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A Randomized Controlled Study of Effects of Dietary Magnesium Oxide Supplementation on Bone Mineral Content in Healthy Girls

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Context: The role of magnesium (Mg) as a determinant of bone mass has not been extensively explored. Limited studies suggest that dietary Mg intake and bone mineral density are correlated in adults, but no data from interventional studies in children and adolescents are available.

Objective: We sought to determine whether Mg supplementation in periadolescent girls enhances accrual of bone mass.

Design: We carried out a prospective, placebo-controlled, randomized, one-year double-blind trial of Mg supplementation.

Setting: The study was conducted in the Clinical Research Centers at Yale University School of Medicine.

Patients or Other Participants: Healthy 8- to 14-yr-old Caucasian girls were recruited from community pediatricians' offices. Dietary diaries from over 120 volunteers were analyzed, and those with dietary Mg intake of less than 220 mg/d were invited to participate in the intervention.

Intervention: Magnesium (300 mg elemental Mg per day in two divided doses) or placebo was given orally for 12 months.

Main Outcome Measure: The primary outcome measure was interval change in bone mineral content (BMC) of the total hip, femoral neck, Ward's area, and lumbar spine (L1–L4) after 12 months of Mg supplementation.

Results: Significantly increased accrual ($P = 0.05$) in integrated hip BMC occurred in the Mg-supplemented *vs.* placebo group. Trends for a positive Mg effect were evident in the pre- and early puberty and in mid-late puberty. Lumbar spinal BMC accrual was slightly (but not significantly) greater in the Mg-treated group. Compliance was excellent; 73% of capsules were ingested as inferred by pill counts. Serum mineral levels, calciotropic hormones, and bone markers were similar between groups.

Conclusions: Oral Mg oxide capsules are safe and well tolerated. A positive effect of Mg supplementation on integrated hip BMC was evident in this small cohort. **(*J Clin Endocrinol Metab* 91: 4866–4872, 2006)**

DIETARY INTAKE, PHYSICAL activity, and genetic factors have been described as potential determinants of osteoporosis in later life (1–6). The dependence of bone mass throughout adulthood upon the attainment of bone mass in late adolescence is not well studied. Nevertheless, the establishment of optimal nutrition during growing years is a targeted strategy for decreasing the incidence of osteoporosis in future decades. Whereas several studies have concentrated on the importance of calcium nutrition on the skeleton in children (7–11), only limited investigations of magnesium (Mg) nutrition have been undertaken in this regard. Mg is an important component of the mineral phase of bone (12–14). Approximately one half of total body Mg is in bone, adsorbed to the hydroxyapatite surface (15, 16). Mg plays a central role in mineral homeostasis, regulating PTH secretion and action (17, 18) and vitamin D activation (19). Mg interacts with the extracellular calcium-sensing receptor on parathyroid and renal tubular cells, providing one direct mechanism by which Mg affects organs essential to mineral homeostasis (20).

Nutritional monitoring programs have consistently demonstrated inadequate dietary Mg intake in young American women. The recommended daily allowance (RDA) for Mg is 240 mg/d for girls aged 9–13 yr and 360 mg/d for girls 14–18 yr old (21); NHANES III (Third National Health and Nutrition Examination Survey) found mean Mg intake in 12–15 yr olds of 206 (±7.6) mg/d (22). Limited human intervention studies indicate decreased bone turnover (23), and improved bone mass with Mg supplementation in targeted groups of adults (24, 25). Furthermore, Mg deprivation in rats during rapid bone growth directly contributes to an osteoporotic phenotype (26). Impaired bone growth with decreased osteoblasts, increased osteoclasts, and loss of trabecular bone occurs in Mg-deprived mice (27).

We therefore hypothesized that Mg undernutrition may contribute to suboptimal attainment of bone mass during

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Abbreviations: BMC, Bone mineral content; BMD, bone mineral density; FEMg, fractional excretion of Mg; RDA, recommended daily allowance; TRP, tubular reabsorption of phosphate.

adolescence, and we designed a pilot study to address this issue. Our Mg supplementation regimen was well tolerated with optimal compliance, and resulted in a favorable incremental gain of bone mineral content (BMC) at the hip in premenarchal girls.

Subjects and Methods

Overview of design

We designed this study to determine whether oral Mg supplementation was safe and acceptable to adolescents. Identification of effect size and determination of compliance with Mg supplements were primary objectives. We directed the study toward 8- to 14-yr-old girls because of the coincident rapid bone accretion and relative Mg undernutrition. Subjects were recruited from local pediatricians. We selected volunteers whose estimated dietary Mg intake was less than 220 mg/d; the major reason for exclusion was a greater Mg intake established by a 3-d dietary record, as described below. Parents were informed of the purpose of the study and dietary Mg status after screening.

The study was a randomized, placebo-controlled, double-blind, yearlong interventional trial of magnesium oxide compared with placebo. Tests were performed in the Clinical Research Centers at Yale University School of Medicine. The study protocol was approved by the Yale Human Investigation Committee. After baseline evaluation, subjects were evaluated at 1, 6, and 12 months after initiating supplementation. Subjects were contacted at 1- to 2-month intervals to assess safety and compliance. If any untoward events occurred during the study, subjects were instructed to contact the study coordinator.

Recruitment and enrollment

After initial contact of eligible subjects by pediatricians via office posting or letter, we explained the project in detail by telephone. If inclusion/exclusion criteria were met, written consent from parents and assent from children were obtained. The eligible study population consisted of premenarchal healthy Caucasian adolescent females, aged 8–14 yr. A registered dietitian interviewed the parent and child to obtain dietary details. After analysis of dietary data, individuals with average Mg intakes less than 220 mg/d were invited to participate in the yearlong supplementation trial. Those participating underwent physical examination by a research nurse trained in pediatric endocrinology. Tanner stage of breast development was recorded.

Inclusion criteria were as follows: Caucasian ethnicity, a ratio of weight-to-height between the third and 97th centiles, and the absence of bone disease. Exclusion criteria were as follows: scoliosis, onset of menses, use of chronic medications (retinoids, thyroid hormone, GH, glucocorticoids, oral contraceptives, anticonvulsants, diuretics, or supplements providing pharmacological dosages of vitamins A or D).

Randomization and intervention

Subjects were randomized in blocks of four to receive either Mg oxide or placebo (1:1 ratio), using a random number table. Study personnel and subjects were blinded to treatment. Mg was supplemented twice daily in a capsule containing powdered magnesium oxide (300 mg of elemental Mg per day). Identically appearing encapsulated methylcellulose powder served as placebo. Capsules were provided in calendar-coded cards with two capsules in each sealed blister. One- to 3-month supplies were distributed throughout the study. Monthly telephone contact by the study coordinator assessed safety and encouraged compliance.

Outcome measures

At entry and after 6 and 12 months of supplementation, densitometric measures of the lumbar spine and hip were performed. BMC was chosen as the primary skeletal outcome variable because it is a direct measure and is not confounded by changes in bone area that occur during growth. Height and weight were recorded, and a complete biochemical profile was obtained at these times and additionally after 1 month of supplementation, as shown in Tables 1 and 5. Follow-up visits and blood sampling generally occurred in the mid-afternoon as to not interrupt school schedules.

Densitometric measures of bone mass accrual

Bone densitometry was performed using dual-energy x-ray absorptiometry (Hologic QDR 4500W bone densitometer; Hologic, Bedford, MA) at four hip sites: femoral neck, trochanter, the intertrochanteric regions of the femoral diaphysis (which taken together are the total hip BMC), and Ward's area. Anterio-posterior scans of the lumbar spine were obtained and were analyzed using pediatric software (Legacy Low Density Spine-revision C; Hologic). All scans were performed by one of two technicians with experience in performing bone densitometry in children. All scans were reviewed to ensure comparable definition of regions of the hip for serial scans within the same subject.

Biochemical assays

Serum and urinary biochemical determinations were performed by the Clinical Chemistry Laboratory at Yale-New Haven Hospital. Total serum and urinary calcium was determined by flame-atomic absorptiometry (model 2380; PerkinElmer, Norwalk, CT). Serum and urinary magnesium, phosphorus, and creatinine were measured using autoanalyzer technology. The urinary bone resorptive marker, NTx (Ostex, Seattle, WA), the N-telopeptide of type I collagen, was assayed by kit methodology. Serum immunoreactive PTH, 25-OHD, and $1,25(OH)_2D$ were measured as described (28), as was serum osteocalcin (29). Tubular reabsorption of phosphate (TRP) was calculated according to the formula:

$$\text{TRP}\ (\%) = 1 - \frac{[\text{Urinary phosphate}] \times [\text{Serum creatinine}]}{[\text{Serum phosphate}] \times [\text{Urinary creatinine}]} \times 100$$

from serum and concurrent timed urine samples.

Nutritional analysis

A questionnaire on general food preferences was used to estimate daily Mg intake. Those with an estimated Mg intake less than 220 mg/d were provided detailed instructions for keeping an ongoing 3-d diet diary for detailed analysis. Instructions for completing the food record were provided in a face-to-face meeting using food models, and printed descriptions of portion sizes. Subjects were asked to record brand names of consumed foods, estimate portion sizes using household measurements, and describe food preparation. Subjects were asked to record their intake over two weekdays and one weekend day.

The completed record was reviewed by the registered dietitian, and any incomplete information was clarified by telephone contact. Results of the food record were provided to subjects, and those with an average Mg intake of less than 220 mg/d were invited to participate. A second 3-d food record was completed by participants midway through the study to assess consistency of intake. Nutrient analysis was performed by a registered research dietitian using the Food Processor Program (ESHA Research, Inc., Salem, OR).

Measures of compliance

Pill counts were performed, and percentage of missed doses was calculated. Fractional excretion of Mg (FEMg), a standard physiological parameter representative of Mg intake, was calculated at baseline and at 1, 6, and 12 months of supplementation, according to the formula:

$$\text{FEM}_g\ (\%) = \frac{[\text{Urinary Mg}] \times [\text{Serum creatinine}]}{[\text{Serum Mg}] \times [\text{Urinary creatinine}]}$$

from serum and concurrent timed urine samples.

Statistical analysis

Statistical analyses for BMC and bone mineral density (BMD) were performed in SAS version 8.2 (SAS Institute Inc., Cary, NC). A P value of 0.1 (one-sided) was used as the level of significance for all tests.

The primary objective of the analysis was to evaluate the magnitude and variability of the incremental BMC changes in the treatment group compared with the placebo group after 12 months of treatment. The secondary objectives were to assess trends in BMC and BMD as related to treatment for each maturity group and for each skeletal site examined.

Maturity groups consisted of a prepubertal-early pubertal group (Tanner stage 1 or 2 at enrollment) and a mid-late pubertal group (Tanner stage 3 or 4 at enrollment). The primary hypotheses were tested using analysis of covariance models with repeated measures over three hip regions [femoral neck, total hip (encompassing the femoral neck, trochanteric, and intertrochanteric regions of the femoral diaphysis), and Ward's area]. In the model, the incremental BMC change from baseline to 12 months was the outcome variable. The treatment (which has two levels) and maturity group (which has two levels) served as fixed effects, and baseline BMC served as a covariate to adjust the baseline effect. Within-subject covariance was adjusted by an unstructured variance-covariance pattern matrix. In addition to the factors and covariates described above, we also tested the following interactions: treatment by maturity group, treatment by location, and treatment by baseline BMC (or BMD). All interactions were tested at the 0.1 level. If none of the interactions was significant, the absolute difference of increase between baseline and the 12-month parameters for treatment and placebo groups was tested by the above-described ANCOVA model in an overall analysis. The associated 95% confidence interval was calculated as well. Least squares means and SE values of BMC (and BMD) increases were calculated in the model and were plotted for each treatment group as a whole as well as for each Tanner group. If a significant treatment and maturity group interaction was found, we applied the same model for each maturity group to assess the treatment effect. Lumbar spine changes were of a markedly different magnitude and were therefore analyzed separately, using similar methodology.

Biochemical data were analyzed using analysis of covariance, employing SAS. Treatment comparisons of these parameters over the time frame of the study were made using a model that accounts for dependence of observations obtained from the same patient by modeling the correlation structure. Treatment, time, the interaction between treatment and time, and baseline levels of the outcome were included in the model as fixed effects. A secondary subgroup analysis was performed examining the effects of treatment and time of therapy with pubertal staging. Where appropriate, result of biochemical data are expressed as least squares means. A one-sided significance level of 0.05 was used to compare treatment *vs.* placebo groups, unless otherwise stated.

TABLE 1. Anthropomorphic data, dietary intake, and biochemistry at enrollment

	Mg-treated (mean ± SD)	Placebo (mean ± SD)
Total no. of subjects	23	27
Anthropometric data		
Age (yr)	10.7 ± 1.3	10.8 ± 1.6
Height (cm)	144.4 ± 9.7	143.8 ± 11.2
Weight (kg)	38.1 ± 8.9	37.2 ± 7.5
BMI (kg/m^2)	18.0 ± 2.4	17.8 ± 1.9
Daily dietary intake		
Energy (kcal)	1554 ± 302	1605 ± 251
Protein (g)	55 ± 15	56 ± 15
Calcium (mg)[a]	786 ± 245	785 ± 279
Magnesium (mg)[b]	184 ± 29	185 ± 26
Phosphorus (mg)[c]	940 ± 240	990 ± 239
Potassium (mg)	1736 ± 363	1773 ± 431
Sodium (mg)	2421 ± 613	2488 ± 718
Vitamin D (IU)[d]	126 ± 58	150 ± 83
Vitamin A (RE)[e]	271 ± 337	236 ± 240
Serum/plasma biochemistry		
Ca (mg/dl)	10.0 ± 0.4	10.0 ± 0.4
Mg (mg/dl)	2.10 ± 0.16	2.13 ± 0.17
P (mg/dl)	4.3 ± 0.5	4.3 ± 0.3
PTH (nlEq/ml)	21 ± 9	34 ± 25
Alkaline phosphatase activity (U/liter)	280 ± 66	271 ± 67
Osteocalcin (ng/ml)[f]	37 ± 11	34 ± 12
Creatinine (mg/dl)	0.7 ± 0.1	0.7 ± 0.1
BUN (mg/dl)	11 ± 3	11 ± 2
25-OHD (ng/ml)	25 ± 4	27 ± 4
1,25(OH)$_2$D (pg/ml)	54 ± 13	56 ± 12
Urine biochemistry		
N-Telopeptide (nmol BCE/mmol Cr)[g]	865 ± 475	734 ± 339
Ca/creatinine (mg/mg)	0.11 ± 0.13	0.09 ± 0.07
TRP (%)	91.7 ± 6.4	91.3 ± 5.0
FEMg (%)	3.7 ± 4.3	3.2 ± 2.8

At baseline, the distribution of anthropomorphic measures, dietary intake, and biochemical parameters between the placebo and Mg-treated groups was comparable ($P > 0.05$). To convert to SI units, multiply calcium value by 0.25 (mmol/liter), magnesium by 0.411 (mmol/liter), phosphorus by 0.323 (mmol/liter), osteocalcin by 0.154 (nmol/liter), creatinine by 88.42 (μmol/liter), BUN by 0.36 (mmol/liter), 25-OHD by 2.5 (nmol/liter), and 1,25-OH$_2$ D by 2.4 (pmol/liter). BUN, Blood urea nitrogen.

[a] The adequate intake for calcium in 9- to 13-yr-old girls is 1300 mg/d (21).

[b] The RDA for magnesium in 9- to 13-yr-old girls is 240 mg/d (21).

[c] The RDA for phosphorus in 9- to 13-yr-old girls is 1250 mg/d (21).

[d] The adequate intake for vitamin D in 9- to 13-yr-old girls is 200 U/d (21).

[e] RE, Retinol equivalents.

[f] Normal range for 8- to 14-yr-old girls is 10–50 ng/ml.

[g] nmol BCE/mmol Cr, Nanomoles of bovine collagen equivalents per millimole of creatinine; normal range for 8- to 14-yr-old girls is 47–2430 (38).

Results

Study population

A total of 122 subjects were screened, 50 subjects enrolled, and 44 completed the study. Dropout rate was four of 27 (15%) for the placebo and two of 23 (9%) for the Mg-supplemented group. Reasons given for withdrawal included moving away, excessive time commitment, and difficulty with compliance with treatment. Anthropomorphic measures, average dietary intake, biochemical variables (Table 1), and bone mass measures were not different between treatment groups at enrollment (Table 2).

Measures of bone mass accrual

In the entire cohort, Mg supplementation in this group of healthy girls with relative Mg undernutrition resulted in an approximately 3% greater increase in the overall hip measures of BMC during the year of therapy compared with placebo (1.05 ± 0.06 g and 0.97 ± 0.06 g, in Mg-treated *vs.* placebo-treated girls, respectively; Fig. 1A and Table 3). This effect of Mg supplementation on BMC was significant ($F_{1,38} = 3.97$; $P = 0.0534$; Table 3). No two-way or three-way interactions of treatment, Tanner group, or location were significant ($P > 0.1$). A significant effect of baseline BMC

TABLE 2. Densitometric measures of bone mass at enrollment

	Mg-treated (mean ± SD)	Placebo (mean ± SD)
BMC (g)		
Lumbar spine	27.31 ± 6.53	28.39 ± 9.15
Ward's area	0.678 ± 0.16	0.719 ± 0.17
Total hip	18.27 ± 4.77	17.87 ± 5.81
Bone area (cm^2)		
Lumbar spine	39.80 ± 5.65	40.70 ± 6.90
Ward's area	1.14 ± 0.10	1.16 ± 0.09
Total hip	25.27 ± 4.23	24.70 ± 5.07
BMD, BMC/bone area (g/cm^2)		
Lumbar spine	0.679 ± 0.082	0.683 ± 0.117
Ward's area	0.592 ± 0.113	0.621 ± 0.124
Total hip	0.700 ± 0.088	0.712 ± 0.117

Measures of bone mass were comparable at baseline between the placebo and Mg-treated groups.

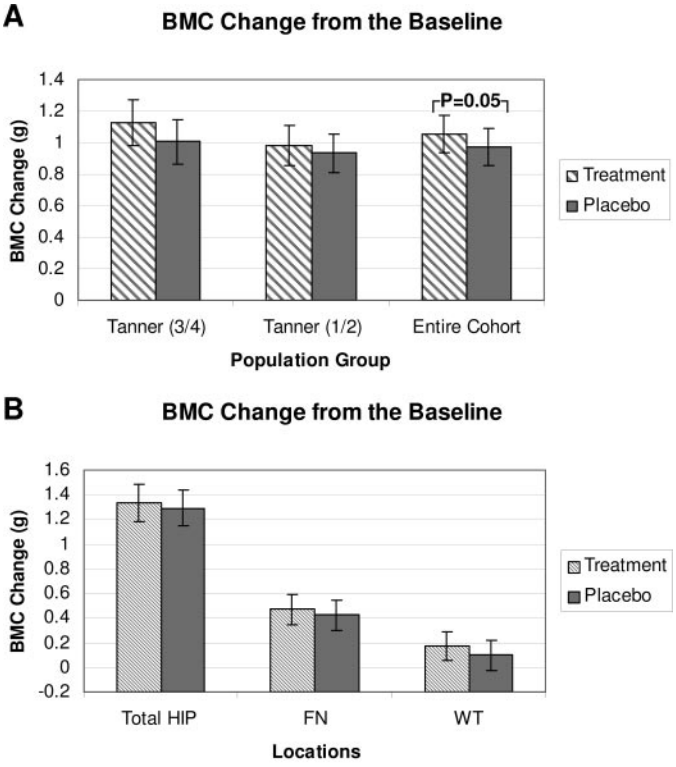

FIG. 1. A, Net change in BMC during the year of the study, over all hip locations measured, expressed in grams. The least square mean of the change in BMC is represented for subjects receiving Mg by the *hatched bars*, and for subjects receiving placebo by the *solid gray bars*. Data for the entire cohort is shown in the *pair of bars on the right*, and by Tanner grouping in the *first and second pairs*. The effect of Mg treatment on overall combined hip BMC measures was greater than that of placebo in the entire cohort ($P < 0.1$). Subgroup analysis by maturity rating confirmed that the direction of the effect on BMC with Mg treatment was evident in both less and more mature girls. B, Net change in BMC during the year of the study by specific anatomical sites. As described above, data are presented as least square means, and subjects receiving Mg are represented by the *hatched bars*, and subjects receiving placebo by the *dark gray bars*. The difference in incremental gain in BMC at each site favored the Mg-supplemented group, although a statistically significant difference could not be shown when each site was analyzed separately. FN, Femoral neck; WT, Ward's triangle area.

($F_{1,52}=9.99$; $P = 0.0026$) indicates that baseline BMC accounts for some of the variance in the change of BMC over the study period. The least square means calculated for both the less mature (Tanner 1 and 2) group (0.98 ± 0.06 g in Mg-treated *vs.* 0.93 ± 0.06 g in placebo) and more mature (Tanner stage 3 and 4) group (1.13 ± 0.07 g in Mg-treated *vs.*1.01 ± 0.07 g in placebo) support a consistent treatment trend across stages of pubertal maturity (Fig. 1A). We then evaluated the treatment effect on BMC at each of the hip regions (total hip, femoral neck, and Ward's area). BMC at each location showed the same treatment trend favoring Mg supplementation as found with the overall combined hip data, although no individual site reached the 0.1 significance level (Table 4 and Fig. 1B). Although the Mg-supplemented group had a slightly greater mean incremental gain in spinal BMC, these differences were not statistically significant (data not shown).

TABLE 3. Combined overall hip measures of bone mass (as change from baseline)

	Least square mean (g)	SE	*P* value
BMC			
Entire cohort			0.0534
Treatment	1.0542	0.06014	
Placebo	0.9688	0.05903	
Tanner 1/2 group			0.2967
Treatment	0.9822	0.06178	
Placebo	0.9324	0.06055	
Tanner 3/4 group			0.0991
Treatment	1.1262	0.0734	
Placebo	1.0052	0.07077	
BMD			
Entire cohort			0.8444
Treatment	0.1163	0.03524	
Placebo	0.1143	0.03513	
Tanner 1/2 group			0.6357
Treatment	0.09897	0.03543	
Placebo	0.1044	0.03531	
Tanner 3/4 group			0.5854
Treatment	0.1337	0.03685	
Placebo	0.1242	0.03657	

BMD was examined using the same methods as performed for BMC. None of the two- or three-way interactions of treatment were significant ($P > 0.1$). There was not a significant treatment effect for BMD in the overall cohort ($P = 0.8444$), although the incremental change in BMD favored the Mg-supplemented group. This observation was similar for the less mature (Tanner stage 1/2, $P = 0.6357$) and more mature (Tanner 3/4) groups ($P = 0.5854$) upon subgroup analysis. Similar results were seen when analyzing BMD corrected for height and BMD corrected for BMI (data not shown). The incremental gain in spinal BMD, as with BMC, was also slightly, but not significantly, greater in the Mg-supplemented group compared with the placebo group.

Biochemical outcomes

Biochemical parameters at 1, 6, and 12 months of therapy are listed in Table 5. No significant effects of Mg supplementation on any of these parameters were evident, except FEMg (see *Safety and compliance*), which was consistently greater during Mg supplementation. A trend toward a greater decrement in urinary excretion of N-telopeptide of type 1 collagen was present at 1 month, suggesting an acute effect of Mg on decreasing bone resorption; however, the absolute excretion of this marker was not different at this or other time points.

TABLE 4. Changes in BMC by individual location

Skeletal location	Least square mean (g)	SE	*P* value
Total hip			0.5769
Treatment	1.3325	0.08194	
Placebo	1.2907	0.07946	
Ward's area			0.1287
Treatment	0.1721	0.03440	
Placebo	0.09825	0.03291	
Femoral neck			0.5515
Treatment	0.4700	0.05477	
Placebo	0.4261	0.05136	

TABLE 5. Biochemistry values through the course of Mg supplementation (mean ± SD)

	Mg-treated (months)			Placebo (months)		
	1	6	12	1	6	12
Serum/plasma						
Ca (mg/dl)	9.9 ± 0.4	10.0 ± 0.4	10.0 ± 0.4	9.9 ± 0.3	9.9 ± 0.4	10.0 ± 0.3
Mg (mg/dl)	2.15 ± 0.15	2.14 ± 0.17	2.13 ± 0.19	2.12 ± 0.16	2.08 ± 0.16	2.10 ± 0.14
P (mg/dl)	4.5 ± 0.6	4.6 ± 0.4	4.3 ± 0.5	4.5 ± 0.6	4.5 ± 0.3	4.3 ± 0.4
ALP (U/liter)	266 ± 68	261 ± 79	260 ± 46	273 ± 61	275 ± 91	255 ± 64
PTH (nlEq/ml)	24 ± 12	23 ± 11	23 ± 12	29 ± 19	30 ± 21	30 ± 23
OC (ng/ml)	33 ± 11	34 ± 12	40 ± 14	33 ± 11	32 ± 9	36 ± 15
Cr (mg/dl)	0.7 ± 0.1	0.7 ± 0.1	0.7 + 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
BUN (mg/dl)	12 ± 3	11 + 3	11 ± 2	13 ± 3	12 + 2	12 + 3
25-OHD (ng/ml)	26 ± 5	29 ± 9	26 ± 3	27 ± 3	27 ± 4	26 ± 4
$1,25(OH)_2D$ (pg/ml)	53 ± 8	54 ± 10	58 ± 6	58 ± 11	59 ± 13	59 ± 9
Urine						
N-Telopeptide (nmol BCE/ mmol Cr)	674 ± 341	761 ± 423	663 + 364	662 ± 349	589 ± 326	599 ± 411
Ca/Cr (mg/mg)	0.09 ± 0.06	0.12 ± 0.10	0.11 ± 0.07	0.10 ± 0.07	0.08 ± 0.07	0.10 ± 0.10
TRP (%)	91.8 ± 4.4	91.6 ± 4.4	92.9 ± 4.8	88.6 ± 4.8	89.9 ± 4.3	91.3 ± 5.5
FEMg (%)[a]	4.3 ± 1.5	4.3 ± 1.7	4.1 ± 1.8	2.7 ± 1.3	2.7 ± 1.0	2.7 ± 1.7

To convert to SI units, multiply calcium value by 0.25 (mmol/liter), magnesium by 0.411 (mmol/liter), phosphorus by 0.323 (mmol/liter), osteocalcin by 0.154 (nmol/liter), creatinine by 88.42 (μmol/liter), BUN by 0.36 (mmol/liter), 25-OHD by 2.5 (nmol/liter), and $1,25\text{-}OH_2$ D by 2.4 (pmol/liter). ALP, Alkaline phosphatase activity; OC, osteocalcin; Cr, creatinine; nmol BCE/mmol Cr, nanomoles of bovine collagen equivalents per millimole of creatinine. BUN, Blood urea nitrogen.

[a] A significant effect of treatment was seen on FEMg ($P = 0.0003$) but was seen for no other variable.

Safety and compliance

Only two subjects reported side effects; both developed loose bowel movements upon starting supplementation. This resolved upon halving the treatment dose with resumption of full dose after 7 d.

Compliance with treatment was approximately 71% for the placebo group and 74% for the Mg-supplemented group and was confirmed by greater FEMg in the Mg-supplemented subjects at all treatment points (Table 5). In three subjects after 6 months of supplementation, we examined intracellular free Mg content of the gastrocnemius muscle, as determined by ^{31}P-nuclear magnetic resonance spectroscopy, adapting the methods of Ryschon *et al.* (30). This methodology uses the chemical shift of ATP peaks in the setting of variable concentrations of intracellular Mg. No differences were evident between Mg-treated or placebo treated subjects (data not shown).

Discussion

This study provides data supporting the hypothesis that Mg supplementation has positive effects on accrual of bone mass in adolescents with suboptimal Mg intake. The incremental gain in overall hip BMC in subjects receiving Mg was significantly greater than in placebo-supplemented subjects. Subgroup analysis of these effects as stratified by maturity (Tanner pubertal stage 1 and 2 girls as a contrast to Tanner stage 3 and 4 girls) was performed, demonstrating that changes favoring Mg supplementation held for each maturity group (although the effects in either group alone did not reach statistical significance). Moreover, analysis of each hip site indicated that change in BMC favored the Mg-treated group for total hip, femoral neck, and Ward's area. The skeletal effects occurred with no major changes in mineral levels or markers of bone turnover. Finally, the Mg-supplemented group had slightly higher (but not statistically significant) incremental gain in spinal BMC and BMD than the placebo group. Daily supplementation of 300 mg of Mg given as two divided doses of encapsulated Mg oxide was safe and well tolerated and met with reasonable compliance. There was no significant difference in weight gain between placebo and Mg-supplemented groups.

Previously correlations of dietary Mg intake with BMD have been demonstrated (31–35). Spinal BMD varied with quartile of Mg intake in premenopausal Scottish women (34); Mg intake in early adolescence correlates with calcaneal bone mass in young adulthood (35), suggesting a role for Mg in bone mineral accretion in early adolescence. Our examination of NHANES data demonstrated an association of dietary Mg and hip BMD in selected groups (*e.g.* younger non-Hispanic white men) (36).

Interventional studies examining Mg effects on bone are limited. Decrements in bone turnover markers are seen by d 5 of Mg supplementation (360 mg/d) in healthy young men (23). In an uncontrolled study of postmenopausal osteoporotic women, Mg supplementation was associated with BMD increases in 60% of those treated (24). A placebo-controlled study of patients with gluten-sensitive enteropathy demonstrated increased BMD after 6 months of Mg supplementation, compared with placebo-supplemented subjects (25). Thus, Mg intervention studies to date have demonstrated positive effects on bone mass, although they have been performed in older populations with underlying illness and not in a healthy young population.

Thus, Mg supplementation may be an important consideration in the periadolescent group, given the suboptimal dietary Mg intake documented in U.S. food surveys (21, 22, 37). We reasoned that early adolescence is an important time to affect Mg intake and therefore designed a pilot study to determine the effects of Mg supplementation in this group. We included only Caucasian females as to limit variance in bone mass explained by gender and race. We enrolled sub-

jects with Mg intake in the lower half of the screened subjects to select for those most likely to respond to the intervention. Subjects were as motivated as could be expected with overall compliance of 73%. The overall dropout rate was 12% and was randomly distributed across treatment and maturity groups.

A primary limitation to this study is its small size. We did not have sufficient preliminary data to predict true sample size requirements and did not have sufficient statistical power to detect changes in solitary anatomical sites. The data should not be overinterpreted given the marginal significance of the differences. However, we suggest that more robust findings would have been possible with a larger study, because the trends favoring Mg supplementation are consistent across all pubertal groups and all anatomical sites studied. Because we studied girls with average Mg intake less than 220 mg/d, we cannot extrapolate the findings to individuals with greater Mg intake, males, or other ethnic groups.

In summary, we have successfully carried out a pilot study demonstrating a positive effect of Mg supplementation for 12 months on accrual of bone mass in peripubertal Caucasian girls selected for suboptimal daily Mg intake. The supplement was well tolerated and safe. This study will serve as a model for designing future studies on skeletal effects of Mg in children.

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