Daily Oral Magnesium Supplementation Suppresses Bone Turnover in Young Adult Males*

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ABSTRACT

This study examined the effects of daily oral magnesium (Mg) supplementation on bone turnover in 12 young (27–36 yr old) healthy men. Twelve healthy men of matching age, height, and weight were recruited as the control group. The study group received orally 15 mmol Mg (Magnosolv powder, Asta Medica) daily in the early afternoon with 2-h fasting before and after Mg intake. Fasting blood and second void urine samples were collected in the early morning on days 0, 1, 5, 10, 20, and 30, respectively. Total and ionized Mg^{2+} and calcium (Ca^{2+}), and intact PTH (iPTH) levels were determined in blood samples. Serum biochemical markers of bone formation (*i.e.* C-terminus of type I procollagen peptide and osteocalcin) and resorption (*i.e.* type I collagen telopeptide) and urinary Mg level adjusted for creatinine were measured. In these young males, 30 consecutive days of oral Mg supplementation had no significant effect on total circulating Mg level, but caused a significant reduction in the serum ionized Mg^{2+} level after 5 days of intake. The Mg supplementation also significantly reduced the serum iPTH level, which did not appear to be related to changes in serum Ca^{2+} because the Mg intake had no significant effect on serum levels of either total or ionized Ca^{2+}. There was a strong positive correlation between serum iPTH and ionized Mg^{2+} (r = 0.699; $P < 0.001$), supporting the contention that decreased serum iPTH may be associated with the reduction in serum ionized Mg^{2+}. Mg supplementation also reduced levels of both serum bone formation and resorption biochemical markers after 1–5 days, consistent with the premise that Mg supplementation may have a suppressive effect on bone turnover rate. Covariance analyses revealed that serum bone formation markers correlated negatively with ionized Mg^{2+} (r = −0.274 for type I procollagen peptide and −0.315 for osteocalcin), but not with iPTH or ionized Ca^{2+}. Thus, the suppressive effect on bone formation may be mediated by the reduction in serum ionized Mg^{2+} level (and not iPTH or ionized Ca^{2+}). In summary, this study has demonstrated for the first time that oral Mg supplementation in normal young adults caused reductions in serum levels of iPTH, ionized Mg^{2+}, and biochemical markers of bone turnover. In conclusion, oral Mg supplementation may suppress bone turnover in young adults. Because increased bone turnover has been implicated as a significant etiological factor for bone loss, these findings raise the interesting possibility that oral Mg supplementation may have beneficial effects in reducing bone loss associated with high bone turnover, such as age-related osteoporosis. (*J Clin Endocrinol Metab* **83:** 2742–2748, 1998)

$\mathbf{M}$AGNESIUM (Mg) is the second most abundant intracellular cation in vertebrates. Mg ion is a critical cofactor in more than 300 enzymatic reactions involving energy metabolism, and protein and nucleic acid synthesis (1). Accordingly, Mg is essential for various normal tissue and organ functions (2). The primary source of Mg in humans is from the diets. The dietary Mg ion is absorbed in the intestine through both active and passive transport systems (3). Excessive Mg is rapidly excreted into the urine (4). During Mg deprivation, the kidney avidly conserves Mg and excretes virtually no Mg in the urine (5). Approximately half of the total Mg in the body of a normal adult human is present intracellularly in soft tissues, and the other half is found in bone, either as exchangeable, surface-bound, divalent cations (2, 6), which may serve as a reservoir for maintaining normal extracellular Mg level, or as an integral component of the hydroxyapatite lattice in bone matrix (6), which may be released during bone resorption. Thus, in addition to the intestine and kidney, the bone is involved in Mg homeostasis.

Past studies with Mg depletion in both humans and animals indicate that Mg may have key regulatory roles in bone and mineral metabolism. It has been shown that Mg is essential for the normal function of the parathyroid glands, vitamin D metabolism, and adequate sensitivity of target tissues to PTH and active vitamin D metabolites (7). Therefore, Mg deprivation is regularly associated with hypoparathyroidism, low production of 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], and end-organ resistance to PTH and vitamin D. The combined effects of Mg deficiency on PTH and 1,25-$(OH)_2D_3$ synthesis and secretion could lead to hypocalcemia without the compensatory increase in PTH secretion (8). These Mg deficiency-associated adverse effects together could impair bone growth and mineralization (9–11) and thereby could reduce bone quality, strength, and density (12–14). Accordingly, Mg deficiency has been suggested to be a potential risk factor for osteoporosis (1, 15, 16). In this regard, women with postmenopausal osteoporosis are frequently associated with low dietary Mg intake (17) and reduced serum and bone Mg levels (18, 19). Two earlier reports, which indicated that Mg repletion in postmenopausal os-

teoporotic women significantly increased bone mass and reduced fracture rate (15, 16), support the contention that Mg deficiency may be associated with osteoporosis. The dietary Mg intake in a large section of population across all ages in Western culture is below the Recommended Dietary Allowance (RDA) for Mg (300–350 mg or 12.5–15 mmol) (20), suggesting that occult Mg deficiency may be relatively prevalent (21). Several recent studies have also indicated that oral Mg supplementation leads to significant increases in bone density in both pre- and postmenopausal women (22–24). These observations together raise the intriguing possibility that Mg supplementation may have potential benefits in the prevention and/or treatment of osteopenia and/or osteoporosis.

Although the adverse effects of Mg depletion on bone metabolism have been extensively investigated in Mg-deficient humans and animals, little is known about the effects of Mg supplementation on bone metabolism in normal human subjects. Moreover, although it appears that Mg repletion was able to increase bone mass in postmenopausal patients (15, 16, 22, 24) as well as in premenopausal women (23), the mechanism by which Mg repletion may cause an increase in bone mass has not been determined. Accordingly, the present study sought to evaluate the effects of daily oral supplementation of a moderate dose of Mg on bone turnover in 12 healthy young adult men with no evidence of Mg deficiency. Twelve healthy males with matching age, height, and body weight were included as controls. Serum biochemical markers of bone formation [*i.e.* C-terminus of type I procollagen peptide (PICP) and osteocalcin] and resorption [*i.e.* type I collagen telopeptide (ICTP)] were monitored as indices of bone turnover. Serum intact PTH (iPTH) and calcium (Ca) were measured to assess effects on Ca metabolism. In this report, we present the first serum biochemical marker evidence suggesting that oral supplementation of a moderate dose of Mg suppresses bone turnover rates in young adult males.

Subjects and Methods

Study subjects

Twelve men, from 27–36 yr of age, were recruited at the University Hospital of the Graz Medical School in Austria. For comparison, 12 age-, height-, and weight-matched male volunteers were included as controls. All participants were in excellent health, were nonsmokers, and did not have a history of drug or alcohol abuse. None of the subjects had abnormal values of serum parameters of hepatic and renal functions or abnormal serum electrolytes and iPTH levels. All subjects were consuming regular diets. Daily dietary intake information was assessed by an extensive dietary recall method (25), in which detailed dietary intakes for 6 consecutive days were obtained for the calculation of daily intakes of calories, protein, sodium, Ca, Mg, and phosphorus with the EWP computer software program using an Austrian nutrient data base (Data Input, Vienna, Austria). All subjects were instructed to abstain from alcohol consumption and excessive physical activities 7 days before and during the study. The study protocol, which followed the Helsinki Declaration as revised in 1989, and the written informed consent were approved by the institutional review board of the University of Graz Medical School. Signed informed consent was obtained from each study subject before participation.

Study protocol

This study consisted of 30 consecutive days of oral Mg supplementation. Starting from the afternoon of day 0 of the study, the subjects in

the Mg-supplemented group received a daily oral dose of 15 mmol Mg in the form of Magnosolv powder (Asta Medica, Wien, Austria), containing 670 mg magnesium carbonate precipitate (equivalent to 169 mg Mg) and 342 mg magnesium oxide (equivalent to 196 mg). The Magnosolv powder (one portion contained 365 mg Mg, 3,703 mg citric acid, 5 mg saccharin-sodium, 60 mg sodium cyclamate, and 500 mg potassium hydrogen carbonate) was dissolved in 250 mL drinking water and was taken unsupervised in the early afternoon with a 2-h fasting period before and after the Mg intake. The 2-h fasting before and after the supplementation was intended to avoid any potential interference of Mg absorption by other food items. The poststudy interview of the study subjects indicated full compliance, and this was supported by the finding of a significant increase in urinary Mg excretion in the test subjects compared to the control group (see *top panel* of Fig. 1). For comparison, the control subjects were asked to drink a glass of water daily in the afternoon with 2-h fasting before and after the intake during the study.

Overnight fasting blood samples and second void urine samples were obtained from each subject between 0800–0900 h on days 0 (baseline, *i.e.* before the first Mg dose), 1, 5, 10, 20, and 30, respectively. Blood pH was measured in freshly obtained arterial blood samples. Levels of ionized Mg (iMg^{2+}) and Ca (iCa^{2+}) ions were measured in freshly obtained venous blood. Levels of total Mg (tMg) and Ca (tCa), iPTH, osteocalcin, PICP, ICTP, and creatinine were measured in serum samples. Mg and creatinine levels were determined in the urine samples.
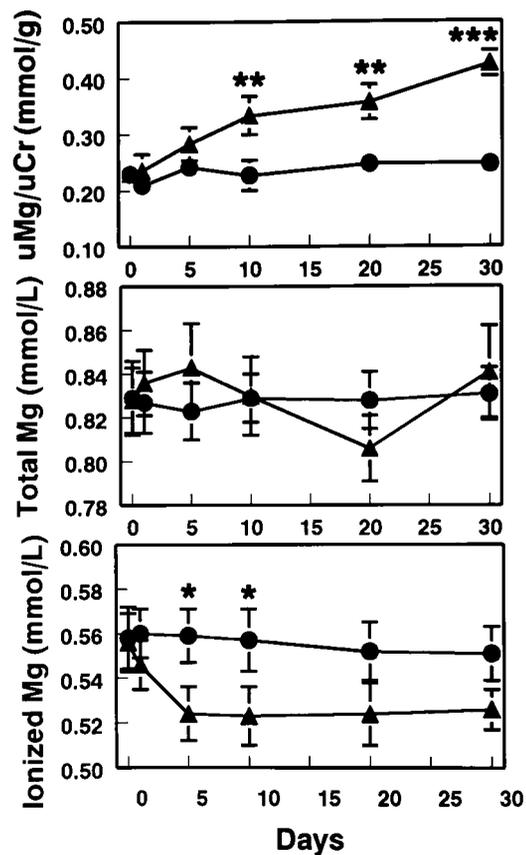

FIG. 1. Effects of daily oral Mg supplementation on urinary Mg excretion (*top panel*) and on serum levels of total Mg (*middle panel*) and ionized Mg^{2+} (*bottom level*). The *closed triangles* are the Mg-supplemented subjects, and the *closed circles* are the control subjects matched in age, weight, and height. The results are shown as the mean ± SEM (n = 12). By two-way ANOVA: a, for urinary Mg excretion, time effect: $P < 0.001$; group effect: $P < 0.001$; and time × group interaction: $P = 0.003$; b, for total Mg, $P = $ NS for both time and group effects and also for the time × group interaction; c, for iMg^{2+}, time effect: $P = $ NS; group effect: $P = 0.002$; and time × group interaction: $P = $ NS. *, $P < 0.05$ **, $P < 0.01$, ***, $P < 0.001$ (pairwise comparison by Fisher's LSD *post-hoc* analysis).

Analytical methods

Arterial samples for measurements of blood pH were handled under anaerobic conditions using a micropuncture set (AVL Microsampler, Medical Instruments, AVL Austria, Graz, Austria), and blood pH was measured within 30 s of collection using an AVL 995-HB automated blood gas analyzer (AVL Austria) (26).

Blood samples for determination of circulating iMg^{2+} and iCa^{2+} levels were drawn into lithium-heparin-containing tubes with an evacuated blood-collecting system (Vacutainer, Becton Dickinson, Rutherford, NJ). The iMg^{2+} was immediately measured in triplicate, using an ion-selective flow-through, liquid membrane electrode (AVL 988–4 Mg Electrolyte Analyzer, AVL Austria) (26). The iCa^{2+} level was determined (in triplicate) immediately on an automated electrolyte analyzer (AVL 988–4 Electrolyte Analyzer) (26). The blood levels of iMg^{2+} and iCa^{2+} were corrected for pH. The reference intervals for iMg^{2+} and iCa^{2+} were 0.4–0.8 and 1.12–1.32 mmol/L, respectively.

Blood samples for serum parameters were collected in evacuated venipuncture tubes in the absence of the additives. Serum samples were prepared after clotting for 1 h at 4 C and were kept in aliquots at −20 C until assay. No serum sample was stored for longer than 2 months. The serum tMg level was measured by the automated xylidyl blue method (27) on the Hitachi 717 spectrophotometer (Boehringer Mannheim, Mannheim, Germany). The tCa was measured with the cresolphthalein complexone method (28) using the Hitachi 717 spectrophotometer. The reference intervals for tMg and tCa were 0.65–1.15 and 2.0–2.6 mmol/L, respectively. Osteocalcin was determined by the OS-TKR-PR RIA kit (CIS Bio-International, ORIS Group, Cedex, France). PICP was assayed by a commercial RIA kit (Orion Diagnostica, Espoo, Finland) as previously described by Melkko *et al.* (29). ICTP was measured with a commercial RIA (Telopeptide, Orion Diagnostica). iPTH was determined with a two-site immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA). The reference interval for iPTH was 10–65 ng/L. Creatinine levels were determined according to the method of Heinegard and Tiderstroem (30). All serum assays were performed in triplicate, and the reported results have passed quality control testings.

Urinary tMg was also measured by the automated xylidyl blue method and was standardized against the urinary creatinine level. The glomerular filtration rate (GFR), a measure of renal function, was calculated according to the equation established by Cockroft and Gault (31), which estimates creatinine clearance from serum creatinine.

Statistical analyses

Results are reported as the mean ± SEM (n = 12 for each). Statistical significance was analyzed with the Systat statistical program (Systat, Evanston, IL) using an IBM-compatible personal computer. The statistical significance of the effect of the supplementation and/or treatment duration was analyzed by two-way ANOVA. Pairwise comparison between groups at each time point was assessed with the Fisher's least significant difference (LSD) *post-hoc* test. Multiple correlation analysis was performed with the Pearson's correlation matrix method. Multiple linear regression and covariance analyses were performed to determine partial correlation coefficient of multiple factors to assess the relative contribution of each corresponding factor to a given effect. The difference was considered significant when $P < 0.05$.

Results

Study subjects

The characteristics of the study subjects and their average daily nutritional intakes are summarized in Table 1. The mean age of the 12 subjects in the Mg-supplemented group was 31 yr. The average height and body weight were 181 cm and 75.2 kg, respectively. The 12 control subjects matched the study subjects in age, weight, and height. There were no baseline differences in the test physiological parameters between the supplemented group and the matched control group. During the study period, there was no significant gain or loss in body weight in either group.

TABLE 1. Subject characteristics and average daily dietary intakes of the study subjects (n = 12)

	Control subjects	Study subjects
Age (yr)	31 ± 3	31 ± 3
Ht (cm)	179 ± 9	181 ± 8
Wt (kg)	74.5 ± 7.4	75.2 ± 10.4
Calorie intake (Cal)	1,995 ± 26	2,019 ± 25
Protein intake (g)	76 ± 5	70 ± 3
Calcium intake (mmol)	21.45 ± 2.05	21.12 ± 1.85
Phosphorus intake (mmol)	45.2 ± 2.9	41.9 ± 1.9
Sodium intake (mmol)	160.9 ± 26.1	156.5 ± 17.4
Average daily dietary Mg intake (mmol)	12.8 ± 0.3	12.9 ± 0.5
Average daily total Mg intake (diet + supplement; mmol)	12.8 ± 0.3	27.9 ± 0.5[a]

Values are the mean ± SEM.
[a] $P < 0.001$.

Dietary analyses revealed that there was no significant difference in the average daily dietary intake of calories, protein, Ca, sodium, and phosphorus between the Mg-supplemented group and the matched control group throughout the study (Table 1). Without considering the supplement, the average daily Mg intakes from dietary sources in the control and the Mg-supplemented group were similar (~13 mmol/day or ~310 mg/day for each group). However, including the supplement, the total daily Mg intake of the study group was twice that of the control group, *i.e.* ~28 *vs.* ~13 mmol/day (Table 1).

Effects of oral Mg supplementation on circulating level of electrolytes and iPTH

Figure 1 shows the effect of the supplementation on Mg homeostasis. The urinary Mg excretion (normalized against urine creatinine) in the supplemented group was significantly elevated (by 2-fold) compared to that in the age-matched controls (*top panel*), confirming the increased Mg intake in the supplemented subjects. Two-way ANOVA indicate that the effects of both treatment group and time were each highly significant at $P < 0.001$, and that there was a significant interaction between Mg supplementation and duration ($P = 0.003$). The *middle panel* of Fig. 1 indicates that the serum tMg level in the supplemented subjects, but not that in the controls, fluctuated in a cyclic manner during the study phase: slightly elevated after 5 days, decreased below the basal level after 20 days, and later again increased on day 30. However, neither the effect of the Mg supplementation nor the duration was significant (by two-way ANOVA). In contrast, despite a significant increase in Mg intake, the blood level of iMg^{2+} in the supplemented group was significantly ($P = 0.002$, by ANOVA) decreased (by as much as 6%) after 5–10 days of Mg supplementation compared to that in the control group (Fig. 1, *bottom panel*). Despite the apparent contrasting effect on the serum level of tMg and the blood level of iMg^{2+}, the serum tMg level correlated strongly and positively with the blood iMg^{2+} level in the supplemented subjects (r = 0.642; $P < 0.001$). A significant positive correlation between serum tMg and blood iMg^{2+} (r = 0.319; $P < 0.01$) was also observed in the age-matched control subjects.

The effects of oral Mg supplementation on the circulating levels of Ca and iPTH are shown in Fig. 2. Two-way ANOVA
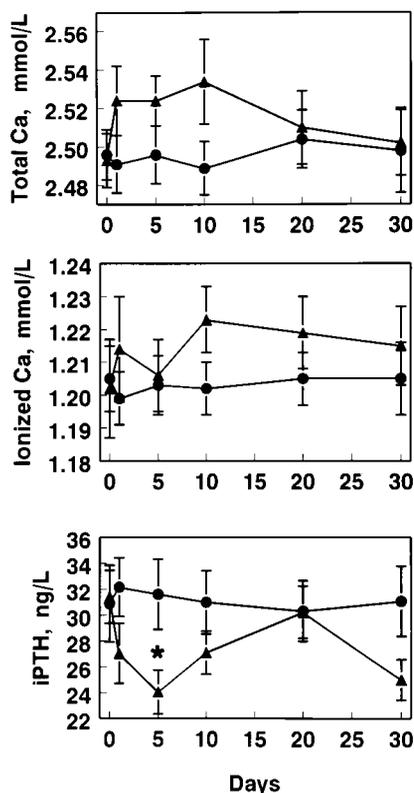
FIG. 2. Effects of daily oral Mg supplementation on the circulating level of tCa (*top panel*), iCa^{2+} (*middle panel*), and iPTH (*bottom level*). The *closed triangles* are the Mg-supplemented subjects, and the *closed circles* are the matched control subjects. The results are shown as the mean ± SEM (n = 12). By two-way ANOVA: a, for tCa, time effect: P = NS; group effect: P = 0.049; and time × group interaction: P = NS; b, for iCa^{2+}, P = NS for both the time and group effect; P = NS for the time × group interaction; c, for iPTH, time effect: P = NS; group effect: P = 0.005; time × group interaction: P = NS. *, P < 0.05; (pairwise comparison by Fisher's LSD *post-hoc* analysis).

reveals that the Mg supplementation had no significant effect on either the serum tCa (*top panel*) or the blood iCa^{2+} (*middle panel*) level (by two-way ANOVA). On the other hand, Mg supplementation significantly (P = 0.005, by ANOVA) suppressed the serum iPTH level by as much as 24% (*bottom panel*), and the suppressive effect seemed to be transient and cyclic in nature (*i.e.* P = 0.02 and 0.062, by Fisher's LSD *post-hoc* test, on days 5 and 30, respectively). Pearson's correlation coefficient and scatter plot (Fig. 3) analyses revealed a strong correlation between the blood iMg^{2+} level (or the serum tMg level) and the serum iPTH level in both the Mg-supplemented subjects and the control subjects. Conversely, there was no significant correlation between the serum levels of iPTH and tCa or between serum iPTH and blood iCa^{2+} (data not shown). Multiple linear regression indicates that only Mg^{2+}, and not Ca^{2+}, displayed a significant partial correlation coefficient (r = 0.396; P = 0.001 for serum tMg; and r = 0.699; P < 0.001 for blood iMg^{2+}) with serum iPTH and that inclusion of the Ca^{2+} in the regression analysis did not improve the overall correlation (r = 0.401; P = 0.004 for tMg and tCa *vs.* r = 0.400; P < 0.001 for tMg alone; and r = 0.703, P < 0.001 for iMg^{2+} plus iCa^{2+} *vs.* r = 0.699; P < 0.001 for iMg^{2+} alone).

Because changes in the blood iMg^{2+} level could have an impact on the acid-base balance and/or renal functions, we also monitored changes in arterial pH and GFR of the participants during the study. The arterial pH and the GFR of the supplemented subjects were not altered significantly with time and also did not differ significantly from those of the control subjects at any time point (data not shown), suggesting that the oral Mg supplementation has no significant adverse effect on the acid-base balance or kidney functions. The supplementation also had no significant effect on circulating levels of creatinine and albumin (data not shown).

*Effects of oral Mg supplementation on serum biochemical markers of bone turnover*

To evaluate whether Mg supplementation had an effect on bone turnover, we monitored the level of serum biochemical markers of bone formation, *i.e.* osteocalcin (*top panel* of Fig. 4) and PICP (*middle panel* of Fig. 4), and resorption, *i.e.* ICTP (*bottom panel* of Fig. 4). Oral Mg supplementation rapidly (within 1–5 days) decreased the serum level of each of the test serum biochemical markers in the supplemented subjects compared to those in the controls: a 15% decrease in serum osteocalcin (P = 0.033, by ANOVA), a 20% decrease in serum PICP (P = 0.007, by ANOVA), and a 30% decrease in serum ICTP (P = 0.004, by ANOVA). The suppression of these markers seemed to be transient, as the reductions were significant only during the first 5–10 days and were not significant after 10–20 days of the supplementation. However, after 20–30 days of supplementation, the serum levels of osteocalcin and PICP were slightly reduced compared to those in the matched control, even though these decreases did not reach a statistical significant level based on Fisher's LSD test. These findings, nevertheless, raise the possibility that the suppressive effect of Mg supplementation on bone turnover serum markers, like that on serum iPTH, might be cyclic.

Pearson's correlation coefficient analysis revealed that serum PICP and osteocalcin, but not serum ICTP, were significantly correlated with the blood iMg^{2+} level (r = −0.353; P < 0.003 for osteocalcin, and r = −0.237; P < 0.05 for serum PICP), but not with Ca^{2+} or iPTH. Multiple linear regression analyses (Table 2) also revealed that blood iMg^{2+}, but not blood iCa^{2+} or serum iPTH, exhibited a significant partial correlation coefficient with both bone formation markers (*i.e.* osteocalcin and PICP) in the Mg-supplemented subjects. Conversely, neither blood levels of iMg^{2+} and iCa^{2+} nor the serum iPTH level displayed a significant partial correlation coefficient with serum ICTP. Covariance analyses confirmed that only the blood iMg^{2+} level, but not the blood iCa^{2+} level or the serum iPTH level, correlated significantly with the two serum bone formation markers (r = −0.274 for PICP, and r = −0.315 for osteocalcin) in the Mg-supplemented subjects.

Discussion

Although Mg repletion has been shown to increase bone mass and reduce the fracture rate in the Mg-deficient osteoporotic as well as premenopausal women (15, 16, 22–24), the issue of whether Mg supplementation also has a beneficial effect on the skeleton in normal, non-Mg-deficient individ-

Control subjects

Mg-supplemented subjects

FIG. 3. Correlation relationship between levels of serum iPTH and circulating tMg (*circles, top panels*) or ionized Mg^{2+} (*triangles, bottom panels*) in the matched control subjects (*open symbols, left panels*) and in the Mg-supplemented subjects (*closed symbols, right panels*). The correlation coefficient of each scatter plot was determined by the Pearson's correlation matrix method.
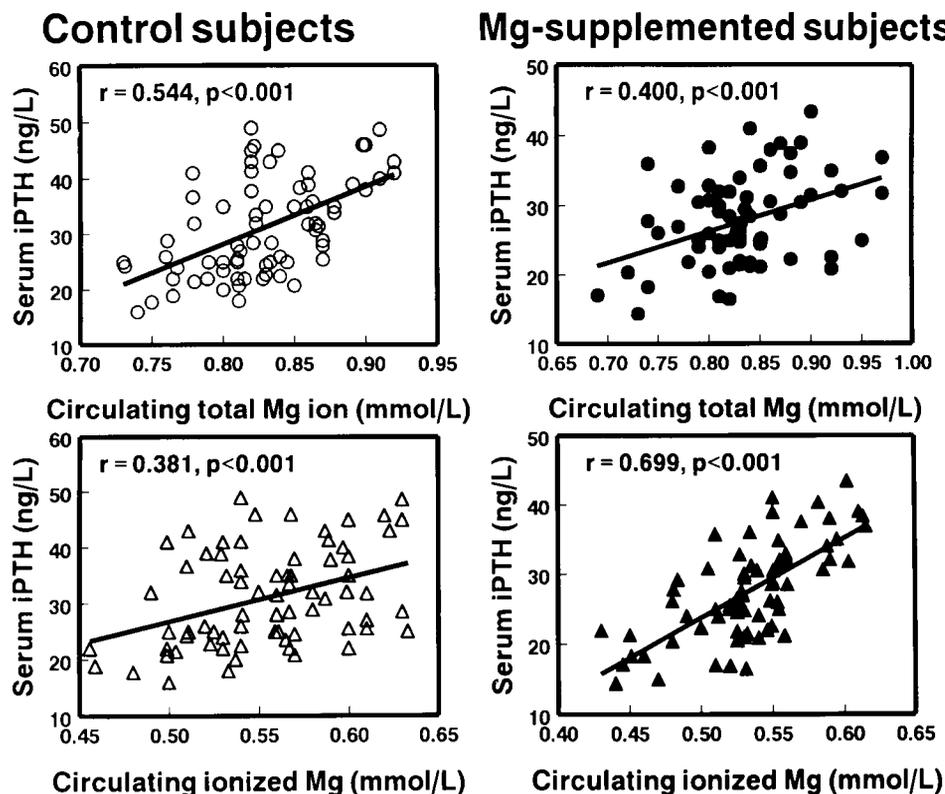
uals has not been evaluated. In this study, we have demonstrated that daily oral supplementation of a moderate dose, *i.e.* 350 mg (~15 mmol), of Mg, given to young adult men as a single dose, significantly reduced serum levels of biochemical markers of both bone formation (*i.e.* osteocalcin and PICP) and resorption (*i.e.* ICTP), compared to those in the control subjects who were matched in age, weight, and height. The subjects in this study were not Mg deficient, because 1) their daily dietary Mg was within the RDA for Mg (300–350 mg); and 2) they excreted a significant amount of Mg in the urine. These subjects were also not malnourished, nor were they deficient in dietary calcium or phosphorus intake. Accordingly, this study provides the first evidence with biochemical markers that oral Mg supplementation in normal, non-Mg-deficient, young adult men suppresses bone turnover.

Recent studies have shown that the Mg balance in Mg-deficient adolescent girls correlated strongly with the Mg intake and absorption (32), and that the serum iMg^{2+} level in pregnant women was proportional to the circulating tMg level (33). A previous study also showed that supplementation of a high dose (4–5 times the RDA) of Mg for 6 days significantly increased serum iMg^{2+} level in normal male volunteers (34). Accordingly, it is intriguing to note that the increased oral intake of Mg in these normal adult males, not only did not increase, but markedly decreased the circulating level of iMg^{2+}, which is the physiologically active form of the element. On the one hand, the serum tMg level in a normal adult is tightly maintained within a narrow range (between 0.7–1.0 mmol/L) (35). Thus, it is not surprising that the serum tMg level of these non-Mg-deficient individuals was not

significantly altered by the increased Mg intake. The observation that there was an apparent cyclic, albeit statistically nonsignificant, fluctuation in the serum tMg level in the supplemented subjects, but not in the matched subjects, is consistent with the premise that the serum tMg level in these individuals was tightly regulated. On the other hand, it is unclear why the daily supplementation of a moderate dose (1 times the RDA) of Mg in this study caused a significant reduction in the blood iMg^{2+} level. These findings are in contrast to the observed increase in serum iMg^{2+} in a previous study in which subjects were supplemented with a high dose (4–5 times the RDA) of dietary Mg (34). We may speculate that the contrasting results are related to the Mg dose. However, we should note that serum iMg^{2+} level was measured in previous studies, whereas the iMg^{2+} level in this study was measured in freshly prepared blood samples. Thus, we cannot ignore the possibility that a significant amount of serum iMg^{2+} might have been released from blood cells during blood coagulation, leading to an artificial increase in the serum iMg^{2+} level. With respect to the observed reduction in blood iMg^{2+} in this study, we speculate that it might be related to an increase in tissue uptake and/or renal excretion of free iMg^{2+}, but not bound Mg (36), or an increase in serum levels of the complexing ions, such as citrate, phosphate, and other anions, leading to the subsequent reduction of the free iMg^{2+} level in blood (1). Much work is needed to definitely determine the cause of the Mg supplementation-associated reduction in the blood iMg^{2+} level. It is also unclear why there was a significant correlation between serum tMg and blood iMg^{2+} levels in the supplemented subjects when the Mg supplementation appeared to
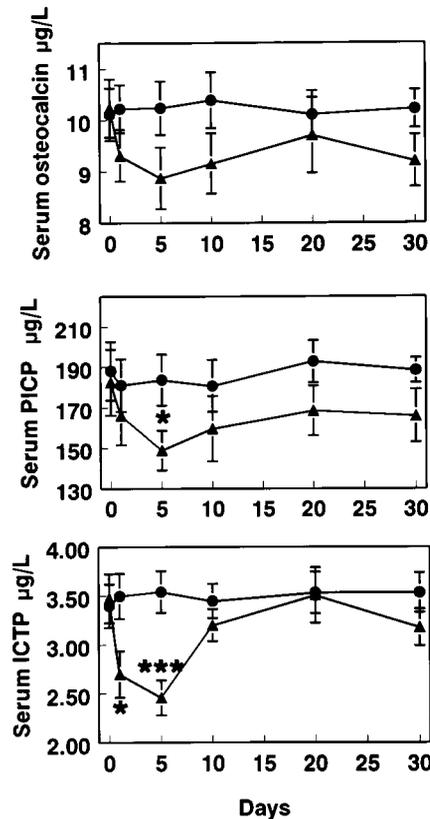
FIG. 4. Effects of daily oral Mg supplementation on serum levels of osteocalcin (*top panel*), PICP (*middle panel*), and ICTP (*bottom panel*). The *closed triangles* are the Mg-supplemented subjects, and the *closed circles* are the control subjects matched in age, weight, and height. The results are shown as the mean ± SEM (n = 12). By two-way ANOVA: a, for osteocalcin, time effect: P = NS; group effect: P = 0.033; time × group interaction: P = NS; b, for PICP, time effect: P = NS; group effect: P = 0.007; time × group interaction: P = NS; c, for ICTP, time effect: P = NS; group effect: P = 0.004; time × group interaction: P = 0.049. *, P < 0.05; ***, P < 0.001 (pairwise comparison by Fisher's LSD *post-hoc* analysis).

TABLE 2. Partial correlation coefficient of circulating iPTH, iMg^{2+}, and iCa^{2+} in multiple correlation analysis of serum bone turnover parameters in the Mg-supplemented subjects

Partial correlation coefficient	iPTH	iMg^{2+}	iCa^{2+}
Osteocalcin	0.161	−0.466	0.026
	p = n.s.	p = 0.005	p = n.s.
PICP	0.248	−0.410	0.192
	p = n.s.	p = 0.012	p = n.s.
ICTP	0.083	0.031	0.068
	p = n.s.	p = n.s.	p = n.s.

Seventy-two data points were used for each parameter.

have differential effects on the serum tMg and blood iMg^{2+} levels. However, we should point out that the number of subjects in this study was rather small (n = 12 for each group). A correlation based on a small sample size should be validated with larger studies.

Regardless of the cause of the reduction of the blood iMg^{2+} level, this study suggests that the reduction of blood iMg^{2+} may be involved in the Mg supplementation-induced suppression of bone turnover, as there was a strong correlation between the blood iMg^{2+} level and the serum biochemical markers in the supplemented subjects. Previous studies of Mg deficiency have suggested that the skeletal effects of Mg may be mediated through the reduction in the synthesis of and responses to PTH and 1,25-$(OH)_2D_3$ (1, 2, 37) as well as through alterations in the calcium homeostasis (38). In this regard, we found that the oral Mg supplementation also reduced the circulating level of iPTH, a finding consistent with the past observations that Mg supplementation may have an inhibitory action on PTH secretion (39, 40). This study suggests that the decrease in serum iPTH level might also be related to the reduction in the blood iMg^{2+} level, as there was a strong, positive correlation between blood iMg^{2+} and serum iPTH. On the other hand, we did not find a significant correlation between serum levels of iPTH and any of the test biochemical markers in the Mg-supplemented subjects, suggesting that the observed suppression of bone turnover might not be related to the reduction in the serum iPTH level. Although serum Ca^{2+} is a known regulator of bone turnover, the lack of a significant correlation between serum bone turnover markers and the circulating level of Ca^{2+} in the supplemented subjects also does not support a major role for serum Ca^{2+} in the suppression of bone turnover. However, we are also mindful that the small sample size in this study might not have the sufficient statistical power to detect the association between the suppression of bone turnover and serum iPTH or Ca^{2+} levels. Nevertheless, based on these correlative findings, we tentatively conclude that the observed suppression of bone turnover may be associated with the reduction in the blood iMg^{2+} level rather than with changes in serum levels of Ca^{2+} or iPTH.

The mechanism by which the reduction in blood iMg^{2+} caused a reduction in bone turnover remains elusive. Considering the critical requirement of iMg^{2+} for numerous biological processes in cells, including bone cells, it is not unreasonable to speculate that Mg^{2+} may have a direct action on bone cells to influence their activities. Accordingly, it has been suggested that Mg^{2+} might act directly on osteoblasts to stimulate cell proliferation (41). The findings that the serum and bone osteocalcin levels in rodents were markedly decreased within 1–2 weeks of feeding a Mg-deficient diet (9, 12), and that the Mg deprivation in rats caused a decrease in the synthesis of bone collagen and alkaline phosphatase (42) are also consistent with a direct action of Mg^{2+} on osteoblasts. However, we should emphasize that there is currently no definitive evidence that iMg^{2+} could act directly on osteoblasts to alter their activities. On the other hand, there is as yet compelling evidence for a direct action of Mg^{2+} on osteoclast activity. The lack of a significant correlation between the blood Mg^{2+} level and the serum bone resorption marker, ICTP, in this study also does not seem to support the contention of a direct action of Mg^{2+} on osteoclast activity. Because of the relatively small sample size of this study, and because the serum ICTP is not a sensitive marker of bone resorption marker, we cannot rule out the possibility that iMg^{2+} could still have a small effect on bone resorption. Accordingly, much additional work is required to disclose the exact mechanism by which iMg^{2+} regulates bone turnover.

Finally, it is intriguing to note that the suppressive effect of Mg supplementation on serum iPTH and biochemical markers

appeared to be transient. The apparent transient nature of these responses raises the interesting possibility that there are counterregulatory mechanisms in normal humans to counteract the effects of the Mg supplementation. In addition, there may be circumstantial evidence supporting the interpretation that the suppressive effects of the Mg supplementation on serum iPTH and biochemical markers might be cyclic in nature. Unfortunately, the duration of this study (*i.e.* 30 days) did not appear to be sufficiently long to allow for a definitive demonstration of the cyclic responses. If the cyclic responses to Mg supplementation is confirmed by subsequent studies, this would suggest that long term Mg supplementation may overcome the putative counterregulatory mechanisms and, as such, may allow for a prolonged suppression of bone turnover in normal non-Mg-deficient individuals.

In summary, we have demonstrated for the first time that daily oral Mg supplementation in normal, non-Mg-deficient, young adults induced a transient suppression of bone turnover. As there is evidence suggesting that adequate Mg intake may be equally as effective as adequate calcium intake in promoting achievement of peak bone mass, we should not overlook the importance of an adequate Mg intake in growing children and young adults. More importantly, because increased bone turnover has been implicated as a potential etiological factor for the bone loss in osteoporotic patients (43), these findings raise an intriguing possibility that daily oral Mg supplementation may be used to suppress bone turnover, which subsequently may lead to reduce bone loss and, thus, may have a potential utility for treatment of osteopenia and/or osteoporosis associated with high bone turnover.

Acknowledgments

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