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Magnesium Deficiency: Possible Role in Osteoporosis Associated with Gluten-Sensitive Enteropathy

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Abstract. Osteoporosis and magnesium (Mg) deficiency often occur in malabsorption syndromes such as gluten-sensitive enteropathy (GSE). Mg deficiency is known to impair parathyroid hormone (PTH) secretion and action in humans and will result in osteopenia and increased skeletal fragility in animal models. We hypothesize that Mg depletion may contribute to the osteoporosis associated with malabsorption. It was our objective to determine Mg status and bone mass in GSE patients who were clinically asymptomatic and on a stable gluten-free diet, as well as their response to Mg therapy. Twenty-three patients with biopsy-proven GSE on a gluten-free diet were assessed for Mg deficiency by determination of the serum Mg, red blood cell (RBC) and lymphocyte free Mg^{2+}, and total lymphocyte Mg. Fourteen subjects completed a 3-month treatment period in which they were given 504–576 mg $MgCl_2$ or Mg lactate daily. Serum PTH, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and osteocalcin were measured at baseline and monthly thereafter. Eight patients who had documented Mg depletion (RBC Mg^{2+} <150 μM) underwent bone density measurements of the lumbar spine and proximal femur, and 5 of these patients were followed for 2 years on Mg therapy. The mean serum Mg, calcium, phosphorus and alkaline phosphatase concentrations were in the normal range. Most serum calcium values fell below mean normal and the baseline serum PTH was high normal or slightly elevated in 7 of the 14 subjects who completed the 3-month treatment period. No correlation with the serum calcium was noted, however. Mean serum 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and osteocalcin concentrations were also normal. Despite only 1 patient having hypomagnesemia, the RBC Mg^{2+} (153 ± 6.2 μM; mean ± SEM) and lymphocyte Mg^{2+} (182 ± 5.5 μM) were significantly lower than normal (202 ± 6.0 μM, $p<0.001$, and 198 ± 6.8 μM, $p<0.05$, respectively). Bone densitometry revealed that 4 of 8 patients had osteoporosis of the lumbar spine and 5 of 8 had osteoporosis of the proximal femur (T-scores ≤ -2.5). Mg therapy resulted in a significant rise in the mean serum PTH concentration from 44.6 ± 3.6 pg/ml to 55.9 ± 5.6 pg/ml ($p<0.05$). In the 5 patients given Mg supplements for 2 years, a significant increased in bone mineral density was observed in the femoral neck and total proximal femur. This increase in bone mineral density correlated positively with a rise in RBC Mg^{2+}. This study demonstrates that GSE patients have reduction in intracellular free Mg^{2+}, despite being clinically asymptomatic on a gluten-free diet. Bone mass also appears to be reduced. Mg therapy resulted in a rise in PTH, suggesting that the intracellular Mg deficit was impairing PTH secretion in these patients. The increase in bone density in response to Mg therapy suggests that Mg depletion may be one factor contributing to osteoporosis in GSE.

Keywords: Gluten-sensitive enteropathy; Magnesium; Malabsorption; Osteoporosis; Sprue

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Introduction

Metabolic bone disease is a frequent complication of intestinal malabsorption disorders. The most common bone disease is thought to be osteomalacia due to

malabsorption of vitamin D [1–3]. Osteoporosis, however, is also common [3–6]. Factors responsible for osteoporosis may include calcium malabsorption, secondary hyperparathyroidism, or malabsorption of other minerals or trace elements [1,2,7].

Patients with malabsorption syndromes are prone to hypomagnesemia, presumably as a result of intestinal malabsorption of magnesium (Mg). Up to 40% of patients with malabsorption syndromes have been shown to be hypomagnesemic [8,9]. The serum Mg concentration, however, does not assess Mg status accurately as only 1% of body Mg is in the extracellular fluid compartment. The prevalence of intracellular Mg depletion has been shown to be much greater than hypomagnesemia in other patient populations at high risk for Mg depletion, such as diabetics and alcoholics [10,11]. The prevalence of intracellular Mg deficiency in malabsorption syndromes, whether treated or not, has not been previously assessed.

Mg deficiency may affect bone and mineral metabolism in several ways. Inhibition of parathyroid hormone (PTH) release and skeletal and renal resistance to PTH action have been documented in hypomagnesemic patients [12,13]. Additionally, decreased serum 1,25-dihydroxyvitamin D concentration and end-organ resistance to vitamin D have been reported [14,15]. Histomorphometric studies in animal models have shown that Mg depletion results in impaired bone growth, decreased osteoblast activity and decreased bone formation [16–18]. Biomechanical testing has demonstrated increased bone fragility [16–18].

We undertook this study to determine the prevalence of Mg deficiency in patients with gluten-sensitive enteropathy (GSE; celiac sprue) on a gluten-free diet and to determine the effect of Mg therapy on bone and mineral metabolism in these subjects.

Methods

Subjects

Twenty-three subjects with biopsy-proven celiac disease were recruited for study from the Celiac Disease Foundation of Southern California. The subjects consisted of 3 men and 20 women with an age range of 31–81 years and a mean age of 56 years. Mean height, weight, body mass index, hemoglobin and serum albumin concentration of these subjects at the initiation of the study are shown in Table 1. Eighteen subjects consented to participate in a 3-month Mg treatment study as described below. Of these, 14 completed the protocol. These subjects consisted of 12 women and 2 men with a mean age of 56 years. All subjects were on a gluten-free diet and had no symptoms of active disease; they were free from any other medical conditions known to affect bone metabolism.

Routine biochemical tests, indices of mineral metabolism and bone mineral density (BMD) are compared with reference normative data. Cellular Mg determinations are compared with those in a normal population consisting of hospital employees and normal volunteers.

Table 1. Baseline parameters at initiation of the study

	Female (n=20)	Male (n=3)
Weight (kg)	59.0±2.2	82.8±1.7
Height (cm)	162.9±1.6	177.7±3.5
BMI (kg/m^2)	22.2±0.7	26.3±1.5
Hemoglobin (g/dl)	13.6±0.3[a]	15.5±0.8
Albumin (g/dl)	4.6±0.1[b]	4.7±0.1

Values are the mean ± SEM.
BMI, body mass index.
[a] Normal range = 11.6–14.9 female; 12.9–16.6 male.
[b] normal range = 3.9–5.0.

Baseline Studies

All 23 subjects had fasting morning serum and urine samples collected. Baseline determination of serum Mg, calcium, phosphate, alkaline phosphatase, osteocalcin, intact PTH, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentrations, and of urine calcium, Mg and creatinine excretion were performed. A complete blood count and biochemistry panel was also obtained. In addition, free (ionized) red blood cell (RBC) and lympocyte Mg^{2+} concentrations, and total lymphocyte Mg content were determined. All subjects were then invited to participate in the protocol described below. Eight subjects who had documented intracellular Mg deficiency (RBC Mg^{2+} <150 μM) underwent BMD measurements of the lumbar spine and proximal femur by dual-energy X-ray absorptiometry (DXA). All were postmenopausal women with a mean age of 61 years, 4 of whom were on estrogen replacement therapy. Five of these subjects also completed a 2-year oral Mg treatment trial (see below).

Three-Month Protocol

After the baseline studies had been completed, 18 subjects were given $MgCl_2$ (Slo-Mag, Searle) or Mg lactate (Niché Pharm) at a starting dose of 336–284 mg of elemental Mg per day in three divided doses. The dosage was adjusted as tolerated to 504–576 mg of elemental Mg per day over 1 month. The recommended dietary allowance (RDA) for Mg is 300–350 mg/day [19]. The initial dose was chosen to approximate the RDA. Since these subjects were Mg depleted and the RDA estimate has been suggested to be lower than actually required [20], we increased the dose to slightly above 500 mg/day. Repeat determinations of the serum and urine biochemistry and calciotropic hormones were obtained at monthly intervals for 3 months. Fourteen subjects completed the study.

Two-Year Mg Treatment Trial

Five subjects continued to take 504–576 mg of elemental Mg per day for 2 years. BMD was determined at 6 months, 1 year and 2 years of therapy. RBC Mg^{2+} and a biochemical panel were also determined at these time points.

Biochemical Methods

Serum and urine creatinine and total serum calcium, phosphate, Mg and alkaline phosphatase were determined using a Technicon SMAC-1 Autoanalyzer. Urine calcium and Mg were determined by atomic absorption spectrophotometry. Serum intact PTH was determined with a Ciba Corning Diagnostics (Medfield, MA) immunochemiluminometric assay as previously described [21], with a normal range of 10–55 pg/ml. Serum 25-hydroxyvitamin D was determined by radioimmunoassay (RIA) using an antibody obtained from INCStar (Stillwater, MN); normal range = 10–50 ng/ml [22]. A non-equilibrium radioreceptor assay was used for measuring 1,25-dihydroxyvitamin D as previously described [23] with a normal range of 15–60 pg/ml. A two-site electroimmunoassay (EIA) specific for human osteocalcin (Biomedical Technologies, Stoughton, MA) was used for the determination of serum osteocalcin. This method has an interassay precision of 10.5%. The assay has a limit of detection of 0.5 ng/ml with a normal range of 2–14 ng/ml. In all instances, each patient's serum was analyzed in the same assay for these parameters. Serum was stored at −70 °C until assay.

Intracellular Mg^{2+} in erythrocytes was measured as previously described [24]. Ten milliliters of heparinized blood is obtained and centrifuged at 2000 rpm for 10 min. The packed cells (4 ml) are decanted into 10-mm NMR glass tubes. The cells are analyzed using ^{31}P NMR spectra at 109 MHz with a Bruker WP-27 NMR spectrometer. The interassay variability of specimens obtained for RBC Mg^{2+} is less than 5%.

To determine intracellular free Mg^{2+} in circulating lymphocytes, cells were prepared from 15 ml of heparinized blood using a Ficoll-Paque (Pharmacia) gradient [11]. The lymphocytes are resuspended in 3.0 ml of a HEPES-buffered balance salt solution (145 mM NaCl, 4 mM KCl, 1 mM NaH_2PO_4, 20 mM HEPES, 9 mM glucose) containing 1 mM calcium. A 1-ml aliquot of lymphocyte suspension (cell blank) is retained for determination of non-specific fluorescence. To the remaining 2 ml of cell suspension is added 4 μl of a 50 μg/50 μl dimethyl sulfoxide (DMSO) solution of the fluorescent probe Mag-fura-2AM (the ester form of Mag-fura-2) (Molecular Probes, Eugene, OR) [25]. To the cell blank is added 2 μl of DMSO. Both treated and blank cell suspensions are incubated with gentle agitation for 15–20 min in the dark at room temperature. The cells are then centrifuged for 7–10 min at 600 *g*. The supernatant is discarded and the pellet resuspended in the HEPES buffer. A second incubation of 30 min is made to allow for hydrolyzation of the acetoxymethyl ester by intracellular esterases. The cells are centrifuged for a second time and resuspended in calcium- and Mg-free Hank's buffered salt solution (HBSS) and transferred to a cuvette for fluorescence measurements. Fluorescence measurements are made at 37 °C. Cells are equilibrated with gentle stirring in a water-jacketed cuvette holder for 5 min prior to measurements. Intracellular fluorescence measurements are obtained in the presence of 200 μM $MnCl_2$, which quenches fluorescence produced by extracellular Mag-fura-2. Minimum and maximal fluorescence measurements are obtained after permeabilizing the cells with digitonin in the presence of 400 mM excess EDTA, pH 8.5, and 4 M excess Mg, respectively. After making the appropriate corrections for the non-specific fluorescence measured in the cell blanks under the same conditions, the intracellular free Mg^{2+} concentration is calculated. To measure intracellular Mg^{2+}, the fluorescence intensity at 510 nm is measured at the two maximal excitation wavelengths: 335 nm and 370 nm. The ratio of fluorescence intensity, 335 nm/370 nm, is proportional to the concentration of Mg^{2+} present in the cell. The concentration of Mg^{2+} in an unknown sample (unk) is calculated from this ratio, the fluorescence measured at the same two wavelengths when the Mag-fura-2 is in the fully bound (max) and fully unbound (min) state, and the dissociation constant according to the following formula (dissociation constant = 1.5 mM):

$$Mg^{2+} = \frac{335\ nm_{(unk)}/370\ nm_{(unk)} - 335\ nm_{(min)}/370\ nm_{(min)}}{335\ nm_{(max)}/370\ nm_{(max)} - 335\ nm_{(unk)}/370\ nm_{(unk)}} \times \frac{370\ nm_{(min)}}{370\ nm_{(max)}} \times 1.5\ mM$$

The intra-assay variation is 6 ± 0.7% based on triplicate determinations of samples from 15 subjects.

Measurement of total Mg content of lymphocytes was performed as previously described [11]. Cell Mg content is measured by atomic absorption spectrophotometry. Lymphocyte protein is measured by the method of Lowry et al. [26] and the results expressed in μg Mg/mg protein.

Bone Densitometry

BMD was determined by DXA (QDR-2000, Hologic, Waltham, MA) [27] in the lumbar (L1–4) vertebral bodies. The mean of these values served as that subject's bone density. BMD was also assessed in the femoral neck and total proximal femur.

Statistical Methods

The *t*-test was used to compare baseline data for cellular Mg from GSE subjects with those from normal controls and a paired *t*-test to compare values of PTH and RBC Mg^{2+} at baseline with those at the 3-month and/or 2-year point. Changes in BMD over the 2-year study

period were analyzed by ANOVA. Values are expressed as the mean ± SEM.

Results

Baseline Metabolic Status

Baseline biochemical indices of the 23 subjects with celiac disease are shown in Fig. 1. The mean serum calcium was within the normal range (9.0 ± 0.3 mg/dl). However, 3 patients had slight hypocalcemia and all but 1 fell below mean normal (9.4 mg/dl), suggesting a mild reduction in the total serum calcium concentration. As shown in Table 1, the mean serum albumin concentration was normal and there was no difference between measured total and albumin-corrected serum calcium concentrations. The mean serum Mg (1.7 ± 0.17 mequiv/l), alkaline phosphatase (71.4 ± 23.0 U/l) and phosphate (3.4 ± 0.4 mg/dl) fell at near mean normal. Only 1 subject had hypomagnesemia. Urinary calcium/creatinine ratio (mg/mg) in the GSE patients was normal (0.053 ± 0.05).

Baseline osteocalcin and calciotropic hormone concentrations in the 14 subjects who completed the 3-month study are shown in Fig. 2. Serum concentrations of PTH, osteocalcin, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D fell within the normal range. It is of note that 2 subjects had a slightly elevated serum PTH concentration and 5 had values in the high normal range. While this observation might be related to lower serum calcium levels, no correlation was found between the serum PTH and calcium concentrations. No subject was found to have either a subnormal serum 25-hydroxyvitamin D level or an elevated serum alkaline phosphatase level.

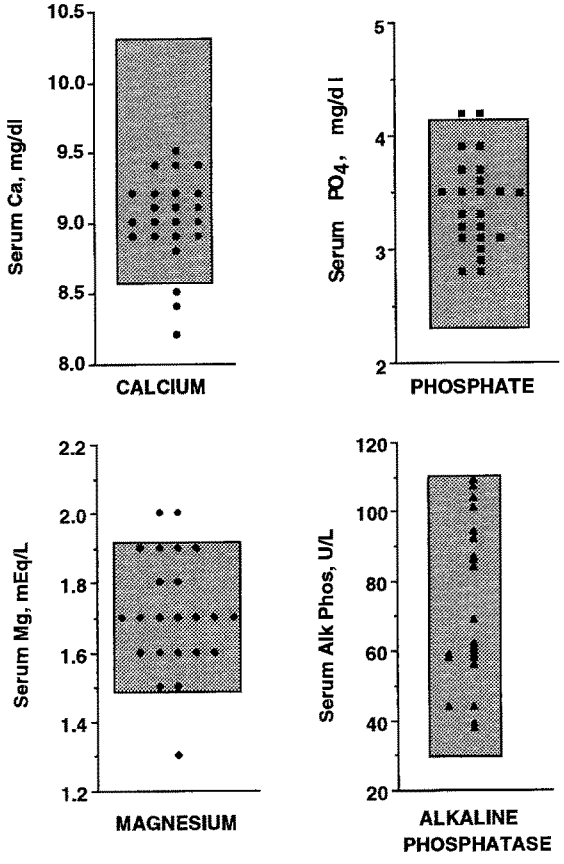

Fig. 1. Baseline serum biochemistry in 23 GSE subjects on a gluten-free diet. The *shaded rectangular boxes* represent the normal range.

Baseline Mg Status

RBC and lymphocyte free Mg^{2+} concentrations are shown in Fig. 3. Despite serum Mg values which fell within the normal range in all but one subject, mean intracellular ionized Mg^{2+} concentrations in both red blood cells (153 ± 6.2 μM) and lymphocytes (182 ± 5.5 μM) were significantly decreased from normal (202 ± 6.0 μM, $p<0.001$, and 198 ± 6.8 μM, $p<0.05$, respectively). Although total Mg content of lymphocytes tended to be lower in the GSE subjects (1.17 ± 0.07 μg/mg protein; $n = 16$), the difference was not statistically different from a normal population (1.23 ± 0.02 μg/mg protein; $n = 49$).

Bone Density

Baseline BMD measurements of the anteroposterior (A-P) lumbar spine, femoral neck and total proximal femur for the 8 subjects who underwent bone densitometry are shown in Fig. 4 and the corresponding *Z*-scores and *T*-score in Fig. 5. Intracellular Mg^{2+}, *T*-scores, and baseline and peak PTH levels for these subjects are shown in Table 2. Four of the 8 subjects had *T*-scores of the A-P lumbar spine ≤ -2.5 and 5 of the 8 subjects had *T*-scores of the femoral neck that were ≤ -2.5. All but two subjects had *T*-scores below 0 and only 4 subjects had *Z*-scores above 0, demonstrating that BMD fell well below that of sex- and age-matched controls and well below that of young normal subjects, establishing a diagnosis of osteoporosis (*T*-score ≤ -2.5) and increased fracture risk in most subjects [28]. There was no significant difference in the *Z*-score for the 4 women on sex-steroid replacement therapy compared to the 4 without for A-P spine (−0.75 vs −0.83), femur neck (−1.3 vs −0.66) or total proximal femur (−0.95 vs −1.74) respectively.

Effect of Mg Supplementation

Four of the 18 subjects who began the 3-month Mg treatment protocol dropped out prior to its completion (3 subjects secondary to gastrointestinal intolerance to the drug and 1 subject secondary to a hip fracture). In the 14 subjects who completed the protocol, there were no significant changes in serum calcium, Mg, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, or osteocalcin concentrations or in urine Ca/Cr ratios during the

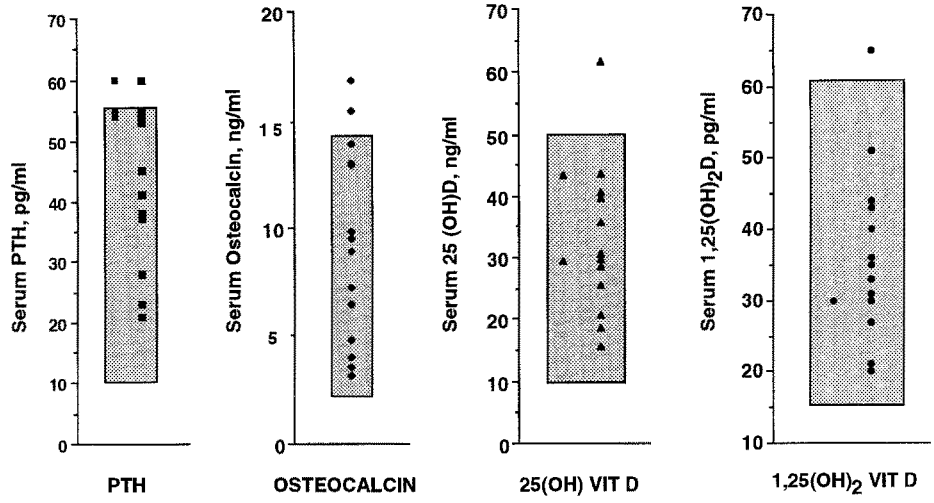

Fig. 2. Baseline PTH, osteocalcin, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in 14 GSE subjects who completed a 3-month Mg treatment study. The *shaded rectangular boxes* represent the normal range.

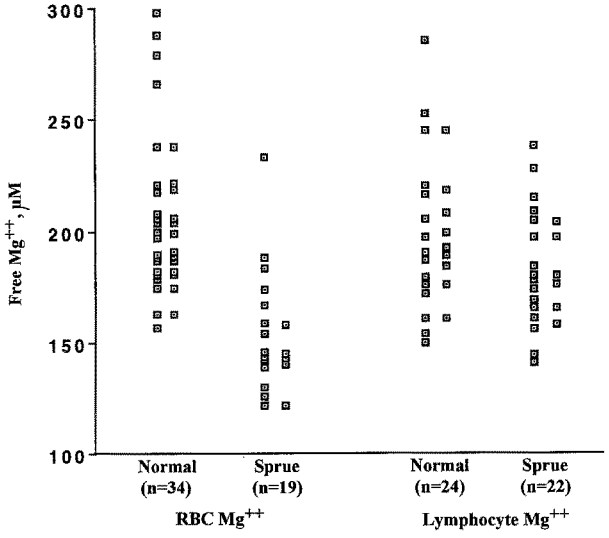

Fig. 3. Significant reduction in RBC (153 ± 6.2 μM; $p<0.001$) and lymphocyte (182 ± 5.5 μM; $p<0.05$) Mg^{2+} concentrations in GSE (sprue) patients compared with normal subjects (202 ± 6.0 μM and 198 ± 6.8 μM respectively).

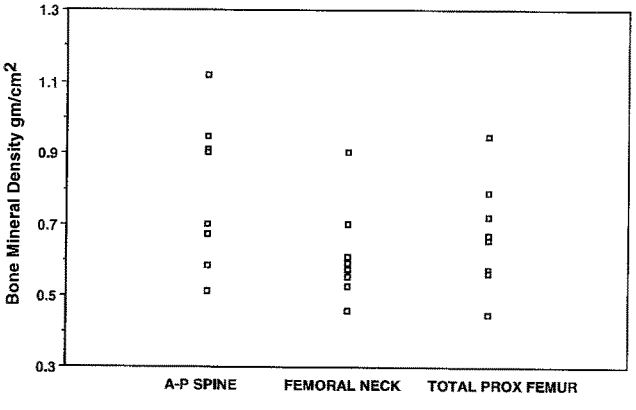

Fig. 4. Bone mineral density of A-P lumbar spine, femoral neck and total proximal femur in 8 postmenopausal GSE, Mg-depleted patients.

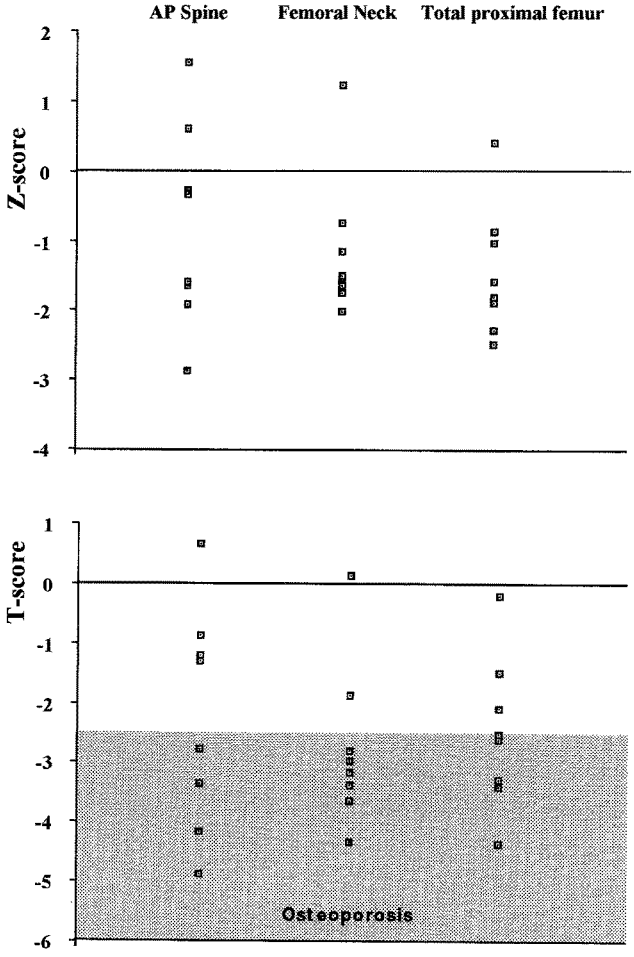

Fig. 5. *Z*-scores and *T*-scores for bone mineral density of A-P lumbar spine, femoral neck and total proximal femur in 8 patients with GSE and intracellular Mg depletion. The *shaded area* denotes a diagnosis of osteoporosis: *T*-score of ≤ -2.5.

3-month course of therapy. The Mg therapy, however, resulted in a significant increase of the mean serum PTH concentration from the baseline value, 44.6 ± 3.6 pg/ml,

Table 2. RBC and lymphocyte Mg^{2+}, *T*-score of A-P lumbar spine and proximal femur, and basal and peak PTH in patients who had BMD measurements and underwent magnesium therapy

Patient	Mg^{2+} (μM)		*T*-score		PTH (pg/ml)	
	RBC[a]	Lymphocyte[b]	A-P spine	Proximal femur	Basal[c]	Peak[d]
1	126	157	−1.31	−2.53	37	97
2	142	214	0.65	−2.30	23	61
3	130	177	−3.37	−2.64	54	54
4	143	144	−1.23	−1.51	51	60
5	144	182	−0.89	−2.10	21	42
6	139[e]	173	−4.19	−4.37	–	–
7	122[e]	153	−2.79	−3.31	60	80
8	146[e]	155	−4.89	−3.41	55	76

[a] Normal = 202 ± 6.0 μM.
[b] Normal = 198 ± 6.8 μM.
[c] Normal = 10–55 pg/ml.
[d] Peak PTH obtained during the first 3 months of Mg therapy.
[e] Three month study only

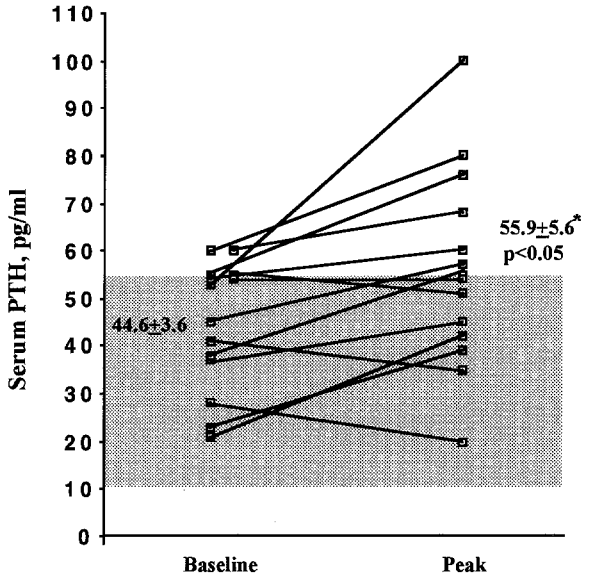

Fig. 6. Serum PTH concentrations at baseline and at peak rise in PTH in response to 3 months of Mg therapy in 14 patients with GSE and reduction in RBC Mg^{2+}.

to the subsequent maximum value obtained for each patient, 55.9 ± 5.6 pg/ml, as shown in Fig. 6 ($p<0.05$). Urine Mg/Cr ratios increased significantly from baseline (0.08 ± 0.07) at 2 months (0.16 ± 0.11; $p<0.05$) and 3 months (0.14 ± 0.08; $p<0.05$), demonstrating compliance with the therapy.

Five subjects completed a 2-year trial of 504–576 mg elemental Mg per day. In these subjects RBC Mg^{2+} rose from 137 ± 3.7 μM at baseline to 193 ± 14 μM at the conclusion of the study ($p<0.02$). Serum PTH rose from 37.2 ± 6.8 pg/ml at baseline to 62.8 ± 9.2 pg/ml at the 3-month point ($p<0.04$) (individual values are shown in Table 2). Bone densitometry was determined at baseline and at 6, 12 and 24 months. Mean BMD increased at all sites at 2 years as shown in Fig. 7. BMD of the A-P lumbar spine fell −0.96% by the 6-month mark; however, it rose by 2.3% (1.3% above baseline) by 24 months. This rise, however, did not reach a level of significance. BMD rose significantly at both the femoral neck and total proximal femur ($p<0.04$, ANOVA). Changes in BMD of the total proximal femur for each subject during Mg therapy are shown in Fig. 8. Three subjects had a marked response to Mg repletion. The increase in BMD at the A-P lumbar spine and proximal femur correlated both with the magnitude of the increase in RBC Mg^{2+} ($r = 0.9833$, $p<0.002$ and $r = 0.984$, $p<0.03$, respectively), and with the peak RBC Mg^{2+} concentration reached ($r = 0.9557$, $p<0.01$ and $r = 0.9037$, $p<0.04$, respectively). The positive significant correlation of the magnitude of the increase in RBC Mg^{2+} and the increase in total hip BMD is shown in Fig. 9

Discussion

In this study we have demonstrated for the first time that vertebral BMD and proximal femur BMD is reduced in GSE. This subnormal BMD is clinically relevant in that approximately one-half of these subjects were noted to be at increased risk of either hip or spine fractures on the basis of *T*-scores ≤ -2.5 [28]. Although all these subjects were postmenopausal women, we found no difference in the reduction of bone mass between those women taking estrogen replacement therapy and those who were not, although the sample size may be too small to find meaningful differences.

The major skeletal disorder in GSE is thought to be osteomalacia due to vitamin D malabsorption and/or interrupted enterohepatic recycling of vitamin D derivatives [1–3]. In *untreated* GSE patients, radiographic evidence of osteomalacia has been reported in 26% of patients [1] and histological evidence has been observed in the majority of patients [1–3]. Biochemical findings of elevated serum alkaline phosphatase and PTH along with reduced serum calcium and 25-hydroxyvitamin D

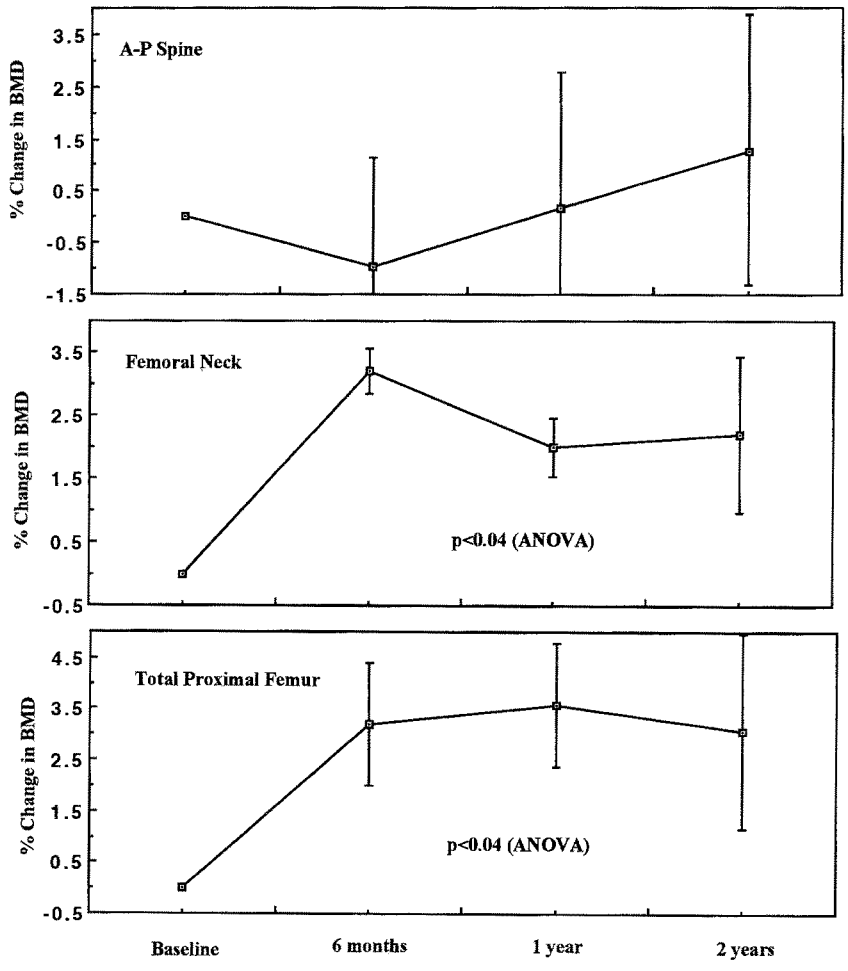

Fig. 7. Percentage change in bone mineral density of the A-P lumbar spine, femoral neck and total proximal femur during 2 years of Mg therapy in 5 Mg-depleted GSE patients.

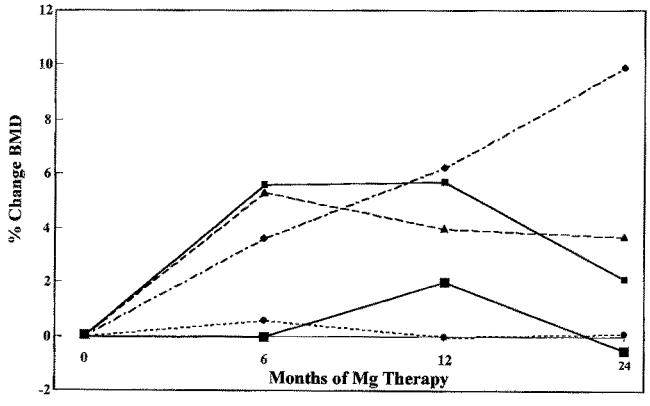

Fig. 8. percentage change in the bone mineral density of the total proximal femur in 5 mg-depleted GSE patients given Mg therapy for 2 years.

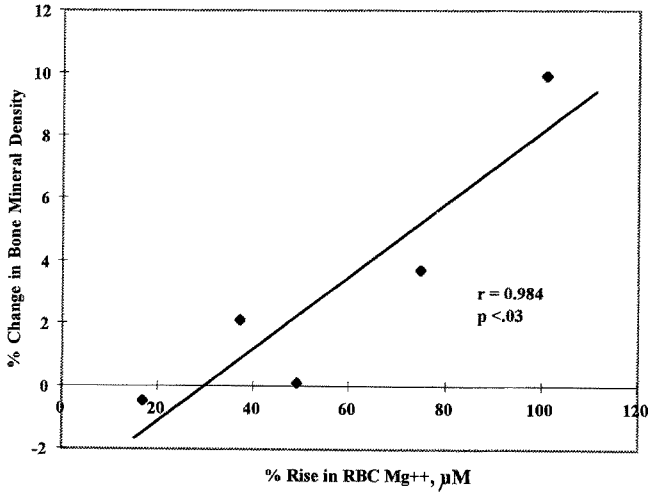

Fig. 9. Significant correlation of the percentage change in bone mineral density of the total proximal femur with the rise in RBC Mg^{2+} in 5 GSE Mg-depleted patients given Mg therapy for 2 years.

are also common [1–3]. However, osteoporosis, as determined by bone densitometry of the distal radius [4,5] or by histomorphometry [3], also occurs frequently. Osteoporosis may be secondary to prolonged calcium deficiency, which may be due to vitamin D deficiency with impaired intestinal calcium absorption or simple dietary calcium deprivation with resultant secondary hyperparathyroidism. Some GSE patients, however, present with osteoporosis without biochemical abnormalities [1,3–6]. In addition, one study reported that the institution of a gluten-free diet and oral 25-hydroxyvitamin D therapy did not result in any change in bone density or any significant improvement of biochemical parameters of mineral metabolism [4]. In our GSE patients *on a gluten-free diet*, we found no evidence of significant hypocalcemia with secondary

hyperparathyroidism, although there appeared to be a slight reduction of the serum calcium and high normal PTH levels in some subjects. Furthermore, there appeared to be no contribution of vitamin D malabsorption with resultant osteomalacia as suggested by normal serum 25-hydroxyvitamin D and serum alkaline phosphatase concentrations. It is possible that some degree of osteomalacia may be observed on bone biopsy without changes in these serum parameters. Other factors, however, may be involved in the development of osteoporosis in GSE.

Mg deficiency is common in malabsorption syndromes, presumably due to intestinal malabsorption of Mg [8,9]. Hypomagnesemia was reported in 40% of such patients on one series [8]. In another study of 20 GSE patients with hypocalcemia, 60% were found to be hypomagnesemic [9]. Since only 1% of the body's Mg is in the extracellular fluid compartment, the serum Mg concentration does not adequately reflect total body Mg stores [10,11]. In patient populations known to be at risk for Mg deficiency, such as those with diabetes mellitus and chronic alcoholics, the prevalence of intracellular Mg depletion is much greater than that of hypomagnesemia [10,11]. Thus, the true prevalence of Mg deficiency in patients with GSE, whether on a gluten-free diet or not, has not previously been assessed. In our study of 23 subjects on a gluten-free diet, the serum Mg concentration fell within the normal range in all but one. Intracellular free Mg^{2+} (the fraction of Mg important for enzyme activity) in RBC and lymphocytes, however, was found to be significantly lower than normal, documenting Mg depletion. Our patients with GSE are highly motivated and compliant with the dietary restriction of their conditions and had no symptomatic or biochemical evidence of active disease, but yet demonstrated significant Mg deficiency. We hypothesize that due to the extreme difficulty of complete avoidance of all gluten-containing products, minor amounts of gluten were ingested by these subjects which then affected the distal small bowel, a common site of pathological involvement in GSE and the main site for Mg absorption, causing Mg malabsorption. In support of this are prior investigations which have demonstrated persistent small intestinal mucosal abnormalities in GSE patients despite rigid institution of a gluten-free diet [29].

The decrease in BMD found in these subjects is undoubtedly multifactorial. It may represent loss of skeletal mass during the more active state of the disease prior to institution of a gluten-free diet. Indeed, children with GSE who were placed on a gluten-free diet early in their disease course did not have any reduction of bone mass compared with matched controls [5]. Malabsorption of other minerals and trace elements may also contribute. We propose that Mg depletion is one contributing factor. Mg deficiency may affect bone and mineral homeostasis in several ways. Mg deficiency impairs secretion of PTH which, when the impairment is severe, may result in symptomatic hypocalcemia [12,13]. This may be why we observed no correlation between the serum Mg and PTH concentrations in our patients. Mg therapy in our patients resulted in a rise in serum PTH. A rise in serum PTH concentration in response to Mg therapy appears to be specific for Mg depletion [30]. Mg administration in normal subjects decreases PTH secretion [30]. These data suggest, therefore, that our GSE patients had a mild impairment of PTH secretion which was reversed with Mg therapy. In addition, renal and skeletal resistance to PTH has been described [12,13]. Serum concentrations of 1,25-dihydroxyvitamin D are also low in severely Mg-deficient humans [14], which may be due to the low serum PTH levels or impaired action of PTH on the renal 25-hydroxyvitamin D-1α-hydroxylase enzyme. End-organ resistance to vitamin D has also been reported [15]. Therefore, impaired intestinal calcium absorption and an inability to adapt to a low calcium diet in terms of increased PTH secretion and 1,25-dihydroxyvitamin D synthesis would contribute to a negative calcium balance and place a patient at risk for osteoporosis, as has been demonstrated in hypomagnesemic diabetic children [31]. PTH and 1,25-dihydroxyvitamin D are also thought to be trophic factors for the osteoblast [32,33], and therefore the low serum levels or skeletal resistance could explain the low bone formation rate observed in experimental Mg deficiency [16–18]. PTH administration has been demonstrated to increase BMD in postmenopausal osteoporosis [34,35], and therefore the rise in PTH in our patients could have contributed to the increase in BMD. Mg depletion in animals resulted in abnormal bone metabolism. Mg-deficient animals demonstrate decreased osteoblast activity, decreased bone formation, and increased skeletal fragility [16–18]. Proliferation of osteoblast-like cells in vitro is directly related to Mg concentration in the medium [36].

In this study we have shown that serum PTH concentrations rise significantly in this population of Mg-depleted subjects when they are given Mg supplementation. This suggests that these patients may have had functional hypoparathyroidism which, when coupled with the direct effect of Mg deficiency on bone metabolism, may result in a state of impaired bone remodelling. The provocative observation in this study is a rise in BMD of the lumbar spine and proximal femur with Mg repletion. The significant correlation of this increase in BMD with the rise in intracellular free Mg^{2+} strongly suggests that the Mg repletion directly or indirectly (i.e. via an increase in PTH) contributed to the increase in bone mass. Mg therapy has previously been shown to enhance bone mass in postmenopausal women [37]. Lacking from our study, however, is a placebo-treated control population, due to the lack of adequate number of subjects willing to participate for a 2-year period. Our data nevertheless suggest that Mg depletion will adversely affect bone and mineral metabolism, and patients at risk for prolonged states of a Mg deficit such as malabsorption syndromes, diabetes mellitus and alcoholism should be monitored for Mg depletion. Larger controlled studies, however, are

needed to better define the role of Mg depletion as a risk factor for osteoporosis.

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