Calcif Tissue Int (1994) 54:44–49

Calcified
Tissue
International

Effects of Magnesium Deficiency on Strength, Mass, and Composition of Rat Femur

M. A. Kenney, H. McCoy, L. Williams

Agricultural Experiment Station, University of Arkansas, Fayetteville, Arkansas 72701, USA

Received: 10 June 1993 / Accepted: 15 July 1993

Abstract. Magnesium (Mg) participates in the normal formation and remodeling of bone. However, little is known about effects of Mg status on the biomechanical function of bone. We examined gross morphometry and composition as well as biomechanical properties of the femurs of male rats fed diets adequate or deficient in Mg. Comparison of deficient animals and controls yielded a number of differences (all significant at $P < 0.05$). Mg-depleted animals exhibited slow growth, inefficient food utilization, and greatly reduced concentrations of Mg in both serum and femur ash. Compared with controls, femurs from depleted animals were shorter, but wet weights, diameters, and midfemoral cross-sectional areas showed no differences. Bone length was reduced to a greater degree than could be accounted for by differences in body weights between the groups. Bones of Mg-deficient rats contained less dry matter and less ash (which contained more Ca/g) than those of controls, along with a higher percentage of moisture. Significantly reduced bone strength in depleted animals was evident from the lighter loads supported at the elastic limit (yield point) and at fracture and from decreased stresses accompanying those loads. Modulus of elasticity, however, was not affected by Mg depletion. Different yield and breaking loads were related to different body weights of groups, but stresses were reduced for deficient bones even after adjusting for body size. Our data establish abnormal biomechanical behavior of cortical bone in Mg-deficient animals and emphasize the importance of measuring such functional properties of bone in the assessment of responses to altered metabolic conditions under experimental conditions.

Key words: Bone — Stress — Elasticity — Mechanical properties — Calcium

Bone mineral provides both rigid support of the animal body and a reservoir of calcium (Ca) and other elements, of which critical concentrations must be maintained in cells and fluids throughout the body. The criterion for adequate support function is the formation and maintenance of sufficient quantity and quality of bone to support the body throughout life and to withstand ordinary stresses to which skeletal components are subjected. Experiments with rats [1–3], mice [3], pigs [4, 5], and geese [5] have assessed bone strength after feeding different levels of Ca. Significant reduction in the peak (breaking) load tolerated was observed at very low Ca intakes except in one small study [5]. Other characteristics, such as stress and stiffness (or modulus of elasticity), responded less consistently to dietary Ca in these experiments. In a study in which Ca and P were varied independently in diets of pigs [4], it was possible to develop a prediction equation for bone-breaking force based on dietary Ca and P and age ($R^2 > 0.8$).

Besides the two major constituents of bone mineral, numerous other nutrients, such as Mg, may affect biomechanical properties through less obvious mechanisms than that for Ca. Not only is Mg a component of bone [6–8], but Mg status also influences osteocyte proliferation, tissue organization, mineralization, and resorption of bone [7, 9–12]. It may interact directly and indirectly with Ca [11, 13], temper the growth of apatite crystals [14, 15], interact with hormonal regulators of bone metabolism [12, 16–20], and may support functioning of osteoblasts [21]. Mg-depleted bones have been described as fragile or osteoporotic or brittle [6, 7], with thinning of the epiphyseal growth plate [22], disproportionately wide femur shafts [9, 10], and abnormal microstructural characteristics [9, 21]. Despite references to weakness of Mg-depleted bones, rigorous testing of biomechanical properties of such bones has not been reported. Information is needed about the influence of Mg on strength of bone to assess the skeletal adequacy of bones of animals that have been subjected to Mg deficiency [10–12, 21–23]. In this study, we determined mass, composition, and biomechanical properties of bone from growing male rats fed either a normal or low concentration of Mg.

Materials and Methods

Care of Animals

Male Sprague-Dawley rats (Hormone Assay Laboratories, Chicago, IL, USA), 32 days old and weighing from 97 to 126 g, were assigned to two groups of 19 animals each. Rats were housed individually in stainless steel wire-mesh cages in an air-conditioned laboratory having 12-hour automatic lighting. Food and distilled water were furnished *ad libitum* and replenished daily. Rats were weighed three times a week and examined daily for general condition and symptoms associated with Mg deficiency.

Rats were assigned to groups fed either an adequate control diet or a similar diet low in Mg. Diets met other recognized nutritional requirements of the rat [24], while providing energy sources resembling those of human diets in the US (40% of energy from blended fat having P/S = 0.4, 40% from carbohydrates, and 20% from protein). The diets contained, in g/kg: vitamin test casein (87% crude protein), 215.0; DL-methionine, 3.0; fat, 190.0; cornstarch, 270.0; sucrose,

Correspondence to: M. A. Kenney

Table 1. Body weight, food efficiency, and concentrations of Mg in serum and femur

Statistic	Diet	No.	Initial body wt	Final body wt	Food efficiency	Serum Mg	Serum Ca	Femur Mg
			g	g	g gain/g food	mEq/liter	mEq/liter	mg/g ash
LSM[a]	Low Mg	19	123	340[b]	0.318[b]	0.70[b]	5.48	0.18[b]
LSM	Control	19	122	433	0.385	1.56	5.39	0.57
SEM[c]			±2	±8	±0.007	±0.02	±0.04	±0.01

[a] Least squares mean (LSM), adjusted for days to sacrifice
[b] Different from controls, $P \leq 0.05$, from F test
[c] Pooled standard error of means from analysis of variance

160.0; AIN-76™ vitamin mix [24], 10.0; choline chloride, 2.0; menadione, 0.001; modified AIN-76 mineral mix [24], 35.0; finely ground cellulose, to make the total of 1000 g. The commercially prepared mineral mix was formulated to provide 50 mg Mg/kg finished ration (i.e., for the low Mg diet). For the control diet, Mg was added in an amount (4.554 g $MgSO_4 \cdot 7H_2O$/kg) calculated to bring its total to 500 mg/kg ration. Atomic absorption analysis showed 81 and 565 mg/kg in the low Mg and control rations, respectively.

The experiment was planned for a period of active bone growth and calcification. Because it was not possible to process tissues from all animals on a single day, four or five rats were selected randomly from each group to be sacrificed on empirically selected days 34, 36, 38, and 42. Ages of all animals, between 66 and 74 days at the end, were within the period of linear change in many bone characteristics [25]. Following an overnight fast, rats were anesthetized with an overdose of sodium pentobarbital. After exsanguination, serum was collected and frozen (−10°C). Femurs were removed, cleaned of adhering soft tissue, and weighed. Left femurs were frozen (−10°C) until determination of dry weight, ashing, and mineral analysis. Right femurs were submerged in physiological saline solution (0.85% NaCl) and frozen until dimensions and mechanical properties could be determined.

Strength Testing

Femur strength was assessed by a 3-point bending (flexure) test. The rationale for selection of a bending test included analogy to conditions in living animals, technical difficulties with compression testing, reduced variability of results compared with that from impact and twisting tests that mimic clinical injuries, and simplicity of the test and its interpretation [26]. It is sensitive and continues to be widely used [1, 4, 5, 27–29]. Recently, some have utilized a 4-point bending device to test bone *in vivo* [30]. The 4-point test subjects a wide area (rather than a single point) to uniform maximal load, permitting identification of the weakest point [31]. The variability in fracture site of individual bones and the possible induction of torsion or shear stresses in irregularly shaped small animal bones, however, may complicate interpretation of the results [30]. Results of 3- and 4-point tests are said to be comparable for small specimens [31]. No single test can assess response to all the causes of fracture in living animals. The choice of test should be governed by the objectives of the study, and probably is less important than rigorous maintenance of constant conditions for handling the bone before and during testing. As a tool for assessing experimental treatment effects, the 3-point test allows the investigator to predetermine a position on the bone at which maximal force will be applied, allowing standardized comparisons across treatment groups even if bones differ in size.

For measurements and mechanical testing, bones were thawed and equilibrated in saline at 27°, and then kept wetted with saline throughout these procedures. The length of the right femur (to the nearest 0.1 mm) and external midshaft diameters (perpendicular and parallel to force to be applied; determined to the nearest 0.001 mm) were measured by micrometer. The flexure test of the femur was performed with a testing machine (Instron Model 1000 Universal Testing Machine, Canton, MA, USA) with 50-kg weigh beam, 2.5 mm/minute crosshead speed, and supports set 11 mm apart. Internal diameters of the bone parallel and perpendicular to the breaking force were measured, using a magnifier with embedded measuring scale, after cutting through the bone shaft near the break with a fine-toothed steel saw. An elliptical shape was assumed in order to calculate cross-sectional area of the bone, a component of equations used to express mechanical properties.

Mathematical expressions of several mechanical properties were calculated [28] from bone dimensions, load applied to the bone during testing, and a tracing of load versus deformation [23] as the load was increased until the bone broke. Yield stress, yield strain, and modulus of elasticity were determined from the initial linear portion of the load-deformation curve [23]. Yield stress for the femur was calculated as the force per unit cross-sectional area, at the point where the curve ceased to be linear and plastic deformation began. Ultimate stress was determined similarly, but based on measurements at bone breakage under peak load.

Chemical Analysis

Left femurs were dried (105°C), weighed, ashed by heating to 600°C, weighed, dissolved in dilute (3 N) hydrochloric acid, and further diluted in lanthanum chloride solution (10 g La/liter) for measurement of Ca and Mg by flame atomic absorption spectrometry. Serum was also analyzed for total Ca and Mg after dilution in lanthanum chloride solution (1 g La/liter).

Data Analysis

Data were analyzed by analysis of variance using the SAS general linear models (GLM) procedure [32]. The model included Mg concentration of diet as a class variable and experimental day as a covariable, because animals were sacrificed at slightly different ages and after correspondingly different lengths of experimental treatment. Results are reported as least squares means, which represent estimations from GLM of treatment effects adjusted to the average length of feeding. Variability is represented by a single "pooled" standard error (SE) derived from the error mean square from the analysis. Differences between means are considered significant only if the F value for treatment effect was associated with probability of 0.05 or less.

To determine whether apparent effects of Mg were independent of differences in growth rates or body sizes, additional analyses included final body weight as a second covariable. From F tests based on type III ('partial') sums of squares [32], effects of Mg, age, and body weight could each be assessed, independent of the other variables.

Results

Mg Status of Animals

Mg deficiency in rats fed the low Mg diet was manifested in several ways. Although body weights did not differ at the beginning of the study, final weights did (Table 1). (All comparisons described as differences are significant at P

Table 2. Femur weights and dimensions

Statistic	Diet	Wet wt (mg)	Length (mm)	Outside diameter midshaft (mm)	Diameter/ length	Area of cross-section of diaphysis (mm^2)
LSM[a]	Low Mg	974	34.1[b]	4.06	0.119[b]	7.86
LSM	Control	1001	36.0	4.02	0.112	7.60
SEM[c]		±21	±0.2	±0.05	±0.001	±0.23
Wt-adjusted LSM	Low Mg	1045[b]	34.6[b]	4.19[b]	0.121[b]	8.30[b]
Wt-adjusted LSM	Control	930	35.5	3.89	0.109	7.18

[a] Least squares mean (LSM), adjusted for days to sacrifice
[b] Different from controls, $P \leq 0.05$, from F test
[c] Pooled standard error of means (SEM) from analysis of variance

≤ 0.05, as indicated in tables, whether or not the P value is repeated in the text.) Mg-depleted animals also used food less efficiently than controls. More specific indicators of Mg deficiency were the dramatic reductions in concentrations of Mg in serum (55%) and in femur ash (68%). In contrast, serum Ca concentration was not significantly affected by diet.

Morphometry of Femurs

Mean values for wet weights of femurs did not differ significantly, but fresh bones were heavier relative to body weight after Mg depletion (Table 2). This indicates that bone mass was affected to a lesser extent than was the rest of the body. Femur length, however, was reduced by the low Mg diet to such a degree that, even when adjusted to the same body weight, bones of deficient animals were still shorter than those of controls.

Despite shortness of Mg-depleted bones, the middiaphyseal diameters (averages of the two perpendicular measurements) were similar for the two groups (Table 2). In fact, when body size differences between the groups were accounted for (in weight-adjusted least squares means), femurs of deficient animals were relatively wider than those of controls. The ratio of diameter to length was greater in Mg deficiency, with or without adjustment for differences in body size. A comparison of inside diameters failed to show a treatment effect. The precision of measuring the small inside diameters, after the bone has been broken, is less than for outside dimensions, reducing the likelihood of detecting a difference. Another parameter relevant to functional integrity of bone is the cross-sectional bone area of the diaphysis. Area was calculated from perpendicular inside and outside diameters, assuming an elliptical shape. The comparison of areas between groups resembled that of outside diameters. No difference in cross-section was detected unless values were adjusted for body size; deficient animals would have manifested greater bone area than controls would if of the same weight.

Bone Composition

Dry bones from Mg-depleted rats weighed significantly less than those of controls (Table 3). Failure to detect a Mg effect in fresh weights, while noting a difference in dry weights, may be due to variability in removal of adhering tissue and in moisture content immediately after cleaning. Such variation should be reduced by drying the bones. However, there was a significantly greater percentage of moisture in bones of deficient animals, indicating that the quantitative effect on the mineral mass was selectively greater than on the organic components. Both fresh and dry weights for bones from depleted rats were heavier than normal in relation to body size.

The concentration of ash in dry bone was reduced by Mg deficiency (Table 3). This and the decrease in dry weight reflect an even greater reduction in the total amount of ash obtained per bone. Because depleted bones were shorter, ash weight was expressed relative to bone length, but ash/mm length also was reduced significantly. Therefore, Mg deficiency reduced the mineral content of femurs, whether expressed per unit bone weight, per unit length, or per whole bone. In contrast to ash, Ca concentration in dry Mg-depleted bones was unaffected. Bone Ca was more concentrated in the ash than normal, indicating a change in composition of the mineral phase.

Biomechanical Properties of Femur

The smaller femurs of Mg-deficient animals reached the elastic limit under a load of 6.55 kg, and fractured with 8.61 kg (Table 4). Both loads were lighter than those supported by bones of controls. This difference in load tolerance occurred in spite of similar diameters and cross-sectional areas of bones in the two groups (Table 3). As bending force increases under stress of increasing body weight [29], loads supported by bones from animals of the same size were estimated by including body weight in the analysis of variance. Weight-adjusted yield and peak loads, at the elastic limit and at breaking, respectively, did not change greatly from original means when adjusted for body size, but could no longer be judged different by the criterion for significance (Table 4).

Stress was determined by expressing the force of a load in relation to moment of inertia, based on bone diameters. Because of inclusion of bone dimensions in the calculation, stress is relatively independent of bone size. Both yield and ultimate stresses, corresponding to the load at the yield point and at fracture, respectively, were significantly lower than those in control bones. The difference between groups persisted after adjusting for differences for body weight. Without a reduction in diameter or cross-sectional area, the inability of the depleted bone to support stress as well as bones of control animals suggests deterioration in bone quality with Mg depletion.

Table 3. Femur composition in Mg deficiency and in control animals

Statistic	Diet	Dry wt			Ash wt			Ca	
		mg	mg/g body wt	Moisture (%)	mg/bone	mg/g dry bone	mg/mm length	mg/g dry bone	mg/g ash
LSM[a]	Low Mg	510[b]	1.51[b]	47.7[b]	309[b]	606[b]	9.05[b]	213	351[b]
LSM	Control	562	1.30	43.8	350	623	9.71	213	341
SEM[c]		±12	±0.02	±0.5	±6	±2	±0.16	±1	±2

[a] Least squares mean (LSM), adjusted for days to sacrifice
[b] Different from controls, $P \leq 0.05$, from F test
[c] Pooled standard error of means (SEM) from analysis of variance

Table 4. Biomechanical properties of right femurs from Mg-deficient and control rats

Statistic	Diet	Yield load	Peak load	Yield stress	Ultimate stress	Modulus of elasticity
		kg	kg	MPa	MPa	MPa
LSM[a]	Low Mg	6.55[b]	8.61[b]	77.8[b]	103[b]	1347
LSM	Control	7.79	11.03	94.6	135	1502
SEM[c]		±0.23	±0.39	±3.9	±7	±79
Wt-adjusted LSM	Low Mg	6.82	8.89	72.7[b]	94[b]	1157[b]
Wt-adjusted LSM	Control	7.51	10.76	99.3	143	1683

[a] Least squares mean, adjusted for days to sacrifice
[b] Different from controls, $P \leq 0.05$, from F test
[c] Pooled standard error of means from analysis of variance

Based on the linear (elastic) portion of the curves, bones of both groups showed similar bending, or strain, at the elastic limit. Young's modulus of elasticity, the stress/strain ratio, describes the stiffness of bone. Elasticity was not significantly affected by diet, unless values were adjusted for size differences. In the latter instance, Mg-depleted bones were significantly less stiff than those of controls. The behavior of depleted bones involved a normal extent of bending, but under a lighter load than predicted to affect control bones (from animals of the same size).

Discussion

These data emphasize the importance of including direct measurements of the mechanical properties of bones in experimental assessments of bone responses to metabolic conditions. There were no differences between controls and Mg-deficient rats for wet femoral weight, midshaft diameter, cross-sectional area of the femur shaft, and for Ca concentration in dry bone. Further, the fraction of body weight attributable to femoral mass and the concentration of Ca in ash were increased for Mg-deficient rats, indicating little negative effect of Mg deficiency on bone growth and calcification. However, disproportionate reduction in femoral length, as previously reported [9, 10], and the lower concentration of ash from bone denote limited growth and suggest impaired mineralization. The literature contains seemingly contradictory findings as to the bone ash and its Ca content in Mg deficiency (see [13]). Much of this confusion may relate to the transitory nature of some of the changes, as animals go through various stages of depletion. Mg-depleted bones have shown diminished rates of both apposition and resorption, which may produce both unmineralized osteoid and dense cortices [9]. Urinary hydroxyproline excretion was reduced coincidentally with maximal bone Ca concentration [13], pointing to slow resorption. Reduced activities of phosphatases in Mg-deficient bones (described as "brittle") [6] may mean altered turnover of bone mineral. Low activity of alkaline phosphatase from depleted bones was stimulated more by addition of Mg *in vitro* than that of controls, indicating a low degree of saturation *in vivo* with that cofactor. An increase in Ca concentration in ash has been attributed by some [6] to replacement of exchangeable adsorbed Mg ions with Ca during Mg depletion. However, that would account for only about 10% of the excess Ca we found in bone ash, consistent with reduced rate of bone resorption relative to accretion.

The reduced Mg and increased Ca concentrations of bone ash indicate qualitative changes in bone mineral. Considerable variation in the qualitative changes reported among various studies have made it difficult to depict Mg deficiency as a static condition. Mg deficiency is dynamic, with manifestations changing during the course of even a few weeks [13, 18]. For example, dry femoral weight was not significantly different after 30 days of feeding 40 mg Mg/kg diet to male rats (115 g initially), even though it had exceeded that of controls after the first 10 days [13]. Our rats were fed more Mg, for a longer time, and showed a decrease in dry weight after 34–42 days. It cannot be determined whether these describe a trend of falling dry weights, after an early increase, that would continue in a longer experiment, or whether other factors led to different responses. Though we found an increased concentration of moisture in whole femurs, others reported reduced water concentration in Mg-sensitive [13] metaphyses of femurs of older rats of a different strain; the length of depletion was similar to ours, but they fed less Mg [18]. The Mg content of hard tissues changes during normal development [8]. Consequently, the timing of Mg deficit in terms of stage of skeletal growth and development may also be critical in determining bone responses to Mg. Other experimental conditions that may influence the responses of

the rat to Mg deficiency include dietary Ca content [12, 16, 33]. It is therefore important that experiments describing Mg deficiency include a variety of measurements so that the stage of deficiency can be characterized.

When we examined mechanical properties of femurs, resistance to stress was lowered in Mg deficiency. Like other skeletal manifestations of Mg deficiency, changes in mechanical properties may vary with experimental conditions. Mildly Mg-depleted rats that grew normally showed normal bone strength except when a high level of nickel (ineffective in Mg-adequate rats) was also fed [23]. The greater degree of Mg depletion in our present study affected bone properties without the addition of a toxic agent.

Reduced stress resistance of femurs in Mg deficiency was accompanied by similar change in ash concentration, showing its relationship to bone mineral content. The modulus of elasticity, which measures stiffness, did not change, despite altered Ca content of bone. Although dietary Ca and P predictably altered bone mineral content, percentage of ash, and breaking load, they were inadequate to predict either modulus of elasticity or stress resistance [4]. Elasticity, in particular, may be determined by the organic matrix interacting with mineral. Until more is known about the roles of organic constituents that may be involved, mechanical properties must be assessed directly.

From data presented here, it seems likely that mild Mg deficiency can adversely affect bone strength without reducing total bone mass (wet tissue basis). However, that same degree of Mg deficiency decreased adversely both the dry bone mass and the femoral ash concentration, components considered important to bone strength; those changes were accompanied by increased water content of the fresh bone.

The progress of experimental Mg depletion usually has been followed by measuring serum Mg and Ca concentrations. Their involvement with bone through endocrine regulation is well recognized, but incompletely understood. Unlike most other mammals, rats have responded to Mg deficiency by becoming hypercalcemic, hypophosphatemic, and hypophosphaturic, suggestive of hyperparathyroidism [17, 18]. Circulating parathyroid hormone concentrations were increased after 5 days of feeding 10 mg Mg/kg diet to male weanling rats [18] and after 14 days of feeding 50 mg Mg/kg diet to male rats of 110 g initial weight [17]. The elevated levels disappeared by 20 days in the latter study and after 5 days in the former. Also, a study with Mg-deficient, male weanling rats (70 mg Mg/kg diet for 2–21 days) showed progressive hypercalcemia accompanied by decreasing activity of the parathyroid glands, as determined from histologic and morphometric examination [9]. It has been suggested that the hypercalcemia that accompanies the early increase in parathyroid hormone (PTH) secretion in Mg-deficient rats causes the parathyroid gland to respond appropriately to the increased blood Ca by reducing its secretion of PTH [18]. Plasma Ca levels remained high in Mg-deficient rats, however, even after PTH fell [17, 18], suggesting that hypercalcemia at that time was due to something other than PTH [18]. This conclusion is consistent with the coexistence of hypercalcemia and evidence of hypoactivity of the parathyroid glands [9]. Furthermore, as in the study reported in this paper, the frequent finding that concentration and/or amount of Ca in bone or bone ash is maintained or increases [9, 13, 19, 34] supports the idea that parathyroid-induced bone resorption does not explain the hypercalcemia of Mg deficiency. Accordingly, reduced sensitivity to PTH, as an alternative to its reduced secretion as an explanation for reduced bone resorption in Mg deficiency [9, 13, 20, 33], lacks credibility in light of evidence that infusion of PTH into parathyroidectomized, Mg-deficient rats brings about expected responses of bone [19] and a rapid decline in circulating PTH with continuing hypercalcemia [17, 18].

The rats in the present study were exposed to Mg deficiency for considerably longer than the 20-day period that reportedly reduced circulating PTH [17]. Although PTH was not measured, the thickening of bones (Table 2) and normalcy of serum Ca (Table 1) indicates that bones may not have been subjected to long-term elevated PTH. Thickened bones could also have resulted from the presence of subperiosteal exostoses and ectopic bone in the marrow. Such abnormalities have been demonstrated using radiographs of Mg-deficient femurs and humeruses [9]. Those histologic studies demonstrated clearly that Mg deficiency resulted in poor mineralization of osteoid tissue in the metaphyses. Reduced bone formation and bone resorption, also noted, possibly resulted from negative effects of Mg deficiency on osteoblasts and osteocytes [9]. A more recent study [21] also showed the Mg deprivation may affect osteoblasts directly and quickly. A reduction in circulating osteocalcin levels in rats after only 2 days of Mg deprivation preceded changes in PTH, 1,25-dihydroxyvitamin D, or calcium. Studies *in vitro* have also demonstrated that Mg concentrations in the models used affected the structure and stability of crystalline biological apatites [14, 15]. Thus, the short, thickened, highly hydrated femurs in our experiment could have become less stress resistant as a result of abnormal bone formation, reduced mineralization, and abnormal mineral composition of the femoral shaft where the breaking force was applied.

In addition to our findings that bones from Mg-deficient rats exhibited reduced tolerance to stress, fragmentary data suggest a possible link between Mg status and bone loss leading to fractures in the elderly human population, of which as many as 20% may be in poor Mg status [35]. Serum Mg was lower in osteoporotic patients than in controls [36]. Dietary Mg (but surprisingly not Ca) was a significant predictor of bone mineral density in Australian women [37]. A recent, apparently successful, attempt to improve bone density in postmenopausal women by Mg therapy (in addition to estrogen) [38] emphasizes the need to understand the effects of Mg status on bone. If a relationship of Mg status to bone loss in the human can be confirmed, serum or blood cell Mg concentration might easily be included in screening to identify individuals at high risk for osteoporosis [36]. Our observation that deficient rats had femurs that were not of normal strength, despite containing a normal concentration of Ca, suggests a need to examine elements such as Mg, in addition to Ca, in evaluating bone status.

References

1. Ferretti JL, Tessaro RD, Audisio EO, Galassi CD (1985) Long-term effects of high or low Ca intakes and lack of parathyroid function on rat femur biomechanics. Calcif Tissue Int 37:608–612
2. Thomas ML, Ibarra MJ, Solcher B, Wetzel S, Simmons DJ (1988) The effect of low dietary calcium and calcium supplementation on calcium metabolism and bone in the immature, growing rat. Bone Miner 4:73–82
3. Wolinsky I (1975) The mechanical strength of femurs of rats and mice fed a calcium-deficient diet—a differential species response. Comp Biochem Physiol 52A:381–383
4. Crenshaw TD (1986) Reliability of dietary Ca and P levels and bone mineral content as predictors of bone mechanical properties at various time periods in growing swine. J Nutr 116:2155–2170
5. Moyle DD, Walker MW (1986) The effects of a calcium-deficient diet on the mechanical properties and morphology of goose bone. J Biomech 19:613–625

6. Lai CC, Singer L, Armstrong WD (1975) Bone composition and phosphatase activity in magnesium deficiency in rats. J Bone Joint Surg 57A:516–522
7. Wallach S (1990) Effects of magnesium on skeletal metabolism. Magnes Trace Elem 9:1–14
8. Robinson C, Weatherell JA (1990) Magnesium in bone and tooth. In: Sigel H, Sigel A (eds) Metal ions in biological systems, vol 26. Marcel Dekker, New York, pp 489–504 (chapter 3)
9. Jones JE, Schwartz R, Krook L (1980) Calcium homeostasis and bone pathology in magnesium-deficient rats. Calcif Tissue Int 31:231–238
10. McCoy H, Kenney MA, Gillham B (1979) Effects of magnesium deficiency on composition and conformation of femur in rats of different ages. Nutr Rep Int 19:233–240
11. Ericsson Y, Luoma H, Ekberg O (1986) Effects of calcium, fluoride and magnesium supplementations on tissue mineralization in calcium- and magnesium-deficient rats. J Nutr 116:1018–1027
12. Welsh JJ, Weaver VM (1988) Adaptation to low dietary calcium in magnesium-deficient rats. J Nutr 118:729–734
13. Rayssiguier Y, Larvor P (1978) Mineral bone composition and some elements of calcium metabolism in magnesium-deficient growing rats. Ann Biol Anim Bioch Biophys 18:157–166
14. Aoba T, Moreno EC, Shimoda S (1992) Competitive adsorption of magnesium and calcium ions onto synthetic and biological apatites. Calcif Tissue Int 51:143–150
15. Bigi A, Foresti E, Gregorini R, Ripamonti A, Roveri N, Shah JS (1992) The role of magnesium on the structure of biological apatites. Calcif Tissue Int 50:439–444
16. Clark I, Rivera-Cordero F (1973) Effects of endogenous parathyroid hormone on calcium magnesium and phosphate metabolism in rats. Endocrinology 92:62–71
17. Rayssiguier Y, Thomasset M, Garel JM, Barlet JP (1982) Plasma parathyroid hormone levels and intestinal calcium binding protein in magnesium-deficient rats. Horm Metab Res 14:379–382
18. Anast CS, Forte LF (1983) Parathyroid function and magnesium depletion in the rat. Endocrinology 113:184–189
19. Hahn TJ, Chase LR, Avioli LV (1972) Effect of magnesium depletion on responsiveness to parathyroid hormone in parathyroidectomized rats. J Clin Invest 51:886–891
20. MacManus J, Heaton FW, Lucas PW (1971) A decreased response to parathyroid hormone in magnesium deficiency. J Endocrinol 49:253–258
21. Carpenter TO, Mackowiak SJ, Troiano N, Gundberg CM (1992) Osteocalcin and its message: relationship to bone histology in magnesium-deprived rats. Am J Physiol 263:E107–E114
22. Messler H, Kühr J, Helbig B, Münzenberg KJ (1989) The influence of magnesium on the endochondral ossification of the growth plate under physiological and experimental conditions. Magnes Res 2:95
23. Kenney MA, McCoy H, Williams L (1992) Effects of dietary magnesium and nickel on growth and bone characteristics in rats. J Am Coll Nutr 11:687–693
24. American Institute of Nutrition (1977) Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. J Nutr 107:1340–1348
25. Sontag W (1992) Age-dependent morphometric alterations in the distal femora of male and female rats. Bone 13:297–310
26. Ascenzi A, Bell GH (1972) Bone as a mechanical engineering problem. In: Bourne GH (ed) The biochemistry and physiology of bone, 2nd ed, vol I. Academic Press, New York, pp 311–352
27. Crenshaw TD, Peo ER, Lewis AJ, Moser BD (1981) Bone strength as a trait for assessing mineralization in swine: a critical review of techniques involved. J Anim Sci 53:827–835
28. Crenshaw TD, Peo ER, Lewis AJ, Moser BD, Olson D (1981) Influence of age, sex and calcium and phosphorus levels on the mechanical properties of various bones in swine. J Anim Sci 52:1319–1329
29. Weir JBV, Bell GH, Chambers JW (1949) The strength and elasticity of bone in rats on a rachitogenic diet. J Bone Joint Surg 31B:444–451
30. Akhter MP, Raab DM, Turner CH, Kimmel DB, Recker RR (1992) Characterization of *in vivo* strain in the rat tibia during external application of four-point bending load. J Biomech 25: 1241–1246
31. McAlister GB, Moyle DD (1983) Some mechanical properties of goose femoral cortical bone. J Biomech 16:577–589
32. SAS Institute Inc (1988) SAS/STAT user's guide, release 6.03, SAS Institute Inc, Cary, NC
33. MacManus J, Heaton FW (1969) The effect of magnesium deficiency on calcium homeostasis in the rat. Clin Sci 36:297–306
34. Smith BSW, Nisbet DI (1968) Biochemical and pathological studies on magnesium deficiency in the rat. I. Young animals. J Comp Pathol 78:149–159
35. Touitou Y, Godard JP, Ferment O, Chastang C, Proust J, Bogdan A, Auzéby A, Touitou C (1987) Prevalence of magnesium and potassium deficiencies in the elderly. Clin Chem 33: 518–523
36. Reginster JY, Strause L, Deroisy R, Lecart MP, Saltman P, Franchimont P (1989) Preliminary report of decreased serum magnesium in postmenopausal osteoporosis. Magnesium 8:106–109
37. Angus RM, Sambrook PN, Pocock NA, Eisman JA (1988) Dietary intake and bone mineral density. Bone Miner 4:265–277
38. Abraham GE, Grewal HG (1990) Effect of total dietary program emphasizing magnesium instead of calcium on the mineral density of calcaneous bone in postmenopausal women on hormonal therapy [abstract]. Magnes Trace Elem 9:327