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Effect of Magnesium Deficiency on Intestinal Calcium Transport in Rats[1, 2] (40307)

HSU-FANG, CHOU, ROBERT H. WASSERMAN, AND RUTH SCHWARTZ

Division of Nutritional Sciences, Cornell University, Ithaca, New York 14853

Intestinal Ca absorption in rats has been reported to be increased (1–4), unaffected (5, 6), and decreased (7) as a result of magnesium depletion. Possible factors accounting for these differences are the degree of magnesium depletion, length of the depletion period, and indirect effects of inanition or alterations in growth rate of the deficient animals. Another factor that makes it difficult to compare findings from different laboratories is the particular technique used to determine Ca absorption. With the exception of two studies (1, 2), in which net Ca absorption was measured by the balance method, previous investigations on intestinal Ca transport in magnesium deficient rats were carried out by means of various *in vitro* techniques (3–7). To obtain information somewhat more applicable to the effects of magnesium depletion in the intact animal while avoiding the inaccuracies of the balance procedure, we used the ligated loop and *in situ* perfusion techniques to estimate Ca fluxes across the small intestine. Both methods were carried out *in vivo* and permit measurement of Ca fluxes in a defined intestinal segment. *In situ* perfusion has the additional advantage of allowing estimation of Ca fluxes at steady state, using serial perfusions in the same animal with solutions of varying Ca concentrations.

Materials and methods. Male Sprague-Dawley rats weighing 120–130 g, were pair-fed in each trial which lasted for either 14 or 28 days. Both the magnesium deficient and control diets were made from a basal diet which has been described in detail elsewhere (8). Suitable quantities of magnesium sulfate were added to the basal diet to final concentrations of 50 ppm Mg^{2+} for the deficient diet and 1000 ppm Mg^{2+} for the control diet. The diets contained 20% casein, 0.6% Ca and 0.4% P. Deionized water was allowed *ad libitum*.

Experiment 1. The rate of Ca absorption across the duodenum and ileum was determined in rats depleted of magnesium for 14 and 28 days. All transport estimates were carried out after an overnight fast. Ca transport was determined by the *in vivo* ligated loop technique described by Wasserman and Taylor (9). Ten centimeters of duodenum and 15 cm ileum were ligated in each rat. Each loop was injected with 0.5 ml of a dosing solution containing 0.1 μCi ^{47}Ca (Cambridge Nuclear, Billerica, MA) in 5 m*M* $CaCl_2$ and 150 m*M* NaCl, pH 7.2. Five, 15, 30, 60, or 90 min after dose injection into the loop, the rats were anaesthetized with ether and the ligated loops were excised. Blood samples, obtained by cardiac puncture, were collected in tubes containing Na_2EDTA. The left tibiae were removed and cleaned of connective tissue and muscle. Radioactivity measurements were made in all intestinal loops and cleaned bones by use of a Nuclear Chicago well-type NaI crystal automatic gamma counter. Ca absorption was calculated by subtracting percent injected ^{47}Ca remaining in the loop from 100%. The difference was designated % ^{47}Ca transferred to the body.

Experiment 2. The *in situ* perfusion technique was used in rats magnesium depleted for 14 days to study Ca fluxes in the duodenum. The procedure has been described in detail by Wasserman *et al.* (10). Each rat was perfused in sequence with five dosing solutions, each containing 150 m*M* NaCl, 0.1–0.4 μCi ^{47}Ca/ml, and 0.5, 1.0, 2.0, 10.0 or 20.0 m*M* Ca. All solutions were adjusted to pH 7.2–7.4. The solutions were perfused into the ligated duodenal loop in order of increasing Ca concentration at a rate of 4–5 ml/hr using a motorized syringe pump (Harvard Apparatus Co., Dover, MA). An initial equilibration period of 40 minutes was allowed for each concentration of Ca perfused, and the outflow solution collected during this period

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was discarded. During the subsequent perfusion, carried out for 30 min, the outflow solutions were collected in graduated tubes. Volumes were measured to obtain estimates of water inflow and outflow rates. Ca influx was calculated as follows (10): Ca infux (lumen to blood) = $(^{47}Ca_i)$ (W_i) − $(^{47}Ca_o)$ $(W_o)/[(SA_i + SA_o)/2] \times L$ where: ^{47}Ca = radiocalcium content of fluids (cpm/ml); SA = specific activity of fluid Ca (cpm/mole); W = rate of water flow (ml/hr); L = length of duodenal segment (cm); i,o = superscripts referring to inflowing and outflowing solutions respectively.

Previous studies showed that calcium is absorbed by two processes, one process is saturable and the other has the characteristics of diffusion (11). In the present series, the rates of passive and saturable diffusion were calculated as follows. A straight line parallel to the diffusional component of each influx curve was drawn through the origin. At 0.5, 1, 2, 10 and 20 m*M* Ca concentrations the values for passive diffusion were subtracted from the corresponding values for Ca influx. The differences, representing the portion of influx due to saturable transport, were plotted against luminal Ca concentration.

Ca and Mg content of blood and tibia. The tibiae were dried at 90° for 3–4 days and ashed at 550° for 16–20 hr in a Thermolyne muffle furnace. The ash was dissolved in 3 ml concentrated HCl. Suitable dilutions of tibia ash or of plasma were analysed for Mg and Ca by atomic absorption using a Perkin-Elmer atomic absorption spectrophotometer, Model 290 B.

Statistical analysis. All analyses were made using the paired *t* test. Levels of significance were based on the differences between pairs of Mg deficient and control rats (13).

Results. Response to magnesium depletion. Weight gains were similar in magnesium deficient and control rats for the initial 10 days of Mg depletion. By day 14, however, the body weights of the magnesium deficient rats were significantly below those of their pair fed controls. By this time plasma Mg concentrations were markedly reduced and remained at this low level throughout the 28 days of depletion (Table I). Plasma Ca levels showed some variability. Mean values were significantly elevated in the rats Mg depleted for 14 days in experiment 2, when there were seven animals per group. No statistically significant differences were seen in rats depleted for 14 or 28 days in experiment 1 when there were only three rats per group. The magnesium content of the tibiae was significantly reduced and the calcium content slightly, but significantly increased after 28 days of lg depletion. (Table II).

Intestinal Ca transport. Intestinal Ca transport (% ^{47}Ca transferred from the lumen to the blood) was consistently less in the magnesium deficient rats than in their pair fed controls. The difference between the two groups was significant after 2 weeks of mag-

TABLE I. BODY WEIGHTS AND CONCENTRATION OF PLASMA Ca AND Mg[a] IN RATS DEPLETED OF MAGNESIUM FOR 14 AND 28 DAYS.

Days of depletion	Parameter	Experiment 1		Experiment 2	
		Control	Mg depleted	Control	Mg depleted
14	Body wt (g)	198 ± 2.9 (23)	192 ± 2.5[b] (23)	224 ± 4.7 (10)	211 ± 4.7[c] (10)
14	Plasma Mg (mg%)	2.54 ± 0.08 (16)	1.21 ± 0.07[b] (16)	2.64 ± 0.14 (6)	1.48 ± 0.07[c] (6)
14	Plasma Ca (mg%)	10.9 ± 0.4 (3)[e]	11.3 ± 0.5 (3)[e]	10.5 ± 0.2 (7)	11.7 ± 0.3[d] (7)
28	Body wt (g)	251 ± 3.1 (23)	220 ± 3.0[b] (23)	—	—
28	Plasma Mg (mg%)	2.43 ± 0.07 (20)	1.04 ± 0.08[b] (20)	—	—
28	Plasma Ca (mg%)	10.4 ± 0.3 (3)[e]	10.4 ± 0.3 (3)[e]	—	—

[a] Values are means ± SEM; figures in parentheses represent number of rats in each group.
[b, c, d] Significantly different from control values, $P < 0.001$, $P < 0.005$ and $P < 0.01$ respectively.
[e] Plasma calcium was measured only in three rats which were not used for calcium absorption measurement.

nesium depletion and further increased after 4 weeks (Fig. 1). Uptake of radioactivity by the tibiae generally reflected differences in intestinal Ca transport (Fig. 2). Almost complete transfer of the injected dose had occurred in the duodenal loop 60 minutes after the dose had been injected; less than 60% had been transferred from the ileum in 90 min.

Unidirectional calcium fluxes at different levels of luminal Ca concentration obtained by the *in situ* perfusion method are shown in Fig. 3. Ca influx was consistently less in magnesium depleted rats than in their pair fed controls at all Ca concentrations. However, the mean values for the data points used to construct Fig. 3, shown in Table III, show statistically significant differences only at luminal Ca concentrations of 0.5, 10 and 20 m*M*. The relationship between Ca influx and luminal Ca concentration was in agreement with the pattern described by Dumont *et al.* (13), suggesting that Ca absorption in the rat duodenum was comprised of at least two components. The curvilinear portion of the transport-concentration curve, at the lower concentration of calcium, suggests the pres-

TABLE II. COMPOSITION OF THE TIBIA IN RATS MAGNESIUM DEPLETED FOR 28 DAYS AND THEIR PAIR FED CONTROLS.[a]

Parameters	Control	Magnesium deficient
Wet wt. (g)	0.55 ± 0.010	0.60 ± 0.010[b]
Dry wt. (g)	0.34 ± 0.006	0.34 ± 0.004
Water (%	38.49 ± 0.65	42.70 ± 0.58[b]
Mg (meq/tibia)	0.098 ± 0.002	0.047 ± 0.001[b]
Mg (meq/g dry wt)	0.30 ± 0.004	0.14 ± 0.002[b]
Ca (meq/tibia)	3.394 ± 0.06	3.60 ± 0.054[c]
Ca (meq/g dry wt)	10.15 ± 0.13	10.45 ± 0.086[d]
Mg and Ca (meq/g dry wt)	10.44 ± 0.13	10.58 ± 0.86

[a] Values are means ± SEM of 23 rats in each group.
[b, c, d] Significantly different from control values, $P < 0.001$, $P < 0.01$ and $P < 0.05$ levels respectively.

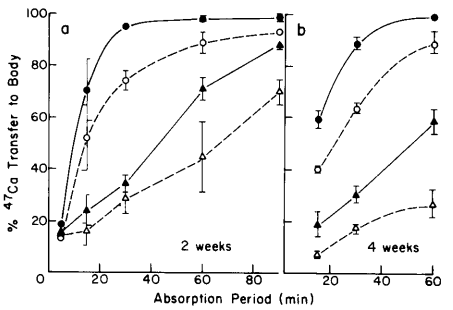

FIG. 1. Effect of Mg deficiency on % ^{47}Ca transferred to body with time. Each point represents mean ± SEM of Mg deficient rats and pair fed controls. (a) 2 weeks Mg depletion, 2–4 rats/time point; (b) 4 weeks depletion, 4–8 rats/time point. ●——● duodenum, control; ○— —○ duodenum, Mg deficient; ▲——▲ ileum, control; △- - -△ ileum, Mg deficient. Overall rate of absorption was significantly reduced in the duodenum of 14-day depleted rats ($P < 0.05$). The decrease was not significant in the ileum. After 28 days the decrease was significant in both segments, $P < 0.01$ in the duodenum, $P < 0.001$ in the ileum.

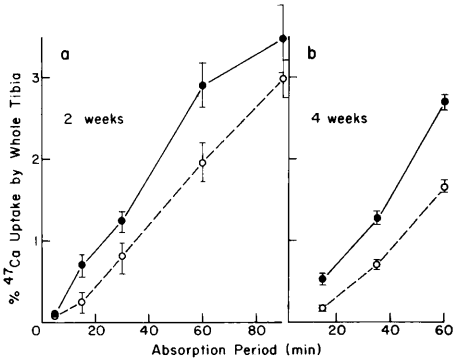

FIG. 2. Effect of Mg deficiency on % ^{47}Ca uptake by the whole tibia with time. Each point represents mean ± SEM of Mg deficient rats and pair fed controls. (a) 2 weeks Mg depletion, 3–4 rats/time point. (b) 4 weeks Mg depletion, 6–8 rats/time point. ●——● control; ○——○ Mg deficient. Overall ^{45}Ca uptake was significantly reduced in Mg deficient rats, $P < 0.01$ after 2 weeks, $P < 0.001$ after 4 weeks of depletion.

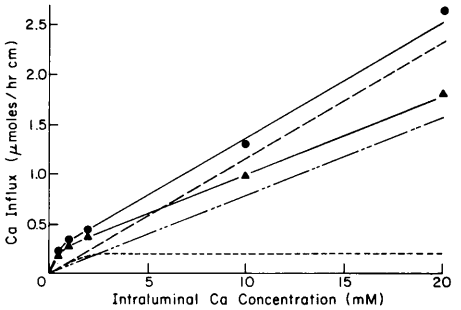

FIG. 3. Effect of 14 days of Mg depletion on Ca transferred from lumen to blood using the in situ perfusion technique. Each point represents the mean obtained in 4–9 rats (mean values ± SEM and the number of rats for each point are shown in Table III). ●——● Pair fed control, total ^{47}Ca transferred ▲——▲ Mg deficient, total ^{47}Ca transferred - - - Pair fed control, linear portion —· ·— Mg deficient, linear portion ----- Mg deficient and pair fed controls, curvilinear portion with plateau.

TABLE III. MEAN ± SEM of Ca TRANSFERRED FROM LUMEN TO BLOOD (μMOLE/HR cm^{-1}).

Ca conc. (m*M*)	*N*	Pair fed control ●—●	Mg deficient ▲—▲
0.5	9	0.22 ± 0.02	0.11 ± 0.01[a]
1.0	8	0.33 ± 0.03	0.27 ± 0.03
2.0	6	0.42 ± 0.06	0.38 ± 0.05
10.0	5	1.30 ± 0.22	0.97 ± 0.16[a]
20.0	4	2.64 ± 0.20	1.82 ± 0.25[a]

[a] Significantly different from control values ($P < 0.05$).

ence of a saturable, carrier mediated mechanism and the linear portion, at higher calcium concentrations, passive diffusion. With this as reference, magnesium depletion appeared to depress passive diffusion of Ca across the duodenal mucosa with also a significant effect at the lowest calcium concentration, 0.5 m*M* (Table III).

Discussion. In the present investigation the depression in Ca transport seen in Mg depleted rats appeared to be entirely due to a decrease in passive diffusion. This finding is in conflict with several previous reports which suggested either an increase or no change in intestinal Ca transport of magnesium-deficient rats (1–5). Of the two previous studies that had shown a decrease in Ca transport (6, 7), one (7) showed an increase in active Ca transport after 10 days of Mg depletion and a significant decrease when Mg depletion was prolonged for 19 days. The transport data in the latter investigation were obtained by an *in vitro* procedure using a modified Ussing apparatus (7). Rats fed adequate magnesium diets showed comparably decreased rates of Ca transport following thyroparathyroidectomy. The authors suggested that both magnesium deficiency and thyroparathyroidectomy depressed Ca transport by alterations in vitamin D metabolism, presumably at the level of regulation of the hydroxylation of 25-OH-D_3 to the 1-hydroxy- or the 24-hydroxy derivatives.

The data reported here do not indicate that Mg depletion of the magnitude or duration applied in this investigation substantially altered vitamin D metabolism. A significant decrease in 1,25-$(OH)_2D_3$ should have decreased intestinal Ca absorption by both passive diffusion and saturable transport. While variability may have obscured the significance of differences in Ca transport of deficient and control rats at the luminal Ca concentrations of 1 and 2 m*M* (Fig. 3), the overall decrease in intestinal Ca transport seen in Mg-depleted animals was small. The Mg deficient diet used in this investigation (50 ppm Mg) was chosen to avoid marked differences in body weights of Mg-depleted and pair fed control rats. Walling *et al.* (7) used a Mg-free diet which probably caused acceleration and enhancement of Mg-depletion and its manifestations, possibly including disturbances in vitamin D metabolism.

Recent findings in this laboratory (14) suggest an explanation for the data reported here which would support the observation of Walling *et al.* (7) that parathyroidectomy and Mg deficiency had similar effects on intestinal Ca transport. Microscopic examination of parathyroid sections removed from Mg deficient rats at intervals from 2 to 21 days of depletion showed progressive manifestations of hypoactivity (14). The same rats consistently exhibited hypercalcemia comparable to that seen in the present investigation in 14 day Mg depleted rats (Table I). Reduction of parathyroid hormone activity is an appropriate response to hypercalcemia. One of the consequences of parathyroid hypoactivity would be depression in intestinal Ca transport.

In conclusion, the decreased rate of intestinal Ca absorption in Mg deficient rats observed in this investigation appears to be due primarily to reduction in the rate of passive Ca diffusion. Among several consequences of magnesium deficiency likely to depress intestinal Ca transport is hypoactivity of the parathyroid glands. This aspect of magnesium deficiency is now under investigation.

Summary. Calcium transport across the duodenum and ileum was measured by an *in vivo* ligated loop technique in Mg depleted rats and rats pair fed a magnesium adequate diet. Intestinal Ca transport and tibial ^{47}Ca uptake were consistently decreased in magnesium depletion. Analysis of Ca fluxes, carried out by *in situ* perfusion, showed a significant decrease in passive diffusion, with less consistent effects on the saturable transport component. Both bone and plasma showed markedly decreased Mg concentration. Tibia Ca levels were slightly but significantly increased and plasma levels were either normal

or slightly, but significantly elevated. The basis for the decrease in Ca transport of Mg depleted rats observed in this investigation is not clear. The data suggest a general alteration in mucosal membrane transport rather than a specific effect on Ca transport *per se.*

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