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Reprod. Nutr. Dev. 42 (2002) 25–33

DOI: 10.1051/rnd:2002003

Original article

Dietary magnesium increases calcium absorption of ovine small intestine in vivo and in vitro

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(Received 25 May 2001; accepted 15 January 2002)

Abstract — The purpose of this study was to investigate whether or not an increase in dietary Mg intake increases Ca absorption in the ovine gastrointestinal tract. In an in vivo experiment, an increase in the infused $MgCl_2$ level (0.0, 25.0 and 75.0 mg Mg·kg BW^{-1}·day^{-1} with 75.0 mg Ca·kg BW^{-1}·day^{-1} as $CaCl_2$) into the rumen for ten days significantly decreased fecal excretion but increased urinary excretion ($P < 0.05$) of Ca in five castrated male sheep. Apparent Ca absorption tended to increase ($P = 0.067$) whilst the retention and plasma concentration of Ca were not changed. In an in vitro experiment with isolated segments from the rumen, upper jejunum, cecum and upper colon under the presence of an electrochemical gradient, the mucosal to serosal Ca flux rate was significantly greater in the presence of 60.0 mM as compared with 1.2 mM $MgCl_2$ ($P < 0.05$). From these results, we conclude that the mucosal Mg has the ability to increase the Ca absorption in the gastrointestinal tract in sheep when the dietary Mg level is raised.

calcium absorption / magnesium / sheep

1. INTRODUCTION

The effects of the dietary composition on the intestinal Ca absorption have extensively been studied in ruminants because milk fever of dairy cows is caused by an imbalance between Ca availability and high Ca demand following the parturition and the onset of lactation (for review, see Breves et al. [6] and Yano et al. [40]). Although milk fever is partly prevented by the limiting of dietary Ca supply [3, 22], the addition of calcium propionate [14] and the acidic treatment of diets [17, 30, 37], its prevention has been incomplete.

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It is well known that small intestine is major site of Ca absorption. It was reported that Ca absorption in the digestive tract occurs mainly in the lower duodenum and upper jejunum in sheep in vivo [1, 31]. Furthermore, the significant net flux rates of Ca were recently reported in the caprine duodenum and jejunum and in the ovine jejunum using the Ussing chamber technique [33]. In addition, it has been recently reported that there existed significant net Ca absorption in the rumen, and this is increased by 1,25 $(OH)_2D_3$ and short-chain fatty acids (SCFAs) [5, 38]. On the contrary, it has been believed that the rumen is the major site of Mg absorption in the ruminant. Martens et al. reported that the mechanism of net Mg absorption in the rumen is dependent on the transepithelial potential difference by the difference of K concentration between mucosal and serosal [25, 26]. Also, it was recently reported the net Mg absorption was influenced by CO_2, SCFAs and phosphate in the rumen [12, 38].

Mg plays the complementary role as a long-term regulatory element together with Ca for various cellular functions [16]. We reported that Mg concentrations in the supernatant of the digesta sampled from the duodenum to ileum increased from 3.9 to 8.2 mM, and an enhanced Mg concentration in the mucosal solution increased net Ca absorption in a concentration-dependent manner in the everted sacs of the caprine ileum [23]. Further, we recently observed that Mg enhanced Ca absorption in everted sacs of the ovine ileum, and that the action was not dependent on the potential difference between mucosal and serosal sides [24]. There are some reports showing that an increased in dietary Mg level enhanced Ca absorption in cattle [20] and human [9]. However, the results of the action of Mg on Ca metabolism reported in many animal species are controversial. When the dietary level of Mg was raised, Ca absorption was increased in human [7], rats [11] and ponies [18], but was not changed in human [35] and sheep [19]. Further, it is recently reported that the apparent calcium absorption of rats fed a diet containing 0.025% Mg was lower than those of rats fed 0.05% Mg, but was higher than those of rats fed 0.15% Mg [36].

The purpose of this study, therefore, was to elucidate whether or not an increase in dietary Mg intake raises Ca absorption in the gastrointestinal tract in vivo, and which parts of the gastrointestinal tract are responsible for the Mg-induced Ca absorption in vitro in sheep.

In an in vivo experiment, we examined the effects of the three Mg levels (0.0, 25.0 and 75.0 mg Mg·kg BW^{-1}·day^{-1}), that were achieved by an infusion of $MgCl_2$ into the rumen, on the Ca metabolism and the plasma Ca concentration. In an in vitro experiment, we examined the effects of increased mucosal Mg concentration on the mucosal to serosal Ca flux rate which was measured with tissue sheets isolated from 4 parts of the gastrointestinal tract (rumen, upper jejunum, cecum and upper colon) of sheep using the Ussing chamber technique.

2. MATERIALS AND METHODS

2.1. Mineral balance studies in vivo

Five castrated male sheep weighing 46–55 kg (4–5 years old) were used in a metabolism cage. They were surgically equipped with a rumen cannula under anesthesia. They were fed timothy hay (2.5% of the body weight (BW)) at 10:00 once a day. They had free access to deionized water. All the experiments were carried out according to the *Guiding Principles for the Care and Use of Animals in the Field of Physiological Science* published by The Physiological Society of Japan and *Guide for the Care and Use of Laboratory Animals* published by Tohoku University. The timothy hay contained 0.18% Mg, 0.24% Ca, 0.27% P and 48.83% TDN on a dry matter basis. Mg levels were altered by a bolus administration

of three doses of $MgCl_2$ (0.0, 25.0, 75.0 mg·kg BW^{-1}·day^{-1} of Mg) into the rumen through a cannula during feeding. In addition, $CaCl_2$ was administered simultaneously with $MgCl_2$ at the dose of 75.0 mg·kg BW^{-1}·day^{-1} of Ca. Animals were finally fed with 39.5, 64.6 and 114.5 mg·kg BW^{-1}·day^{-1} of Mg, respectively, with 128.8 mg·kg BW^{-1}·day^{-1} of Ca. Therefore, the average dietary intake was 2.1, 3.4, and 6.1 g·day^{-1} of Mg and 6.8 g·day^{-1} of Ca. Each diet was fed for 10 days and the total feces and urine were collected on day 9 and 10. The blood samples were taken through the jugular vein in heparinized syringes before feeding on the last day for each diet. The fecal samples prepared by the wet-ashing method with HNO_3 and HClO were dissolved in 6 N HCl. The urine samples (2 mL) were mixed with 1 N HCl (1 mL). The plasma samples (3 mL) were mixed with 1 N HCl solution (1.5 mL), boiled and were centrifuged at 1 000 × *g* for 10 min after the addition with 10% trichloric acetic acid solution (1.5 mL). The supernatant of the samples was analyzed for Ca and Mg by atomic absorption spectrophotometry (AA-610, Shimazu, Japan). Apparent absorption and retention of the mineral were determined by the following equations:

Apparent absorption = Intake – Fecal excretion,

Retention = Intake – (Fecal excretion + Urinary excretion).

2.2. In vitro Ca absorption in tissue sheets of the gastrointestinal tract with the Ussing chamber

The tissue sheets from the gastrointestinal tract (rumen (ventral sac), upper jejunum (3.0–4.0 m from the pylorus), cecum and upper colon) from five sheep were obtained at a local abattoir. The tissue sheets, after washed fully with physiological saline solution, were attached on the Ussing chamber (exposed area 1 cm^2) to measure the mucosal to serosal Ca flux rate (Jms) in the presence of an electrochemical gradient. After 30-min preincubation, the mucosal side of the tissue was bathed with 5 mL of 10 mM Ca-containing HEPES-buffered saline solution (HBS, pH 6.5) whilst the serosal side was bathed with 5 mL of Ca-free HBS (pH 7.4) at 37 °C under 100% O_2. Serosal solution of 500 μL was sampled three times at 30-min intervals to measure Ca concentration. Jms was calculated by the following equation: Jms (nmole·cm^{-2}·h^{-1}) = Vs·Δ[Ca]s·A^{-1}·ΔT^{-1}, where Vs is the volume of the medium in serosal side (5 mL), Δ[Ca]s is the difference of serosal Ca concentration between samples 1 and 2 (or samples 2 and 3), A is the exposed area of segment (1 cm^2) and ΔT is the time interval of the sampling (30 min). The result of Jms was calculated as the mean of the two values.

2.3. Solutions

2.3.1. Serosal solution

Serosal solution contained (mM): 125.0 NaCl, 4.7 KCl, 1.2 $MgCl_2$, 10.0 sodium gluconate, 10.0 glucose, 20.0 HEPES (pH 7.4).

2.3.2. Mucosal

The 1.2 mM $MgCl_2$ solution contained (mM): 125.0 NaCl, 4.7 KCl, 1.2 $MgCl_2$, 10.0 $CaCl_2$, 10.0 glucose, 20.0 HEPES. The 60.0 mM $MgCl_2$ solution contained (mM): 55.0 NaCl, 4.7 KCl, 60.0 $MgCl_2$, 10.0 $CaCl_2$, 10.0 glucose, 20.0 HEPES (pH 6.5).

Osmolality of all solutions was adjusted to 300–310 mOsm·kg^{-1} H_2O.

2.4. Statistics

The results are represented as the mean ± SEM. The mean values were analyzed by paired *t*-test or one-way analysis of variance (ANOVA) followed by Duncan multiple range test [41].

3. RESULTS

3.1. Balance study

The body weight of animals did not change during the period of the balance experiment. Diarrhea was not observed during the experiment. Table I shows effects of the dietary Mg level on Ca and Mg balances in sheep ($n = 5$). A fecal Ca excretion of sheep with administration of 0.0 mg·kg^{-1} BW·day^{-1} Mg level was 137.2 ± 5.6 mg·kg^{-1} BW·day^{-1}. An increase in the dietary Mg levels significantly decreased the fecal Ca excretion (121.2 ± 5.5 and 110.6 ± 3.7 mg·kg^{-1} BW·day^{-1}; $P < 0.05$, Duncan test). In contrast, an urinary Ca excretion of sheep administered of 0.0 mg·kg^{-1} BW·day^{-1} was 6.3 ± 1.3 mg·kg^{-1} BW·day^{-1}, and an increase in the dietary Mg levels significantly increased the urinary Ca excretion (16.6 ± 3.1 and 25.7 ± 3.3 mg·kg^{-1} BW·day^{-1}; $P < 0.05$, Duncan test). When the dietary Mg level was raised, the apparent Ca absorption tended to increase from –18.6 ± 8.5 to 3.0 ± 4.0 mg·kg^{-1} BW·day^{-1} ($P = 0.067$, ANOVA). However, an increase in the dietary Mg levels did not influence the Ca retention (from –24.9 ± 8.5 to –21.0 ± 4.4 mg·kg^{-1} BW·day^{-1}) ($P = 0.906$).

When the Mg intakes were increased, a fecal Mg excretion of sheep was significantly raised from 19.9 ± 1.5 to 36.5 ± 0.8 and 66.9 ± 0.2 mg·kg^{-1} BW·day^{-1} ($P < 0.05$, Duncan test). Urinary Mg excretion was also significantly increased from 10.5 ± 1.6 to 18.6 ± 3.6 and 33.3 ± 5.0 mg·kg^{-1} BW·day^{-1} ($P < 0.05$, Duncan test). The apparent Mg absorption was also significantly raised from 13.6 ± 2.4 to 21.6 ± 2.7 and 37.9 ± 5.7 mg·kg^{-1} BW·day^{-1} ($P < 0.05$, Duncan test). However, an increase in the dietary Mg levels did not influence the Mg retention (from 2.7 ± 3.4 to

Table I. Effects of Mg levels on Ca and Mg balances in sheep.

	Ruminal infusion of Mg (mg·kg^{-1} BW·day^{-1})			ANOVA (*P*-value)
	0.0	20.0	75.0	
Ca				
Intake	118.6 ± 4.3	116.9 ± 4.7	113.6 ± 6.5	0.796
Fecal excretion	137.2 ± 5.6^{a}	12l.2 ± 5.5^{b}	110.6 ± 3.7^{b}	0.009
Urinary excretion	6.3 ± 1.3^{a}	16.6 ± 3.1^{b}	25.7 ± 3.3^{c}	0.001
Apparent absorption	–18.6 ± 8.5	–4.4 ± 4.1	3.0 ± 4.0	0.067
Retention	–24.9 ± 8.5	–21.0 ± 4.4	–22.6 ± 4.9	0.906
Mg				
Intake	32.0 ± 3.2^{a}	55.8 ± 3.4^{b}	103.4 ± 4.8^{c}	0.001
Fecal excretion	l9.9 ± 1.5^{a}	36.5 ± 0.8^{b}	66.9 ± 0.2^{c}	0.001
Urinary excretion	10.5 ± 1.6^{a}	18.6 ± 3.6^{a}	33.3 ± 5.0^{b}	0.003
Apparent absorption	13.6 ± 2.4^{a}	21.6 ± 2.7^{a}	37.9 ± 5.7^{b}	0.003
Retention	5.0 ± 2.9	2.7 ± 3.4	6.0 ± 2.4	0.722

Values (mg·kg^{-1} BW·day^{-1}) are means ± SEM for five sheep.
The *P*-values smaller than 0.001 are represented as 0.001.
Means not sharing a common letter (a, b and c) in the same row are significantly different ($P < 0.050$, Duncan test).

Table II. Effects of Mg levels on plasma Ca and Mg concentrations (mM) in sheep.

	Ruminal infusion of Mg (mg·kg^{-1} BW·day^{-1})			ANOVA (*P*-value)
	0.0	20.0	75.0	
Ca	2.78 ± 0.08	2.83 ± 0.07	2.80 ± 0.06	0.904
Mg	0.94 ± 0.05^{a}	0.95 ± 0.04^{a}	1.11 ± 0.05^{b}	0.002

Values (mM) are mean ± SEM for five sheep.
Means not sharing a common letter (a and b) in the same row are significantly different ($P < 0.050$, Duncan test).

6.0 ± 2.4 mg·kg^{-1} BW·day^{-1}; $P = 0.722$, ANOVA).

Table II shows the effects of the dietary Mg levels on plasma Ca and Mg concentrations of sheep. An increase in the dietary Mg levels did not influence plasma Ca concentrations although plasma Mg concentration was significantly greater when 75.0 mg Mg·kg^{-1} BW·day^{-1} of Mg was administered (1.11 ± 0.05 mM; $P < 0.05$, Duncan test).

3.2. In vitro Ca absorption study

We examined the effect of changing the serosal Mg concentration on mucosal to serosal flux rates (Jms) of Ca in the segment of the rumen, upper jejunum, cecum and upper colon of sheep (Fig. 1, $n = 5$). An increase in mucosal Mg concentration from 1.2 to 60.0 mM raised Jms for Ca in all the segments from different parts of the gastrointestinal tract (rumen, 18.0 ± 5.4 vs. 30.2

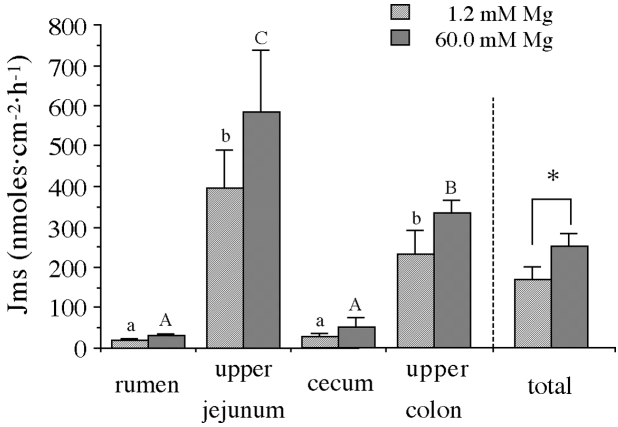

Figure 1. The effect of an increase in mucosal Mg concentration on the mucosal to serosal Ca flux rate (Jms) in the segments isolated from 4 parts of the gastrointestinal tract (rumen, upper jejunum, cecum, upper colon) of sheep. The results are shown as the mean ± SEM ($n = 5$). The segments fixed on the Ussing chambers were incubated in 10.0 mM Ca-containing HBS (pH 6.5) on the mucosal side and in Ca-free HBS (pH 7.4) on the serosal side as described in Materials and Methods. The asterisks represent a significant difference between the values for 1.2 and 60.0 mM Mg ($P < 0.050$, paired *t*-test). Means not sharing a common letter (a and b, or A, B and C) are significantly different between the values for 1.2 or 60.0 mM Mg treatment, respectively, of the 4 parts of the gastrointestinal tract ($P < 0.050$, Duncan test).

± 5.3; upper jejunum, 395.6 ± 93.2 vs. 584.3 ± 152.9; cecum, 27.8 ± 6.9 vs. 51.2 ± 22.9; upper colon, 230.4 ± 59.6 vs. 334.4 ± 29.4 nmole·cm^{-2}·h^{-1}). There was a significant increase in the combined mean of Jms in 4 different segments when treated with two different Mg concentrations (168.0 ± 30.2 vs. 250.3 ± 33.3 nmole·cm^{-2}·h^{-1}; $P < 0.05$, paired *t*-test). When the mucosal Mg concentration was 1.2 mM Mg, Jms of the upper jejunum and the upper colon was significantly greater than that of the rumen and the cecum ($P < 0.05$, Duncan test). On the contrary, when the mucosal Mg concentration was raised to 60.0 mM, the Jms of the upper jejunum was significantly greater than that of the other 3 different segments ($P < 0.05$, Duncan test).

4. DISCUSSION

In the present study, we examined whether or not an increase in dietary Mg intake changes Ca metabolism in sheep in vivo. We took the samples for mineral balance studies during a short period. Most cows are rapidly in negative Ca balance during the early days of lactation [27]. In addition, the contents of intestine are stable after about 7 days from alteration of diet. We think that Ca balance in a short period after about 10 days from the alteration of Mg diet is important for basic research to prevent milk fever. In this experiment, animals were finally given with 39.5, 64.6 and 114.5 mg·kg BW^{-1}·day^{-1} of Mg, respectively, with 128.8 mg·kg BW^{-1}·day^{-1} of Ca. That is, the average dietary intake was 2.1, 3.4, and 6.1 g·day^{-1} for Mg and 6.8 g·day^{-1} for Ca. As dietary requirement for Mg and Ca is from 0.12 to 0.18% and from 0.20 to 0.82%, respectively, of dry matter in sheep [28], calculated average dietary intake is from 1.6 to 2.4 g·day^{-1} for Mg and from 2.7 to 10.9 g·day^{-1} for Ca. These values were comparable with those in the present study.

An increase in the dietary Mg level significantly decreased the fecal Ca excretion and increased the urinary Ca excretion in vivo (Tab. I). The apparent Ca absorption tended to increase when the dietary any Mg level was raised (Tab. I). In addition, plasma Ca concentrations were not changed by the diet Mg levels (Tab. II). These results suggest that an increase in the mucosal Mg concentration raises the Ca absorption from the gastrointestinal tract when the dietary Mg level is raised. These findings also imply that an increased urinary Ca excretion may be to maintain plasma Ca concentration at the constant level in spite of an increased Ca absorption from the gastrointestinal tract in sheep. But an increase in the apparent Ca absorption was not significant when the dietary any Mg level was raised.

To maintain normal plasma Ca concentration, Ca balance is mainly controlled by resorption of bone Ca store, intestinal Ca absorption and urinary Ca excretion. Parathyroid hormone (PTH) enhances the bone Ca mobilization and the renal 1,25 $(OH)_2$ D_3 production, and decreases the urinary Ca excretion. Also, 1,25 $(OH)_2$ D_3 increases bone Ca mobilization and the intestinal Ca absorption to increase blood Ca concentration. We did not measure these hormones controlling Ca balance in this experiment. It was reported that hypermagnesemia decreased PTH concentration in the serum of goat [39] and rat [13]. Also, it was reported that there is a significant negative relationship between PTH and Mg concentrations in the blood of hemodialysis patients not receiving vitamin D_3 [29]. Further, it was reported that an increase in dietary Mg decreased plasma PTH concentration in rat [13]. When dietary Mg level was raised in the present experiment, hypermagnesemia may also decrease plasma PTH concentration.

It was reported that an increase in dietary Mg decreased Ca concentration in plasma in sheep [10] and cattle [8]. The mechanism of this effect would involve a decrease in PTH concentration coursed by an increase in dietary Mg. The decreased PTH level leads

to a reduction in the renal 1,25 $(OH)_2$ D_3 level, which decreases the bone Ca mobilization and increases the urinary Ca excretion. An increase in dietary Mg level did not change plasma Ca concentration in our experiment. Under our experimental conditions, an increase in Ca absorption induced by Mg would exceed a decrease in Ca absorption induced by a decrease in PTH concentration. In other words, Mg induces Ca absorption by a different mechanism as compared with PTH and/or 1,25 $(OH)_2$ D_3-mediated Ca absorption. In addition, a decrease in PTH concentration in blood would lead to an increase in urinary Ca excretion from kidney to maintain blood Ca concentration in this experiment. However the difference between our experimental conditions and other researcher's experimental conditions is not clear. It may be due to basal condition of Ca metabolism depending on aging, sex and breeding.

In the present experiment, an increased dietary Mg level did not influence the Ca retention in vivo (Tab. I). The negative values for the Ca retention observed in the present experiment may be due to aging and scarcity of exercise. Five castrated male sheep used in the experiment were 4–5 years old. It is reported that the Ca retention became negative when sheep get older than 16 months of age [4]. It is, also, known that aging decreases the calcium absorption in human [2]. In addition, the sheep were fed in the metabolism cage during the experiment. It is well known that prolonged inactivity induces a rise in the urinary calcium excretion. For example, scarcity of exercise showed the negative values for Ca retention [21], whilst exercise increased bone mass in human [32, 34].

It was reported that there existed significant net flux rates of Ca in the caprine duodenum and jejunum and in the ovine jejunum [33], and that the major site of Ca absorption in the digestive tract is the lower duodenum and upper jejunum in sheep [1, 31]. It was also reported that the Ca absorption in the small intestine was slightly raised when the dietary Mg level was increased from 0.1% to 0.2% of dry matter in sheep attached with abomasal and ileum cannulae [15]. In our experiment, an increase in the mucosal Mg concentration (60.0 mM) significantly increased the mean Jms of 4 different segments of the gastrointestinal tract, and Jms for the upper jejunum was greatest among the segments used (Fig. 1). Our results from in vitro experiments supported these previous results. It is, therefore, clear that the major site of Mg-induced Ca absorption is the small intestine. However, mucosal Mg concentration (60.0 mM) used in the present study was clearly higher than the physiological status although we reported that an increase in Mg concentration (from 1.2 to 60.0 mM) in the mucosal solution increased net Ca absorption in a concentration-dependent manner in the everted sacs of the caprine ileum [23]. Therefore, it is likely that higher Mg concentration at the luminal side should be an inevitable condition for the increased Ca absorption in vivo.

In the in vitro study, the mucosal Ca concentration was adjusted at 10 mM because the mucosal Ca concentration was about 2–14 mM in digesta sampled from the rumen to the upper jejunum of goats [23]. We are unable to demonstrate whether the active Ca transport or the passive Ca transport is responsible for the Mg-induced Ca absorption because there still exists a Ca concentration difference and an electrical potential difference between the mucosal and serosal sides. In the future, we need to measure the net Ca flux rates in the absence of an electrochemical gradient under short circuit condition to understand whether or not Mg accelerates the active Ca transport via the intracellular pathway.

We conclude that the mucosal Mg has the ability to increase the Ca absorption in the gastrointestine of sheep when the dietary Mg level was raised.

ACKNOWLEDGMENTS

The authors gratefully thank Dr. H. Yajima (Meiji Milk Products Co., LTD, Japan) for teaching us the usage of the Ussing chamber, and Messrs. Y. Ohtomo, M. Kawai, G. Furukawa and T. Seno (Tohoku University, Japan) for taking care of animals. The authors also thank Dr. Sravan C. Goparaju (AIST, Japan) for his advice on the preparation of this manuscript. A part of this study was financially supported by Mercian Corporation Co. LTD (Japan).

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