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Effects of magnesium citrate and phytin on reducing urinary calcium excretion in rats

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Summary. The aim of this study was to determine and compare the effects of both magnesium citrate and phytin on reducing urinary calcium excretion under high-calcium-diet conditions during single and combined treatments. An animal experiment was carried out over a period of 4 weeks in 35 male rats. Urinary calcium excretion was reduced significantly by magnesium citrate and/or phytin in rats fed on high-calcium diets. The hypocalciuric effect of magnesium citrate was more evident than that of phytin. Urinary magnesium excretion was high in all experimental groups. However, the urinary magnesium/calcium ratios showed a consistent increase only in the groups treated with magnesium citrate. Urinary citrate excretion showed a relative increase with the introduction of magnesium citrate plus phytin; however, in both the high-calcium-diet group and the magnesium-citrate group this was found to be reduced. Urinary phosphate excretion was slightly higher in the groups treated with phytin. There was no definite difference in urinary oxalate concentration between the groups. No significant change was noted in the serum concentration of calcium, magnesium, or phosphate.

It is generally accepted that the calcium-containing stone is the most common variety of stone. A high urinary calcium level is common in patients with renal calcium stones, and the majority of patients appear to have intestinal hyperabsorption of calcium. It has been shown that if urinary calcium excretion can be reduced and maintained at near normal levels in these patients, the incidence of stone formation can be reduced.

Increased fluid intake and restriction of dietary calcium are important means of reducing calcium excretion; however, restriction of dietary calcium intake over long periods is difficult to maintain, and the long-term results are not encouraging. Some drugs, such as sodium cellulose phosphate, orthophosphate, and thiazicles, have been shown to be effective means of reducing urinary calcium excretion in hypercalciuric patients and, hence, the incidence of recurrent stone formation [1–3]. However, these drugs may induce side effects.

During the last decade, the experimental and clinical hypocalciuric effects of rice bran (or wheat bran) have been reported [4–8]. Phytin (inositol hexaphosphate) proves to be the most effective substance of rice-bran treatment and plays an active role in reducing the intestinal absorption of calcium. In the intestine, phytic acid combines with calcium to form an insoluble salt, calcium phytate, which in turn reduces urinary calcium excretion.

It is known that magnesium and citrate are undoubtedly the most evaluated inhibitors of calcium oxalate and calcium phosphate crystallization and crystal growth [9]. It appears likely that combined treatment with magnesium and citrate alters the composition of urine in a way that might be more favorable than that achieved using treatment with either substance alone. Magnesium citrate may be considered an excellent choice of medical therapy in recurrent idiopathic calcium urolithiasis. Surprisingly, little is actually known about the influence of magnesium citrate on urinary calcium excretion. Morevoer, no report on the hypocalciuric effect of magnesium citrate and its mechanism has yet been published.

To confirm the hypocalciuric effect of magnesium salts, we carried out experiments and made comparisons using magnesium citrate and phytin in rats on a high-calcium diet.

Materials and methods

A total of 35 male Wistar rats (body wt., approx. 150 g) were acclimated for 1 week and then randomly divided into 5 experimental groups. Each group consisted of seven rats and the following diets were offered: group 1, normal calcium diet (0.95 g calcium/100 g diet); group 2, high-calcium diet (1.60 g calcium/100 g); group 3, high-calcium diet supplemented with phytin (3 g phytin/100 g); group 4, high-calcium diet supplemented with magnesium citrate (20 mmol magnesium citrate/100 g); and group 5, high-calcium diet supplemented with magnesium citrate and phytin (3 g phytin and 20 mmol magnesium citrate/100 g). All the diets were given in powdered form, which could easily be combined with experimental additives; the rats had access to drinking water ad libitum.

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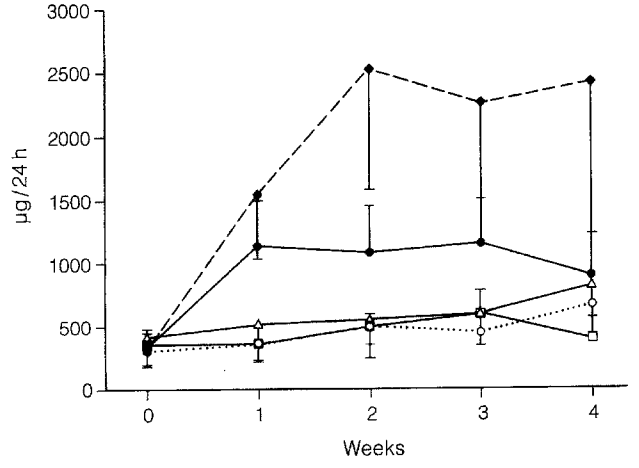
The rats were weighed weekly, and 24-h urine samples were collected weekly using metabolic cages. Urine specimens were collected in flasks that contained 100 µl 20% chlorhexidine gluconate to prevent bacterial contamination and dissolution of calcium crystals. The urinary volumes and pH values were measured and recorded (samples were then acidified so as to make the pH lower than 2.00), and the samples were subsequently stored at −20°C until analysis. Blood samples were drawn from the venii plex orbitalis posterior every 2 weeks with the animals under ether anesthesia.

All parameters were determined with a colorimeter (calcium, by Lancer; magnesium and phosphorus, by Merckotest; citrate and oxalate, by Boehringer-Mannheim). Statistical analyses were performed using the Student-Newman-Keuls (SNK) test, and a confidence level of >95% was considered significant in the animal experiments.

Results

Both phytin and magnesium citrate, mixed in the powdered food, were accepted by the rats without apparent distaste or impairment of general health. The average body weight gain observed during the course of the experiment was the same for all groups (approx. 30 g). As compared with the normal-calcium-diet control group, the urinary volume was relatively high in both the high-calcium-diet control group and the phytin-plus-magnesium-citrate group.

The weekly urinary excretion of calcium as measured for each group is shown in Fig. 1. Rats fed on high-calcium diets excreted significantly more calcium than did those fed on normal diets. Urinary calcium excretion was



1. Week: Group	1	2	3	4	5
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3. Week: Group	1	2	3	4	5
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2. Week: Group	1	2	3	4	5
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4. Week: Group	1	2	3	4	5
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2	*		*	*	*
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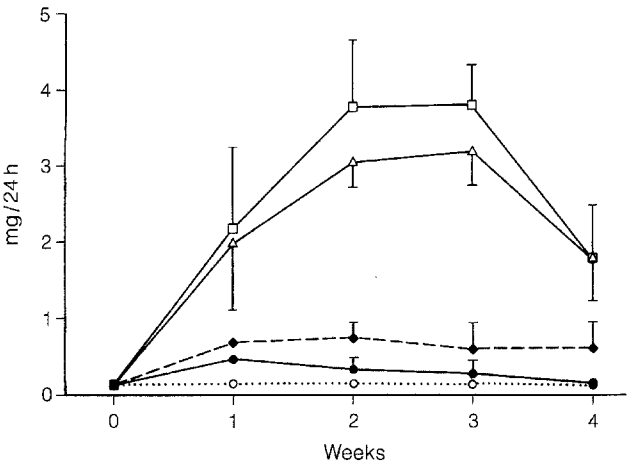
Fig. 1. Urinary calcium excretion in rats (mean ± SE). * Significant difference ($P < 0.05$). ·····○····· Group 1; −−◆−− group 2; —●— group 3; —□— group 4; —△— group 5



1. Week: Group	1	2	3	4	5
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3. Week: Group	1	2	3	4	5
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2. Week: Group	1	2	3	4	5
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4. Week: Group	1	2	3	4	5
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4	*		*	*	*
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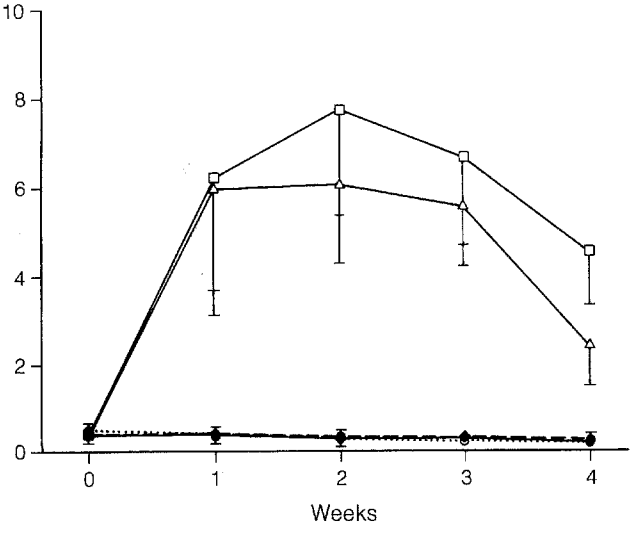
Fig. 2. Urinary magnesium excretion in rats (mean ± SE). * Significant difference ($P < 0.05$). ·····○····· Group 1; −−◆−− group 2; —●— group 3; —□— group 4; —△— group 5



1. Week: Group	1	2	3	4	5
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3. Week: Group	1	2	3	4	5
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2. Week: Group	1	2	3	4	5
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4. Week: Group	1	2	3	4	5
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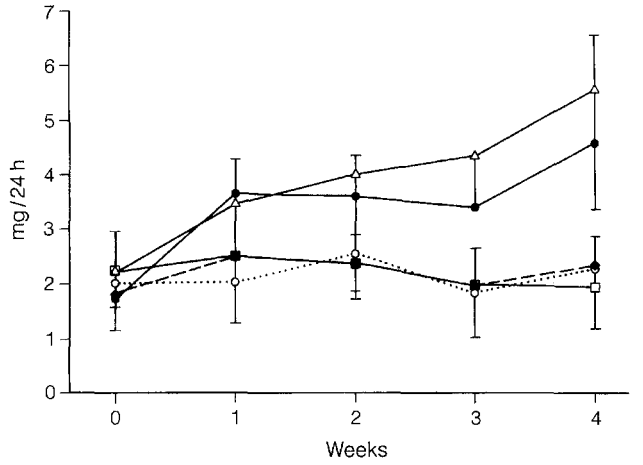
Fig. 3. Urinary magnesium/calcium ratios in rats (mean ± SE). * Significant difference ($P < 0.05$). ·····○····· Group 1; −−◆−− group 2; —●— group 3; —□— group 4; —△— group 5



1. Week:						3. Week:					
Group	1	2	3	4	5	Group	1	2	3	4	5
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Group	1	2	3	4	5	Group	1	2	3	4	5
1						1					
2						2					
3	*					3	*	*		*	
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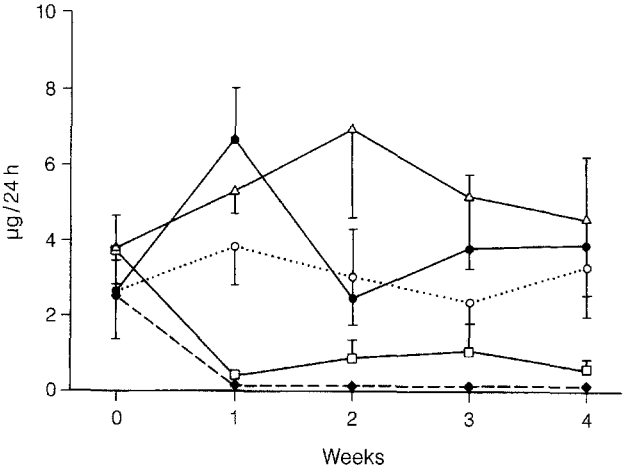
Fig. 4. Urianry phosphorus excretion in rats (mean ± SE). * Significant difference ($P < 0.05$). ·····○····· Group 1; −−◆−− group 2; —●— group 3; —□— group 4; —△— group 5



1. Week:						3. Week:					
Group	1	2	3	4	5	Group	1	2	3	4	5
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2. Week:						4. Week:					
Group	1	2	3	4	5	Group	1	2	3	4	5
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3		*				3		*		*	
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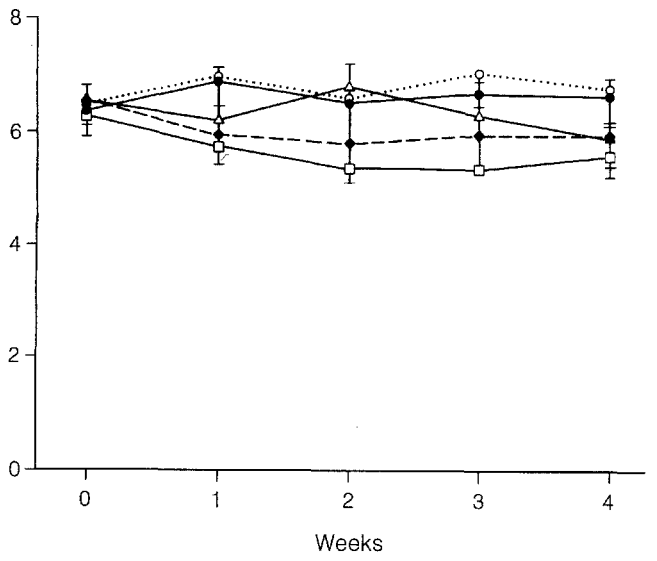
Fig. 5. Urinary citrate excretion in rats (mean ± SE) * Significant difference ($P < 0.05$). ·····○····· Group 1; −−◆−− group 2; —●— group 3; —□— group 4; —△— group 5



1. Week:						3. Week:					
Group	1	2	3	4	5	Group	1	2	3	4	5
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2. Week:						4. Week:					
Group	1	2	3	4	5	Group	1	2	3	4	5
1		*		*	*	1		*		*	*
2						2					
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Fig. 6. Urinary pH (mean ± SE). * Significant difference ($P < 0.05$). ·····○····· Group 1; −−◆−− group 2; —●— group 3; —□— group 4; —△— group 5

reduced significantly by the addition of magnesium citrate and/or phytin in the high-calcium-diet groups and was maintained on a normal level or a gradually achieved normal level. The hypocalciuric effect of magnesium citrate was better than that of phytin. Our study revealed that there was no synergy between magnesium citrate and phytin.

The weekly urinary excretion of magnesium as measured for each group is shown in Fig. 2. Urinary magnesium excretion was markedly increased ($P < 0.05$) in both the magnesium-citrate and the phytin-plus-magnesium-citrate groups and was only slightly increased in the high-calcium-diet control and phytin groups.

The urinary magnesium/calcium ratios increased consistently during the experiment in the groups treated with magnesium citrate and with phytin plus magnesium citrate (Fig. 3). Moreover, the ratios of the former group were higher than those of the latter ($P < 0.05$).

The weekly urinary excretion of phosphorus as determined for each group is shown in Fig. 4. There was a continuous increase in urinary phosphorus excretion in the groups treated with phytin.

The weekly urinary excretion of citrate as measured for each group is shown in Fig. 5. Urinary citrate excretion was significantly decreased in the high-calcium-diet control group and the group treated with magnesium citrate, whereas it was increased in the group receiving phytin

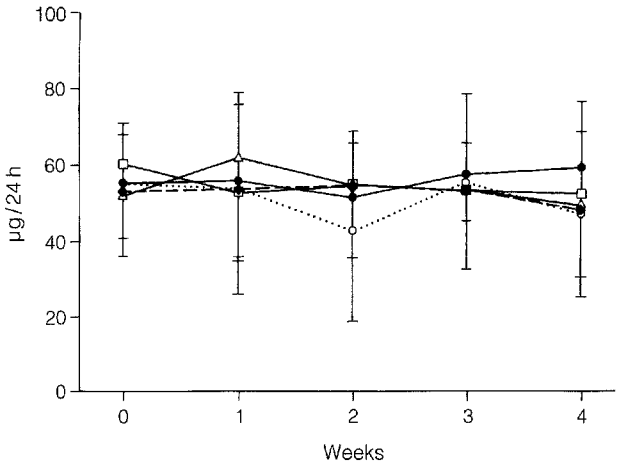

Fig. 7. Urinary oxalate excretion in rats (mean ± SE). There is no signfiicant difference between the groups. ·····○····· Group 1; --♦-- group 2; —●— group 3; —□— group 4; —△— group 5

plus magnesium citrate. The same change was noted in pH. The urinary pH in the high-calcium-diet control group and the group receiving magnesium citrate was found to be decreased (Fig. 6). The observed rise in citrate concentration was presumably associated with the increased alkalinity of the urine, for it is known that the administration of alkalines results in pronounced elevations of urinary citrate. However, no makred difference in urinary oxalate concentration was observed among the various groups (Fig. 7). It seems that the urinary oxalate concentration is not affected by these various diets.

No significant change was observed in the serum concentration of calcium, magnesium or phosphate (Table 1).

Discussion

Magnesium and citrate are two potent inhibitors of calcium-salt crystallization and crystal growth. Selective treatment of urinary stone disease with magnesium and citrate has revealed varying results. However, the major purpose of this paper is to explore the role that magnesium citrate may play in the treatment of absorptive hypercalciuria and in the prevention of calcium stone disease by reducing urinary calcium excretion.

The results of the present study clearly demonstrate that magnesium citrate can exert a considerable influence on urinary calcium excretion. Urinary calcium excretion was reduced significantly by the addition of magnesium citrate and/or phytin in the high-calcium-diet groups and was then maintained on a normal level. The effect of magnesium citrate was better than that of phytin.

Phytin has been known to be an important constituent of bran. When given orally, phytin is thought to combine with dietary calcium to form nonabsorbable calcium phytate in the intestinal tract. Absorption of calcium is thus impaired and the concentration of calcium in the urine falls [5, 7]. Dietary calcium may displace magnesium from phytin to form an insoluble salt in the intestines, which is then excreted. Displaced magnesium may be absorbed into the blood and then be excreted together with the urine [7]. This process was also confirmed by our experimental results.

Magnesium and calcium metabolism are closely related. The intestinal absorption and renal excretion of the two ions are interdependent. We believe that the major hypocalciuric effect of magnesium citrate is most likely due to the competition of magnesium and calcium for absorption in the intestine. Magnesium absorption primarily takes place in the distal region of the small intestine [10, 11]. Although the interrelationship between magnesium and calcium absorption has been known for a long time, it nevertheless has been a subject of much controversy. However, it has been confirmed that magnesium balances in animals and humans show a reciprocity between magnesium and calcium [12]: a high intake of magnesium decreases calcium absorption, and a low intake of magnesium enhances the absorption of calcium. Furthermore, the kidney is the main regulator of both the serum concentration and the total body content of magnesium. The diffusiable fraction of magnesium is filtered by the glomerulus and reabsorbed in the tubules, with 60%–75% of the magnesium that is filtered at the glomerulus being reabsorbed within the loop of Henle, especially within the thick ascending loop [13]. Due to a common mechanism of renal calcium and magnesium handling, this proved to be true: the addition of magnesium or calcium was found

Table 1. Serum biochemistry studies in rats, showing no significant difference between the groups

		Group 1	Group 2	Group 3	Group 4	Group 5
Serum calcium:	Pre	9.34 ± 0.85	9.59 ± 1.63	10.01 ± 1.65	9.52 ± 1.15	9.16 ± 0.78
	2 weeks	9.76 ± 2.19	10.37 ± 1.52	11.65 ± 1.61	11.47 ± 1.99	9.96 ± 0.73
	4 weeks	8.76 ± 1.56	8.81 ± 2.35	9.78 ± 1.69	9.57 ± 2.02	9.19 ± 1.15
Serum magnesium:	Pre	1.59 ± 0.44	1.81 ± 0.41	1.87 ± 0.52	1.71 ± 0.51	1.53 ± 0.40
	2 weeks	1.81 ± 0.92	1.90 ± 0.74	1.85 ± 0.48	1.88 ± 0.37	1.92 ± 0.49
	4 weeks	1.77 ± 0.31	1.79 ± 0.66	1.69 ± 0.55	1.67 ± 0.66	1.80 ± 0.66
Serum phosphorus:	Pre	4.49 ± 1.06	3.68 ± 1.28	3.84 ± 0.98	3.77 ± 0.84	3.09 ± 0.61
	2 weeks	4.33 ± 1.66	3.78 ± 1.27	4.48 ± 0.75	4.05 ± 0.92	3.83 ± 0.91
	4 weeks	3.04 ± 1.04	3.15 ± 0.97	3.16 ± 1.08	3.36 ± 0.87	3.33 ± 0.97

Data represent mean values ± SE (mg/dl). *Pre,* Pretreatment values

to result in an interaction between the respective transport rates and in competition for excretion at the thick ascending loop of Henle [12].

Recent experience suggests that magnesium supplementation in the form of magnesium citrate might be more effective than that in either the oxide or hydroxide form because magnesium citrate has a greater propensity for aqueous solubility and absorption, thus providing more bioavailable magnesium [14–16]. The hypocalciuric effect of magnesium oxide or hydroxide is not so evident.

Calcium-citrate complexing is enhanced by the greater abundance of ionized citrate that exists in an alkaline environment. Another hypocalciuric effect of magnesium citrate may be attributable to the observation that citrate combines with dietary calcium in the intestine to form the nondissociating, readily dissolving, and poorly absorbable calcium-citrate chelate complex [17, 18]:

$$Ca^{2+} + Citrate^{3-} \leftrightarrow Ca\text{-}Citrate^{-}.$$

According to this equation, the increase in citrate concentration decreases the concentration of the ionized calcium. The intraluminal level of citrate determines the amount of intraluminal ionized calcium, and the luminal concentration of ionized calcium determines the mucosal transport of calcium. Since citrate combines with calcium in the intestine to reduce the amount of free calcium that can be absorbed, as a result, the urinary concentration of calcium is decreased while the large amounts of intestinal citrate are excreted and small amounts of free citrate remain available for absorption. This process was confirmed by our experimental results. The reason why the urinary citrate excretion was increased in rats fed on phytin plus magnesium citrate is not entirely clear. It is probable that phytin combines with calcium in the intestine does more readily than magnesium citrate. Moreover, the urinary citrate concentration is influenced by the urinary pH: in an alkaline environment the citrate concentration rises, whereas in a more acidic environment it falls [19]. This may also help explain our findings.

According to our results, it seems that there was no additive hypocalciuric effect between magnesium citrate and phytin. The urinary magnesium/calcium ratio increased consistently only in the groups receiving magnesium citrate. However, as with phytin alone, we observed no effect on the change in the magnesium/calcium ratios.

Our data show some discrepancies as compared with the experimental data reported by Ogawa and co-workers [9]. These investigators reported that when magnesium citrate was given to rats, both the urinary calcium excretion and the urinary calcium concentration were higher, whereas the magnesium/calcium ratio remained mostly unchanged. Because of relative differences in diet content, the rats fed on a calcium-oxalate lithogenic diet were found to be normal-calciuric. However, our rats that were fed on a high-calcium diet were found to be hypercalciuric. Thus, the urinary calcium excretion in the aforementioned experiment was not influenced by magnesium citrate, in contrast to our findings.

The urinary phosphorus excretion in the high-calcium-diet groups treated with phytin showed a significant increase. The source of the urinary phosphorus is presumed to be the absorbable phosphorus produced by the hydrolysis of phytate in the intestine.

The urinary oxalate concentration in all experimental groups remained unchanged. In our experiment, phytin and/or magnesium citrate had no effect on oxalate metabolism. However, other investigators [5, 6, 8, 20–22] have reported that treatment with rice bran or wheat bran shows a tendency to influence urinary levels of oxalate. We believe that phytin, as the inositolhexaphosphatic salt, is an important constituent of bran for interfering with the intestinal absorption of calcium but not of oxalate.

The reasons for the observed increase in urinary magnesium excretion in the high-calcium control group might have been that the high-calcium diet caused a high calcium content in the intestine and a restrained excretion of magnesium from the intestine, leading to an increase in the amount of magnesium excreted in the urine. Magnesium citrate and/or phytin had no effect on the serum concentration of calcium, magnesium, or phosphorus.

In summary, we believe that the role of magnesium citrate in preventing calcium stone formation involves not only an inhibition of the nucleation, agglomeration and growth of calcium crystals but also a reduction in the urinary excretion of calcium. The association of decreased urinary concentrations of calcium and increased urinary levels of magnesium results in an increased magnesium/calcium quotient and, hence; in a lower risk for the formation of urine supersaturated with calcium salts. Therefore, the question arises as to whether magnesium citrate might be used in the treatment of patients with idiopathic hypercalciuria to prevent recurrent calcium stone formation; further studies into this problem would be considered necessary.

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