equivalent mineral density values were calculated using the mean Hounsfield unit of the circular region of interest and the regression equation from the QCT data versus the corresponding concentration of K_2HPO_4, a direct linear relationship ($r^2 = 0.998$).

Cylindrical cancellous bone specimens (12.7 mm diameter X 10 mm) were obtained from 8 bones, corresponding to the scanned regions (Fig. 1). This destructive technique was limited to 8 bones to reduce the number

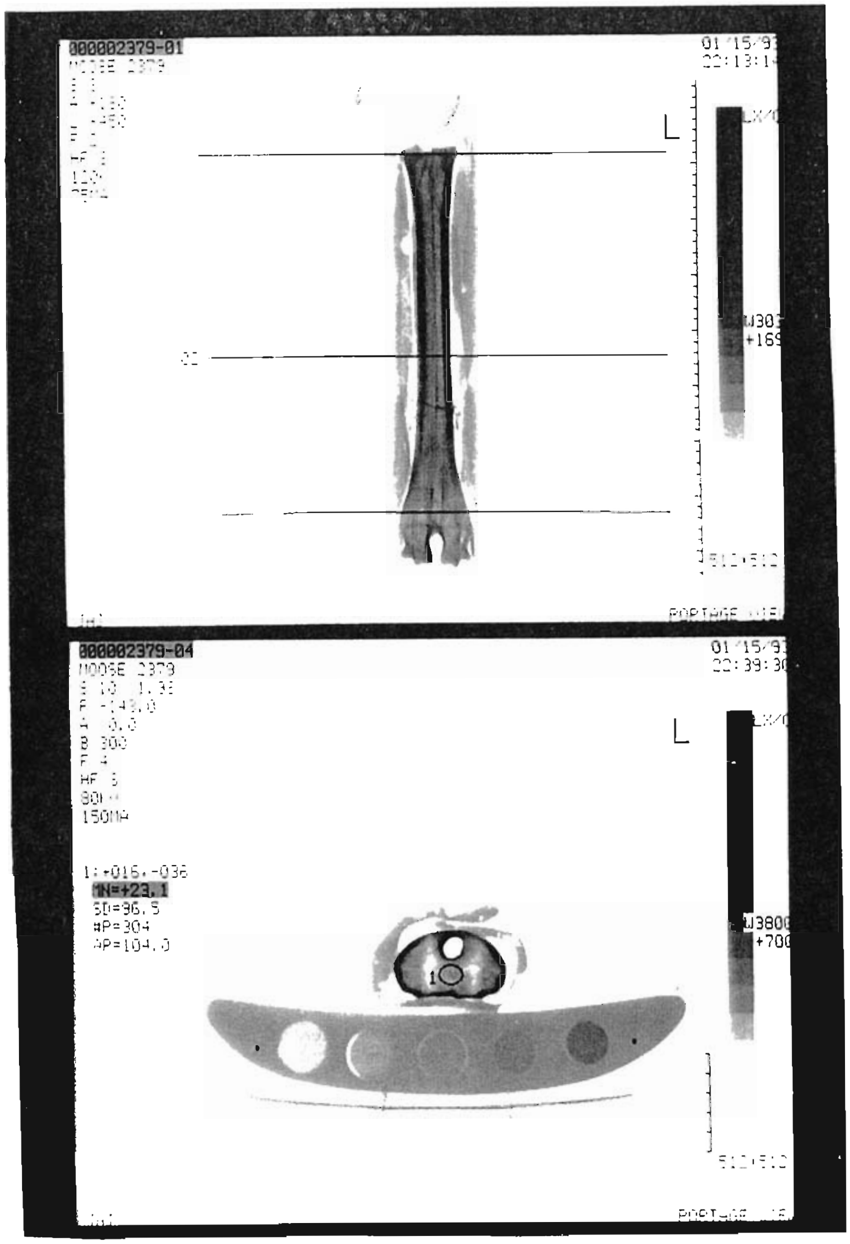


Fig. 1. Quantitative Computed Tomography (QCT) scan layout for moose metatarsal bones collected in Isle Royale National Park, MI, from skeletal remains. Bone density mineral equivalent values in milligrams per cubic centimeter were obtained for a region of interest in the distal cross-section, from the same anatomical position as specimens cut for the measurement of mechanical properties.

of bones sacrificed from the archival collection. Location of the specimens was standardized using plastic templates. Transverse cuts were made with a band saw; care was taken to ensure that the orientation was consistent. Two specimens were cut from each resulting bone slab, one on each side of the distal metatarsal foramen. A 0.5 inch diamond tip drill press cylinder saw was used with water irrigation to cool the blade and reduce damage to the trabeculae. Each end of the cylindrical specimen was carefully milled to ensure smooth and parallel surfaces. Before testing, each specimen was weighed, measured, and photographed under transmitted light microscopy. The apparent density of cancellous bone was determined using weight divided by volume of the overall physical dimensions.

Compression tests to failure were done on 16 specimens using an Instron Tension/Compression machine with a 1,000 pound load cell. Data were collected using a computer data acquisition system that records voltage versus time. An amplifier was used to obtain better resolution and a higher voltage range. The system was recalibrated prior to each testing period to ensure accuracy. The influence that strain rate has on the biomechanical properties was determined at 2 different strain rates, 0.2 in/min and 0.02 in/min. The original voltage/time data were converted to stress/strain data and graphed on Templegraph software. Load was measured by the calibrated load cell of the test system and specimen deformation was measured after compression with calipers. Modulus of elasticity and maximum stress were determined for each specimen. The slope of the load deflection curve was used for calculating the elastic modulus and maximum stress was calculated from the peak load. Mechanical properties were compared to bone mineral density using regression analysis. The level of statistical significance was 0.05.

RESULTS AND DISCUSSION

Modulus of elasticity of trabecular bone was significantly correlated with the equivalent mineral density as measured by quantitative computed tomography (Fig. 2) at the lower strain rate of 0.02 in/min ($r^2 = 0.948$, $P = 0.0001$), and at a higher strain rate of 0.2 in/min ($r^2 = 0.887$, $P = 0.0005$). The linear

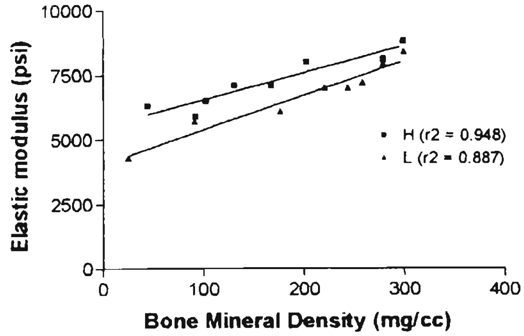


Fig. 2. Relationships of modulus of elasticity of metatarsal cancellous bone to bone mineral density determined by quantitative computed tomography, at high strain rate (H), $r^2 = 0.948$, $P = 0.0001$, and at low strain rate (L), $r^2 = 0.887$, $P = 0.0001$, from moose skeletal remains collected in Isle Royale National Park.

relationship between elasticity and bone density presented here falls within the range of power of 1-3 as demonstrated by Carter and Hayes (1976), Rice *et al.* (1988), and Lotz *et al.* (1990). Bone mineral density values for the cancellous bone specimens in this study ranged from 25 - 298 mg/cm^3 in contrast to the values of high density of compact bone which ranges from 1,500 - 2,000 mg/cm^3 (Hindelang 1996). As in these previous studies, the lack of homogeneity of trabecular bone suggests that the modulus relationships are likely to vary as a function of bone architecture.

Maximum stress showed an increasing trend, although not statistically significant, as density increased (Fig. 3), and values under high strain rate were consistently higher than those tested under low strain rate, demonstrating the significant energy storage capacity of cancellous bone (Hvid *et al.* 1983) which increases with higher loading speeds (Carter and Hayes 1976) and is influenced by the anisotropic properties (Williams and Lewis 1982).

Apparent density (density determined by weight and measurement in mg/cm^3) and QCT bone mineral equivalent density

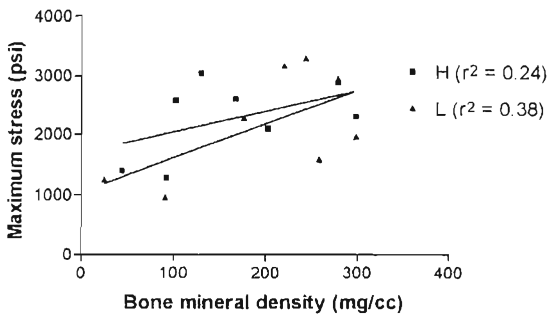


Fig. 3. Relationships of maximum stress of metatarsal cancellous bone to bone mineral density determined by quantitative computed tomography, at high strain rate (H), $r^2 = 0.24$, $P = 0.218$, and at low strain rate (L), $r^2 = 0.38$, $P = 0.0166$, from moose skeletal remains from Isle Royale National Park.

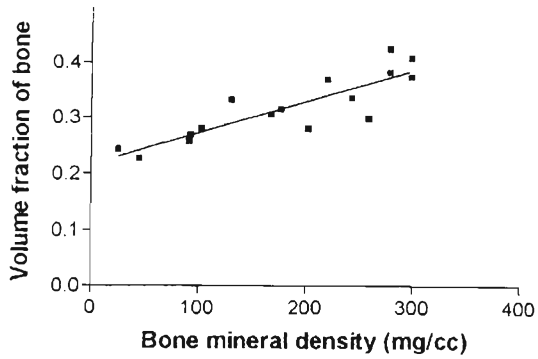


Fig. 5. Relationship between volume fraction of bone and QCT bone mineral equivalent of moose metatarsal cancellous bone ($r^2 = 0.749$, $P = 0.001$). Volume fraction of bone is indicative of the width and spacing of trabeculae, the supporting and shock-absorbing structures of cancellous bone.

(Fig. 4) were significantly correlated ($r^2 = 0.748$, $P < 0.0001$), consistent with McBroom *et al.* (1985), Lang *et al.* (1988), and Lotz *et al.* (1990). Our findings support the concept that good correlations between QCT number and density suggest that CT values are useful in predicting mechanical properties for cancellous bone (Rho *et al.* 1995).

The relationship between volume fraction of bone and QCT bone mineral equivalent (Fig. 5) was also significant ($r^2 = 0.749$, $P = 0.0001$). Volume fraction of bone is indicative of the width and spacing of trabeculae, and is important in predicting the mechanical behavior of cancellous bone (Pugh *et al.* 1973). Microscopic examination of the structure of the bone specimens before testing revealed that strong specimens had uniform structures with trabeculae of similar thickness and spacing, while weak specimens had decreased trabecular width and increased trabecular spacing (Fig. 6).

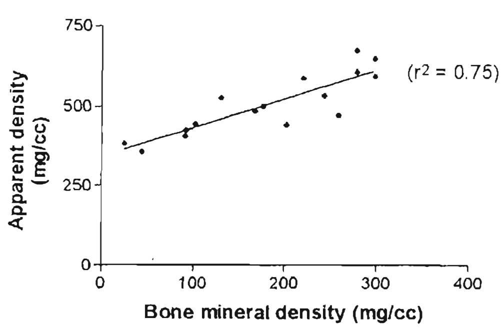
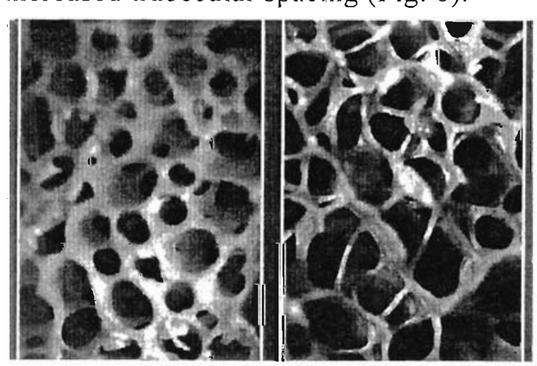


Fig. 4. Relationship between apparent density and QCT bone mineral equivalent density of moose metatarsal cancellus bone, $r^2 = 0.748$, $P < 0.0001$, indicating the usefulness of QCT values in predicting biomechanical performance of bone.



2315 2335

Fig. 6. Trabecular microstructure of moose cancellous bone specimens (x 40) from the distal metatarsus from skeletal remains collected on Isle Royale. Sample 2315 has uniform structures with solid trabeculae of uniform thickness and spacing, while sample 2335 has increased trabecular spacing with decreased and fractured trabeculae.

When subjected to biomechanical testing, specimens with strong trabecular microstructure performed far better than specimens with loss of trabecular connectivity (Fig 7). Trabecular perforation during bone resorption prevents new bone formation from occurring because the framework is lost, thus buckling fractures occur when horizontal supports of vertical rods are unable to withstand the stress (Pugh *et al.* 1973, Carter and Hayes 1976, Gibson 1985, Marcus 1991).

The biomechanical properties exhibited by the specimens are indicative of the condition of the animal at the time of death (Table 1). Moose that died of malnutrition tended to have the poorest bone mineral status and biomechanical performance and those killed by wolves had lower BMD and strength than moose that died of other causes (Hindelang 1996). In this small sample size, no significant trend in age or sex was found, but the collection process for the bones was biased toward the old and weak and not

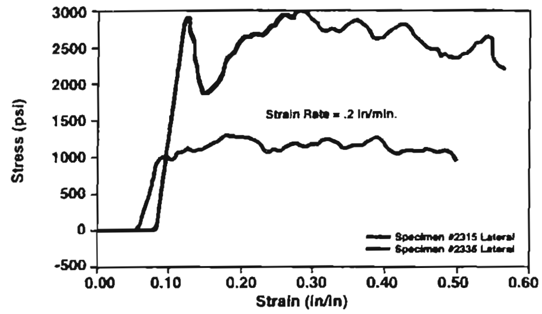


Fig. 7. Stress/strain curves for moose metatarsal cancellous bone specimens subjected to biomechanical testing. Specimen 2315 (top) is representative of a specimen with strong trabecular connectivity able to withstand maximum stress of nearly 3,000 psi before failure, while specimen 2335 (bottom) represents bone with increased trabecular spacing, thinning, and fracture at less than 1,500 psi.

Table 1. Summary of results of trabecular bone biomechanical properties and bone mineral density of metatarsal bones from moose that died in Isle Royale National Park.

Spec	Sex	Age	CD	BMD	Dns	VolFx	Rate	E	Str
1	F	14	W	278	675	0.426	0.20	8100	2888
2	M	14	W	102	446	0.281	0.20	6500	2573
3	M	14	W	167	486	0.306	0.20	7100	2598
4	M	15	M	92	427	0.269	0.20	5900	1280
5	F	8	M	202	443	0.280	0.20	8000	2102
6	M	14	M	130	527	0.332	0.20	7100	3041
7	M	13	M	44	359	0.226	0.20	6300	1396
8	F	15	W	298	594	0.375	0.20	8800	2302
9	M	9	W	258	474	0.299	0.02	7200	1590
10	F	14	W	278	608	0.384	0.02	7900	2953
11	M	15	M	91	408	0.257	0.02	5700	954
12	F	8	M	220	587	0.370	0.02	7000	3165
13	M	14	M	25	385	0.243	0.02	4300	1247
14	M	14	M	243	533	0.337	0.02	7000	3289
15	M	13	M	176	501	0.316	0.02	6100	2271
16	F	15	W	298	650	0.410	0.02	8400	1962

Note: CD = Cause of death, W = wolves, M = malnutrition. BMD = bone mineral density, mg/cm^3. Dns = apparent density, mg/cm^3. VolFx = volume fraction of bone. Rate = strain rate, in/min. E = elastic modulus, psi. Str = maximum stress, psi.

necessarily representative of the whole population. Areas for further study include age- and sex-related differences in cortical and cancellous bone on a large sample size. This study has implications for the understanding of moose bone fragility related to mechanical and geometric properties such as elasticity and trabecular microstructure in routine activities of daily living (foraging, ambulating, and bedding-down) as well as when subjected to stressors such as kicking predators, running, and falling.

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