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REVIEW: CURRENT TOPICS

Magnesium deficiency and osteoporosis: animal and human observations

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Abstract

Although osteoporosis is a major health concern for our growing population of the elderly, there continues to be a need for well-designed clinical and animal studies on the link between dietary magnesium (Mg) intake and osteoporosis. Relatively few animal studies have assessed the skeletal and hormonal impact of long-term low Mg intake; however, these studies have demonstrated that Mg deficiency results in bone loss. Potential mechanisms include a substance P-induced release of inflammatory cytokines as well as impaired production of parathyroid hormone and 1,25-dihydroxyvitamin D. Abnormal mineralization of bones may also contribute to skeletal fragility. Clinical studies have often varied greatly in study design, subject age, menopausal status and outcome variables that were assessed. Most studies focused on female subjects, thus pointing to the great need for studies on aging males. According to the U.S. Department of Agriculture, the mean Mg intake for males and females is 323 and 228 mg/day, respectively. These intake levels suggest that a substantial number of people may be at risk for Mg deficiency, especially if concomitant disorders and/or medications place the individual at further risk for Mg depletion. In this paper, we will review animal and human evidence of the association of Mg deficiency with osteoporosis and explore possible mechanisms by which this may occur.

Keywords: Magnesium; Osteoporosis; Parathyroid hormone; 1,25$(OH)_2$-vitamin D; Substance P; Tumor necrosis factor

1. Introduction

Magnesium (Mg) is the fourth most abundant cation in the body and the second most prevalent intracellular cation [1,2]. In terms of its physiological role, Mg is essential for many enzymatic reactions [3]. Mg has two general interactions: first, it can bind to an enzyme substrate, thereby forming a complex with which the enzyme interacts, as in the reaction of kinases with magnesium ATP^{2-} (MgATP). Second, Mg binds directly to the enzyme and alters its structure and/or serves a catalytic role (e.g., exonuclease, topoisomerase and RNA and DNA polymerases) [4,5]. Overall, the predominant action of Mg is related to ATP utilization; it exists in all cells primarily as MgATP. Mg deficiency has been associated with hypertension, cardiac arrhythmias, myocardial infarction, hypokalemia and hypocalcemia [6]. Approximately 50–60% of Mg is in the skeleton, and dietary Mg deficiency has also been implicated as a risk factor for osteoporosis [6]. The current recommended daily allowance for Mg for adult males and females is 420 and 320 mg/day, respectively [7]. According to the U.S. Department of Agriculture, the mean Mg intake for males and females is 323 and 228 mg/day, respectively [8]. These intake levels suggest that a substantial number of people may be at risk for Mg deficiency, especially if concomitant disorders and/or medications place the individual at further risk for Mg depletion. Unfortunately, the mechanistic results of low Mg intake have been more thoroughly investigated in tissues such as muscles than they have been in bones. This is due in part to the complex hormonal regulatory mechanisms that function to maintain bone homeostasis and is also due to the difficulties of investigating osteoblast and osteoclast functions in vitro in ways that are directly translatable to the in vivo response. Thus, the review presented here builds upon a small body of investigative animal research and clinical studies to present findings on the effect of low Mg intake on bone and mineral metabolism.

2. Animal models

Experimental research studies on Mg deficiency have usually used a rat model with severe restriction of Mg intake

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ranging from 0.2 to 8 mg/100 g chow (normal=50–70 mg/100 g chow) [9–14]. In animal studies using young growing animals exposed to Mg deficiency, epiphyseal and diaphyseal growth plates are thinned and there is a decrease in the number and organization of chondrocytes [11–13]. We have recently assessed the effect of a more moderate dietary Mg restriction, 10% of the nutrient requirement (NR), on the growth plate and articular cartilage in rats following a 6-month dietary Mg restriction [15,16]. Histomorphometry demonstrated decreased distal femur articular cartilage chondrocyte density and decreased tibial growth plate width. Growth plates of low-Mg animals showed reduced chondrocyte column formation. Matrices of both articular and growth plates in treated animals displayed decreased glycoprotein content. Immunolocalization of SOX9 was decreased in both the articular and growth plate cartilage in treated animals compared with controls, suggesting that Mg intake at this reduced level causes cartilage changes that may be secondary to reduced levels of the SOX9 transcription factor [16].

In terms of bone mass, a reduction of trabecular bone mass has been demonstrated in rats and mice by histomorphometry and microcomputed tomography at very low dietary Mg intakes [10–12,14]. A similar finding has now been reported at a dietary Mg intake of 10% of NR [15]. This, in part, appears to be secondary to reduced osteoblastic bone formation as assessed by quantitative histomorphometry [11,13,17]. In addition, reduction in serum and bone alkaline phosphatase [12,18,19] and osteocalcin [10,17,20] as well as a decrease in collagen formation [21] support a reduction in osteoblast function. Osteoclast activity and number, however, appear to be increased in rats and mice at dietary intakes of 0.2% [11,13] and 10% [15] of NR. Therefore, there appears to be an uncoupling of osteoblast and osteoclast activities.

Bones from Mg-deficient animals have been described as brittle and fragile [19,22]. Biomechanical studies have demonstrated increased skeletal fragility in rats and pigs [10,23–26].

3. Human observations

3.1. Epidemiological studies

Epidemiological studies provide a link associating dietary Mg inadequacy to osteoporosis; however, differences in study populations and study designs often make such clinical data difficult to interpret and compare.

In premenopausal women, one study found a significant correlation between the bone mineral density (BMD) of the lumbar spine and Mg intake [27]. Another study of premenopausal women found a positive correlation between the BMD of the forearm, but not of the femur or spine, and Mg intake [28]. In a third study of premenopausal women, a significant relationship between dietary Mg intake and the rate of change of the BMD of the lumbar spine over a 1-year period was observed [29]. In preadolescent girls, Mg intake was positively related to quantitative ultrasound properties of the bone; this finding suggests that Mg was important in skeletal growth and development [30].

A cross-sectional study of premenopausal and postmenopausal women did not find a correlation between Mg intake and BMD in the distal forearm [31]. However, a longitudinal observation over 4 years in this same study population demonstrated that loss of bone mass was inversely related to Mg intake. Another study of postmenopausal women did show a significant positive correlation between the BMD of the forearm and Mg intake [32]. Moreover, in older females in a cross-sectional study and a 2-year longitudinal study, a positive correlation between the BMD of the hip and Mg intake was present in the cross-sectional analysis but not in the longitudinal assessment data [33].

A positive correlation between Mg intake and appendicular BMD was found in women aged 43–80 years [34]. In a study of women 45–55 years old, higher intakes of Mg were associated with higher bone mass of the forearm (but not of the femoral neck or hip) [35]. However, such a correlation was not found in women who recently underwent menopause [28]. In addition, a cross-sectional study combining premenopausal and postmenopausal women aged 28–74 years found no such correlation with BMD at the lumbar spine, femoral neck or total body calcium [36].

There are only a few epidemiological studies on Mg intake and bone density in male subjects. In a cross-sectional study, a significant correlation between Mg intake and BMD at the hip in elderly men aged 69–97 years was observed [33]. A subsequent longitudinal analysis over a 4-year period showed less bone loss in men with higher intakes of Mg, potassium, fruits and vegetables [37]. In another study in men aged 61–81 years, no correlation was present between mean Mg intake and appendicular BMD. Analysis of a subset of these men taking Mg supplements, however, did identify a significant correlation between Mg intake and appendicular BMD [34].

In summary, these epidemiological studies link dietary Mg intake to bone mass but point to the need of additional well-designed studies that would include both male and female subjects. Exceptions to the link between dietary Mg intake and bone mass appear to include women in the early postmenopausal period, in which the effect of acute sex steroid deficiency may mask the effect of dietary factors such as Mg. In addition, diets deficient in Mg are usually also deficient in other nutrients, especially calcium, which also affect bone mass. Further investigations are needed to provide a firm relationship between dietary Mg inadequacy and osteoporosis.

3.2. Effect of dietary Mg on bone turnover

"Bone markers" are the biochemical products released into circulation during the process of bone formation (e.g., serum osteocalcin and bone-specific alkaline phosphatase) or resorption (e.g., urine deoxypyridinoline and *N*-telopeptide

of collagen cross-links). Elevations in these markers reflect an increase in bone turnover and have been used in various studies to predict bone loss [38].

In two of the epidemiological studies cited above, markers of bone turnover were included in the study design. When no correlation was found between BMD and dietary Mg intake, serum osteocalcin did not correlate with Mg (or any other nutrient) intake [36]. In another study, although serum osteocalcin was not associated with dietary intake of Mg or other nutrients, Mg intake was negatively correlated with urinary excretion of pyridinoline and deoxypyridinoline; this finding suggests that a low-Mg diet was associated with increased bone resorption [35].

3.3. Mg status in patients with osteoporosis

While most body Mg, 50–60%, resides in the skeleton, and skeletal Mg reflects Mg status, studies have reported low, normal or high bone Mg content in osteoporosis [39–45].

Few studies that assessed Mg status in patients with osteoporosis have been performed. Low serum values of Mg, copper and zinc were found in osteoporotic subjects compared with nonosteoporotic postmenopausal women [46]. In another study involving perimenopausal and postmenopausal women, women with severe osteoporosis had significantly lower serum ionized Mg levels [47]. In patients with Crohn's disease, higher serum Mg predicted higher BMD at the femur; however, a negative correlation was observed in the spine [48]. Family members affected with an autosomal dominant form of primary hypomagnesemia due to renal Mg wasting demonstrated significant reductions in serum and lymphocyte Mg concentrations as well as significantly reduced BMD in the lumbar spine and proximal femur [49]. These data suggest that hypomagnesemia may be associated with low bone mass.

Mg tolerance testing and serum Mg levels were techniques used to assess elderly osteoporotic subjects aged 70–85 years compared with nonosteoporotic subjects [50]. Both groups had normal serum Mg concentrations. The Mg tolerance test, however, revealed a significantly greater retention in the osteoporotic patients, 38%, as compared with 10% in the control subjects, suggesting Mg deficiency. [The Mg tolerance test is based on the amount of Mg excreted into the urine following an intravenous infusion of Mg. Normal subjects usually retain less than 20% of the infused Mg load. Greater than 20% retention is suggestive of Mg deficiency and greater than 50% retention confirms Mg deficiency. While this is a good indicator of Mg status, it is cumbersome because it requires an intravenous infusion and 24-h urine collections. In addition, any disorder (e.g., diabetes mellitus) or drug (e.g., diuretics) that causes urine Mg loss would negate the usefulness of this test.] In contrast, 12 younger osteoporotic women aged 55–65 years had significantly lower serum Mg concentrations than control subjects, but no difference in the Mg tolerance test results was observed [39]. Red blood cell Mg was found to be significantly lower in postmenopausal women who had at least one vertebral fracture as compared with subjects with degenerative osteoarthritis; however, no difference in plasma Mg was found [40]. In a second report, however, Mg levels in postmenopausal women with vertebral crush fracture had a significantly lower serum Mg but no difference in red blood cell Mg compared with nonosteoporotic women [51].

In summary, Mg status has been assessed in very few osteoporotic patients. Low serum and red blood cell Mg concentrations, as well as high retention of parenterally administered Mg, have suggested a Mg deficit. These results, however, are not consistent from one study to another and are often difficult to compare. Similarly, while low skeletal Mg content has been observed in some studies, others have found normal or even high Mg content.

3.4. Effect of Mg therapy on osteoporosis

The effect of dietary Mg supplementation on bone mass in patients with osteoporosis has not been extensively studied. Administration of 600 mg of Mg per day over 6–12 months to 19 patients demonstrated that the BMD of the calcaneus increased by 11% compared with a 0.7% rise in that of control subjects [45]. All subjects were postmenopausal, undergoing sex steroid replacement therapy and received 500 mg of calcium per day as well as many other dietary supplements, thus making it difficult to conclude that Mg alone was the sole reason for the increase in bone mass. In a retrospective study, administration of 200 mg of Mg per day, given to postmenopausal women, was observed to produce a small nonsignificant 1.6% rise in the bone density of the lumbar spine; no change was seen in the femur [52]. A 2-year trial prospective study in which postmenopausal osteoporotic women were given 250 mg of Mg per day, increasing to a maximum of 750 mg/day for 6 months depending on tolerance, was conducted [53]. All subjects were given 250 mg of Mg per day from months 6 to 24. Age-matched subjects served as controls. At 1 year, there was a significant 2.8% increase in the bone density of the distal radius. Twenty-two of the 31 subjects had an increase in bone density while that of 5 did not change. No significant effect of Mg supplementation was shown at 2 years, although only 10 subjects completed the trial. In a small uncontrolled trial, a significant increase in the bone density of the proximal femur and lumbar spine was found in celiac sprue patients who received approximately 575 mg of Mg per day for 2 years [54]. These subjects had shown evidence of reduced free Mg in red blood cells and peripheral lymphocytes.

In summary, the effect of Mg supplements on bone mass has generally led to an increase in BMD, although study design variations limit the usefulness of some of this clinical information.

4. Potential mechanisms for Mg-induced bone loss

A number of potential mechanisms may contribute to Mg-deficiency-induced bone loss. These are illustrated in Fig. 1.

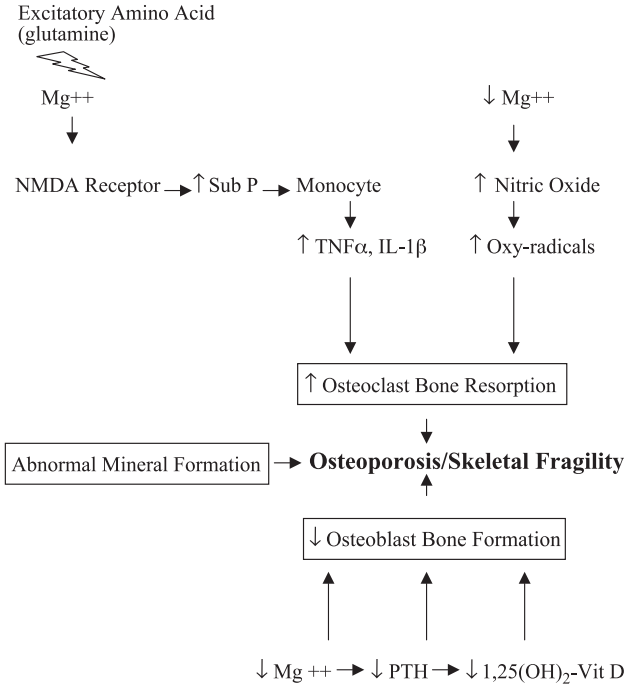

Fig.1. Potential mechanisms for Mg-deficiency-induced bone loss include the following. (1) Increased release of substance P as low Mg enhances the stimulation of the NMDA receptor by excitatory amino acids. Substance P then stimulates TNFα and IL-1β production by monocytes that in turn activate osteoclasts. (2) The development of oxidative stress during Mg depletion may increase oxyradicals that also may induce osteoclastic bone resorption. (3) Osteoblast bone formation may decrease due to loss of a mitogenic effect on this cell or by decreased PTH secretion and/or decreased 1,25(OH)$_2$-vitamin D production. (4) The formation of larger and more perfect crystals may also contribute to skeletal fragility.

4.1. Direct effect of low Mg on cell function

Mg plays a crucial role in cell proliferation and function. Cells are unable to proliferate in the absence of extracellular Mg because of the resultant reduction in DNA, RNA and protein synthesis [55,56]. In a recent study, differentiation of malignant cell lines was shown to be influenced by extracellular Mg concentrations [57,58].

Approximately 50–60% of body Mg is in the bones [59]. The majority of Mg in bones is incorporated into the hydroxyapatite crystal. One third is surface limited and appears to serve as a reservoir for maintaining extracellular Mg concentration. Experimental Mg deficiency results in a 30–40% fall in bone Mg content [11,13,15].

Mg appears to be important in bone cell activity. It is mitogenic for osteoblasts in culture [60] and its depletion causes cellular growth inhibition in vitro [61]. Thus, Mg deficiency may directly result in a decrease in osteoblastic bone formation. We have observed a decrease in the osteoblast number of Mg-deficient rodents [11,13,15]. In other studies, serum and bone alkaline phosphatase and osteocalcin and bone osteocalcin mRNA were reduced; this finding suggests that there was a decrease in osteoblastic function [12,17,19,20]. Decreased collagen formation, sulfation of glycosaminoglycans [17] and decreased tetracycline labeling [13] have also been observed. In a recent study of less severely depleted rats, osteoblast number did not differ from control subjects; markers of osteoblastic bone formation (serum alkaline phosphatase and osteocalcin) were reduced, however, suggesting the presence of impaired osteoblast activity [15]. A recent study has suggested that Mg results in an increase in osteoblast adhesion to bioceramic surfaces via an integrin-mediated mechanism [62]. These above-cited studies suggest that variation in Mg concentration may directly affect osteoblast function and bone metabolism.

4.2. Effect on parathyroid hormone and 1,25(OH)$_2$-vitamin D

Calcium is the major regulator of parathyroid hormone (PTH) secretion. Acute changes in extracellular Mg concentrations will influence PTH secretion in a manner qualitatively similar to calcium by binding to the calcium-sensing receptor. Mg deficiency, however, perturbs mineral homeostasis, and hypocalcemia is a prominent manifestation of Mg deficiency [6,63,64]. One major cause for hypocalcemia is impaired parathyroid gland function [6,63]. Most patients with hypocalcemia due to Mg deficiency have low or inappropriately normal serum PTH levels. The administration of Mg will result in an immediate rise in the serum PTH level. The presence of normal or elevated serum concentrations of PTH in the face of hypocalcemia suggests that there may also be end-organ resistance to PTH action [63]. Skeletal resistance to exogenous PTH in hypocalcemic Mg-deficient patients has been reported [65]. Similarly, urinary excretion of cyclic adenosine monophosphate (AMP) and/or phosphate in response to PTH in such patients has been observed [6,63].

The mechanism for impaired PTH secretion and action in Mg deficiency remains unclear. It has been suggested that there may be a defect in the second messenger systems in Mg depletion. Adenylate cyclase has been universally found to require Mg for cyclic AMP generation both as a component of the substrate (MgATP) and as an obligatory activator of enzyme activity [66,67]. PTH has also been shown to activate the phospholipase C second messenger system. Mg depletion could perturb this system via several mechanisms because a Mg-dependent guanine nucleotide regulating protein is involved in activation of phospholipase C; Mg has also been shown to be a noncompetitive inhibitor of inositol 1,4,5-trisphosphate-induced calcium release [68,69].

Mg is also important in vitamin D metabolism and/or action [70]. Patients with hypocalcemia and Mg deficiency have been reported to be resistant to pharmacological doses of vitamin D, 1α-hydroxyvitamin D and 1,25-dihydroxyvitamin D [1,25(OH)$_2$-vitamin D] [70,71]. The exact nature of altered vitamin D metabolism and/or action in Mg deficiency is unclear. Serum concentrations of 1,25(OH)$_2$-vitamin D have been found to be low or low–normal in most hypocalcemic Mg-deficient patients [70,71]. We have also reported that serum 1,25(OH)$_2$-vitamin D is profoundly

lowered in Mg-depleted rats [11,15]. Because PTH is a major trophic for 1,25$(OH)_2$-vitamin D formation, the low serum PTH concentrations could explain the low 1,25$(OH)_2$-vitamin D levels. Mg is also known to support 25-hydroxy-1α-hydroxylase in vitro [72], suggesting that Mg deficiency may directly impair the ability of the kidney to synthesize 1,25$(OH)_2$-vitamin D. Because both hormones are trophic for the osteoblast, these changes in PTH and 1,25$(OH)_2$-vitamin D levels may contribute to impaired bone formation.

4.3. Abnormal bone crystal formation and mineralization

Mg directly affects the process of mineralization. It is known to inhibit hydroxyapatite crystal growth in solution [73,74]. It also binds to the surface of apatite crystals and retards their formation and growth [73,74]. When rats are fed excess Mg, the mineral crystals in their bones are smaller than those in control animals [75], whereas crystal size and perfection are significantly increased in Mg-deficient rats [10]. Postmenopausal women with osteoporosis and documented Mg deficiency were found to have larger and more perfect crystals in the trabecular bone as assessed on infrared spectrophotometry [76]. Another study demonstrated decreased bone Mg and high crystallinity index in senile osteoporosis and type 2 diabetes, whereas increased bone Mg and a low crystallinity index were found in women undergoing hormone replacement therapy [39]. Therefore, there appears to be an inverse correlation between bone Mg and crystallinity index. The size of mineral crystals influences mechanical properties. When crystals are excessively large, bones may be brittle and not able to bear a normal load [73–76].

Mg deficiency has also been observed to delay the onset of mineralization of newly formed cartilage matrix and bones [77]. Whether this is due to the effect on crystal formation or to some other process in mineralization is unclear. These abnormalities, however, could contribute to reduced bone mass and skeletal fragility induced by Mg deficiency.

4.4. Magnesium-deficiency-induced increase in inflammatory cytokines

We have observed that Mg-deficient rats and mice demonstrated an increase in osteoclast number, surface area covered by osteoclasts and eroded surface [11,13,15]. This finding suggests that increased bone resorption was a major factor causing the decrease in bone mass. A study in humans found that dietary Mg intake was the strongest predictor of urinary deoxypyridinolium excretion; that is, a low Mg intake was associated with elevated urinary deoxypyridinoline [35].

The potential mechanism(s) of the increase in osteoclastic bone resorption is unclear at present. Mg has been shown to inhibit the *N*-methyl d-aspartate (NMDA) receptor [78]; reduction of extracellular Mg lowers the threshold level of excitatory amino acids necessary to activate this receptor. Activation of the NMDA receptor induces the release of neurotransmitters such as substance P [79]. Dietary Mg deficiency produces elevated serum levels of neuropeptides such as substance P within 1–3 days of low Mg diet introduction in rodents [79,80] and is followed by release of proinflammatory cytokines [interleukin (IL)-1β and tumor necrosis factor (TNF)-α] within the first week of dietary Mg depletion [79]. Substance P is known to be released at nerve endings in bones and to enhance osteoclastic bone resorption [81,82]. We have observed localized increased levels of substance P, TNF-α and IL-1β in cellular elements of the bone marrow microenvironment of Mg-deficient mice and rats using immunohistochemical techniques [13,15]. This localized cytokine increase may contribute to increased osteoclast numbers and bone resorption, thus explaining the uncoupling of bone formation and bone resorption observed in Mg-deficient rats [11,15] and mice [13]. These cytokines have also been proposed to contribute to an increase in osteoclastic bone resorption in postmenopausal women [83]. Mg deficiency is also associated with oxidative stress [84,85]. There is a stimulation of nitric oxide and an increase in oxyradical production. The final effector molecule in osteoclastic bone resorption appears to be the receptor activator of nuclear factor-κB ligand [86]. Recently, induced oxidative stress in rats resulted in increased bone resorption [87] and markers of oxidative stress predicted BMD in women [88], suggesting that this may be another mechanism for Mg-deficiency-induced bone loss.

5. Conclusions and outlook

The relatively small body of basic science and clinical data that are available in the literature on the effects of long-term low-Mg dietary intake have been reviewed here. Fundamental questions can now be formulated to answer questions on how low Mg influences bone mass, bone turnover, the bone-related hormones and cytokine levels. For instance, what dietary Mg intake is required to maintain skeletal health? In addition, the impact of other common nutrient deficiencies such as calcium and vitamin D on low dietary Mg-induced bone loss has not been investigated. The potential role of inflammatory cytokines in mediating the adverse effect of Mg deficiency on bones must be investigated. The importance of elucidating the role of low Mg intake on the development of osteoporosis cannot be overly stressed because the aging population at risk for this disease continues to grow and contribute to health care costs.

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References

[1] Maguire ME, Cowan JA. Magnesium chemistry and biochemistry. Biometals 2002;15:203–10.
[2] Wolf FI, Cittadini A. Chemistry and biochemistry of magnesium. Mol Aspects Med 2003;24:3–9.

[3] Cowan JA. Structural and catalytic chemistry of magnesium-dependent enzymes. Biometals 2002;15:225–35.
[4] Cowan JA. Introduction to the biological chemistry of magnesium. In: Cowan JA, editor. The biological chemistry of magnesium. New York: VCH Publishers; 1995. p. 1–24.
[5] Black CB, Cowan JA. Magnesium dependent enzymes in nucleic acid biochemistry (chapter 6) and magnesium dependent enzymes in general metabolism (chapter 7). In: Cowan JA, editor. The biological chemistry of magnesium. New York: VCH Publishers; 1995. p. 137–58.
[6] Rude RK. Magnesium deficiency: a heterogeneous cause of disease in humans. J Bone Miner Res 1998;13:749–58.
[7] Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington (DC): National Academy Press; 1997. p. 190–249.
[8] Cleveland LE, Goldman JD, Borrude LG. Data tables: results from USDA's 1994 continuing survey of food intakes by individuals and 1994 diet and health knowledge survey. Beltsville (MD): Agricultural Research Service, U.S. Department of Agriculture; 1994.
[9] Kenney MA, McCoy H, Williams L. Effects of magnesium deficiency on strength, mass and composition of rat femur. Calcif Tissue Int 1994; 54:44–9.
[10] Boskey AL, Rimnac CM, Bansal M, Federman M, Lian J, Boyan BD. Effect of short-term hypomagnesemia on the chemical and mechanical properties of rat bone. J Orthop Res 1992;10:774–83.
[11] Rude RK, Kirchen ME, Gruber HE, Meyer MH, Luck JS, Crawford DL. Chronic dietary-induced magnesium deficiency alters bone and mineral homeostasis in the rat. Magnes Res 1999;12:257–67.
[12] Mirra JM, Alcock NW, Shils ME, Tannenbaum P. Effects of calcium and magnesium deficiencies on rat skeletal development and parathyroid gland area. Magnesium 1982;1:16–33.
[13] Rude RK, Gruber HE, Wei LY, Frausto A, Mills BG. Magnesium deficiency: effect on bone and mineral metabolism in the mouse. Calcif Tissue Int 2003;72:32–41.
[14] Gruber HE, Rude RK, Wei LY, Frausto A, Mills BG, Norton HJ. Magnesium deficiency: effect on bone mineral density in the mouse appendicular skeleton. BMC Musculoskelet Disord 2003;4(17 Apr):7.
[15] Rude RK, Gruber HE, Norton HJ, Wei LY, Frausto A, Mills BG. Bone loss induced by dietary magnesium reduction to 10% of nutrition requirement in the rats is associated with increased release of substance P and tumor necrosis factor-α. J Nutr 2004;134:79–85.
[16] Gruber HE, Ingram J, Norton HJ, Wei LW, Frausto A, Mills BG, et al. Alterations in growth plate and articular cartilage morphology are associated with reduced SOX9 localization in the magnesium-deficient rat. Biotech Histochem 2004;79(1):45–52.
[17] Carpenter TO, Mackowiak SJ, Troiano N, Gundberg CM. Osteocalcin and its message: relationship to bone histology in magnesium-deprived rats. Am J Physiol 1992;263:E107–14.
[18] Loveless BW, Heaton FW. Changes in the alkaline phosphatase (EC 3.1.3.1) and inorganic pyrophosphatase (EC 3.6.1.1) activities of rat tissues during magnesium deficiency. The importance of controlling feeding pattern. Br J Nutr 1976;36:487–95.
[19] Lai CC, Singer L, Armstrong WD. Bone composition and phosphatase activity in magnesium deficiency in rats. J Bone Joint Surg 1975;57: 516–22.
[20] Creedon A, Flynn A, Cashman K. The effect of moderately and severely restricted dietary magnesium intakes on bone composition and bone metabolism in the rat. Br J Nutr 1999;82:63–71.
[21] Trowbridge HO, Seltzer JL. Formation of dentin and bone matrix in magnesium-deficient rats. J Periodontal Res 1967;2:147–53.
[22] Duckworth J, Godden W, Warnock GM. The effect of acute magnesium deficiency on bone formation in rats. Biochem J 1940; 34:97–108.
[23] Kenney MA, McCoy H, Williams L. Effects of dietary magnesium and nickel on growth and bone characteristics in rats. J Am Coll Nutr 1992; 11:687–93.
[24] Miller ER, Ullrey DE, Zutaut CL, Baltzer BV, Schmidt DA, Hoefer JA, et al. Magnesium requirement of the baby pig. J Nutr 1965;85:13–20.
[25] Heroux O, Peter D, Tanner A. Effect of a chronic suboptimal intake of magnesium on magnesium and calcium content of bone and on bone strength of the rat. Can J Physiol Pharmacol 1974;53:304–10.
[26] Smith BS, Nisbet DI. Biochemical and pathological studies on magnesium deficiency in the rat. I: young animals. J Comp Pathol 1968;78:149–59.
[27] New SA, Bolton-Smith C, Grubb DA, Reid DM. Nutritional influences on bone mineral density: a cross-sectional study in premenopausal women. Am J Clin Nutr 1997;65:1831–9.
[28] Angus RM, Sambrook PN, Pocock NA, Eisman JA. Dietary intake and bone mineral density. Bone Miner 1988;4:265–77.
[29] Houtkooper LB, Ritenbaugh C, Aickin M, Lohman TG, Going SB, Weber JL, et al. Nutrients, body composition and exercise are related to change in bone mineral density in premenopausal women. J Nutr 1995; 125:1229–37.
[30] Wang MC, Moore EC, Crawford PB, Hudes M, Sabry ZI, Marcus R, et al. Influence of pre-adolescent diet on quantitative ultrasound measurements of the calcaneus in young adult women. Osteoporos Int 1999;9:532–5.
[31] Freudenheim JL, Johnson NE, Smith EL. Relationships between usual nutrient intake and bone-mineral content of women 35–65 years of age: longitudinal and cross-sectional analysis. Am J Clin Nutr 1986;44: 863–76.
[32] Tranquilli AL, Lucino E, Garzetti GG, Romanini C. Calcium, phosphorus and magnesium intakes correlate with bone mineral content in postmenopausal women. Gynecol Endocrinol 1994;8:55–8.
[33] Tucker KL, Hannan MT, Chen H, Cupples LA, Wilson PWF, Kiel DP. Potassium, magnesium and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women. Am J Clin Nutr 1999;69:727–36.
[34] Yano K, Heilbrun LK, Wasnich RD, Hankin JH, Vogel JM. The relationship between diet and bone mineral content of multiple skeletal sites in elderly Japanese-American men and women living in Hawaii. Am J Clin Nutr 1985;42:877–88.
[35] New SA, Robins SP, Campbell MK, Martin JC, Garton MJ, Bolton-Smith C, et al. Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable consumption and bone health. Am J Clin Nutr 2000;71:142–51.
[36] Michaelsson K, Holmberg L, Mallmin H, Wolk A, Bergstrom R, Ljunghall S. Diet, bone mass, and osteocalcin: a cross-sectional study. Calcif Tissue Int 1995;57:86–93.
[37] Tucker KL, Hannan MT, Kiel DP. The acid–base hypothesis: diet and bone in the Framingham Osteoporosis Study. Eur J Nutr 2001;40: 231–7.
[38] Endres D, Rude RK. Mineral and bone metabolism. In: Burtis CA, Ashwwod ER, editors. Tietz fundamentals of clinical chemistry. Philadelphia (PA): WB Saunders; 2001. p. 795–821.
[39] Cohen L, Laor A, Kitzes R. Magnesium malabsorption in postmenopausal osteoporosis. Magnesium 1983;2:139–43.
[40] Reginster JY, Maertens de Noordhout B, Albert A, Dupont-Onkelinx A, Franchimont P. Serum and erythrocyte magnesium in osteoporotic and osteoarthritic postmenopausal women. Magnesium 1985;4:208.
[41] Manicourt DH, Orloff S, Brauman J, Schoutens A. Bone mineral content of the radius: good correlations with physicochemical determinations in iliac crest trabecular bone of normal and osteoporotic subjects. Metabolism 1981;30:57–62.
[42] Milachowski K, Moschinske D, Jaeschock RR. Die bedeutung des magnesiums beider medialen schenkelhasfrakur des alter menschen. Magnes-Bull 1981;3:90–102.
[43] Basle MF, Mauras Y, Audran M, Clochon P, Rebel A, Allain P. Concentration of bone elements in osteoporosis. J Bone Miner Res 1990;5:41–7.
[44] Burnell JM, Baylink DJ, Chestnut III CH, Mathews MW, Teubner EJ. Bone matrix and mineral abnormalities in postmenopausal osteoporosis. Metabolism 1982;31:1113–20.
[45] Abraham GE. The importance of magnesium in the management of primary postmenopausal osteoporosis. J Nutr Med 1991;2:165–78.

[46] Gur A, Colpan L, Nas K, Cevik R, Sarac J, Erdogan F, et al. The role of trace minerals in the pathogenesis of postmenopausal osteoporosis and a new effect of calcitonin. J Bone Miner Metab 2002;20:39–43.
[47] Brodowski J. Levels of ionized magnesium in women with various stages of postmenopausal osteoporosis progression evaluated on the densitometric examinations. Przegl Lek 2000;57:714–6.
[48] Habtezion A, Silverberg MS, Parkes R, Mikolainis S, Steinhart A. Risk factors for low bone density in Crohn's disease. Inflamm Bowel Dis 2002;8:87–92.
[49] Kantorovich V, Adams JS, Gaines JE, Guo X, Pandian MR, Cohn DH, et al. Genetic heterogeneity in familial renal magnesium wasting. J Clin Endocrinol Metab 2002;87:612–7.
[50] Cohen L, Kitzes AL. Bone magnesium, crystallinity index and state of body magnesium in subjects with senile osteoporosis, maturity-onset diabetes and women treated with contraceptive preparations. Magnesium 1983;2:70–5.
[51] Reginster JY. Serum magnesium in postmenopausal osteoporosis. Magnesium 1989;8:106.
[52] Eisinger J, Clairet D. Effects of silicon, fluoride, etidronate and magnesium on bone mineral density: a retrospective study. Magnes Res 1993;6:247–9.
[53] Stendig-Lindberg G, Tepper R, Leichter I. Trabecular bone density in a two year controlled trial of peroral magnesium in osteoporosis. Magnes Res 1993;6:155–63.
[54] Rude RK, Olerich M. Magnesium deficiency: possible role in osteoporosis associated with gluten-sensitive enteropathy. Osteoporos Int 1996;6:453–61.
[55] Rubin H. Central role for magnesium in coordinate control of metabolism and growth in animal cell. Proc Natl Acad Sci U S A 1975;72:3551–5.
[56] Wolf FI, Cittadini A. Magnesium in cell proliferation and differentiation. Front Biosci 1999;4:607–17.
[57] Di Francesco A, Desnoyer RW, Covacci V, Wolf FI, Romani A, Cittadini A, et al. Changes in magnesium content and subcellular distribution during retinoic acid-induced differentiation of HL60 cells. Arch Biochem Biophys 1998;360:149–57.
[58] Covacci V, Bruzzese N, Sgambato A, Ganapathi R, Cittadini A, Wolf FI. Effect of extracellular magnesium on topoisomerase II activity and expression in human leukemia HL-60 cells. J Cell Biochem 2000; 78:325–33.
[59] Wallach S. Effects of magnesium on skeletal metabolism. Magnes Trace Elem 1990;9:1–14.
[60] Liu CC, Yeh JK, Aloia JF. Magnesium directly stimulates osteoblast proliferation. J Bone Miner Res 1988;3:S104.
[61] Sgambato A, Wolf FI, Faraglia B, Cittadini A. Magnesium depletion causes growth inhibition, reduced expression of cyclin D1, and increased expression of P27 in normal but not in transformed mammary epithelial cells. J Cell Physiol 1999;180:245–54.
[62] Zreiqat H, Howlett CR, Zannettino A, Evans P, Schulze-Tanzil G, Knabe C, et al. Mechanism of magnesium-stimulated adhesion of osteoblastic cells to commonly used orthopaedic implants. J Biomed Mater Res 2002;62:175–84.
[63] Rude RK, Oldham SB, Singer FR. Functional hypoparathyroidism and parathyroid hormone end-organ resistance in human magnesium deficiency. Clin Endocrinol 1976;5:209–24.
[64] Rude RK, Oldham SB, Sharp CF, Singer FR. Parathyroid hormone secretion in magnesium deficiency. J Clin Endocrinol Metab 1978;48: 800–6.
[65] Estep H, Shaw WA, Watlington C, Hobe R, Holland W, Tucker SG. Hypocalcemia due to hypomagnesemia and reversible parathyroid hormone unresponsiveness. J Clin Endocrinol 1968;29:842–8.
[66] Maguire ME. Hormone-sensitive magnesium transport and magnesium regulation of adenylate cyclase. Trends Pharmacol Sci 1984;5:73–7.
[67] Cech SY, Broaddus WC, Maguire ME. Adenylate cyclase: the role of magnesium and other divalent cations. Mol Cell Biochem 1980;33: 67–92.
[68] Litosch I. G protein regulation of phospholipase C activity in a membrane-solubilized system occurs through a Mg^{2+}- and time-dependent mechanism. J Biol Chem 1991;266:4764–71.
[69] Volpe P, Alderson-Lang BH, Nickols GA. Regulation of inositol 1,4, 5-trisphosphate-induced Ca^{2+} release. I: effect of Mg^{2+}. Am J Physiol 1990;258:C1077–85.
[70] Rude RK, Adams JS, Ryzen E, Endres DB, Niimi H, Horst RL, et al. Low serum concentrations of 1,25-dihydroxyvitamin and in human magnesium deficiency. J Clin Endocrinol Metab 1985;61:933–40.
[71] Fatemi S, Ryzen E, Flores JF, Endres DB, Rude RK. Effect of experimental human magnesium depletion on PTH secretion and $1,25(OH)_2$-vitamin D metabolism. J Clin Endocrinol Metab 1991;72: 1067–72.
[72] Fisco F, Traba ML. Influence of magnesium on the in vitro synthesis of 24,25-dihydroxyvitamin D3 and 1a,25-dihydroxyvitamin D3. Magnes Res 1992;5:5–14.
[73] Salimi MH, Heughebaert JC, Nancollas GH. Crystal growth of calcium phosphates in the presence of magnesium ions. Langmuir 1985;1:119–22.
[74] Wilson JW, Werness PG, Smith LH. Inhibitors of crystal growth of hydroxyapatite: a constant composition approach. J Urol 1986;134: 1255–8.
[75] Burnell JM, Liu C, Miller AG, Teubner E. Effects of dietary alteration of bicarbonate and magnesium on rat bone. Am J Physiol 1986;250: f302–7.
[76] Cohen L, Kitzed R. Infrared spectroscopy and magnesium content of bone mineral in osteoporotic women. Isr M Med Sci 1981;17:1123–5.
[77] Schwarty R, Reddi AH. Influence of magnesium depletion on matrix-induced endochondral bone formation. Calcif Tissue Int 1979; 29:15–20.
[78] McIntosh TK. Novel pharmacologic therapies in the treatment of experimental traumatic brain injury: a review. J Am Chem Soc 1993; 89:2719–25.
[79] Weglicki WB, Dickens BF, Wagner TL, Chemielinska JJ, Phillips TM. Immunoregulation by neuropeptides in magnesium deficiency: ex vivo effect of enhanced substance P production on circulation T lymphocytes from magnesium-deficient mice. Magnes Res 1996;9: 3–11.
[80] Kramer JH, Phillips TM, Weglicki WB. Magnesium-deficiency-enhanced post-ischemic myocardial injury is reduced by substance P receptor blockade. J Mol Cell Cardiol 1997;29:97–110.
[81] Goto T, Yamaza T, Kido MA, Tanaka T. Light- and electron-microscopic study of the distribution of axons containing substance P and the localization of neurokinin-1 receptor in bone. Cell Tissue Res 1998;293:87–93.
[82] Broome CS, Whetton AD, Miyan JA. Neuropeptide control of bone marrow neutrophil production is mediated by both direct and indirect effects on CFU-GM. Br J Haematol 2000;108:140–50.
[83] Kimble RB, Srivastava S, Ross FP, Matayoshi A, Pacifici R. Estrogen deficiency increases the ability of stromal cells to support murine osteoclastogenesis via an interleukin-1 and tumor necrosis factor-mediated stimulation of macrophage colony-stimulating factor. J Biol Chem 1996;271:28890–7.
[84] Hans CP, Chaudhary DP, Bansal DD. Effect of magnesium supplementation on oxidative stress in alloxanic diabetic rats. Magnes Res 2003;16:13–9.
[85] Mak T, Kramer JH, Weglicki WB. Suppression of neutrophil and endothelial activation by substance P receptor blockade in the Mg-deficient rat. Magnes Res 2003;16:91–7.
[86] Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Boyle WJ, Riggs BL. The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. J Bone Miner Res 2000;15:2–12.
[87] Isomura H, Fujie K, Shibata K, Inoue N, Iizuka T, Takebe G, et al. Bone metabolism and oxidative stress in postmenopausal rats with iron overload. Toxicology 2004;197:93–100.
[88] Basu S, Michaelsson K, Olofsson H, Johansson S, Melhus H. Association between oxidative stress and bone mineral density. Biochem Biophys Res Commun 2001;288:275–9.