Nutrition and magnesium absorption

Voeding en magnesiumabsorptie

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Proefschrift
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Zonder de hulp, steun en kritiek van velen was het tot stand komen van dit proefschrift niet mogelijk geweest. Vanaf deze plaats wil ik iedereen die mij heeft geholpen tijdens mijn onderzoek en de voorbereiding van dit proefschrift hartelijk bedanken.

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1. De rat is niet in alle gevallen een goed model voor de mens.
   Dit proefschrift

2. Bij het gebruik van laag-energetische vetvervangers zou niet alleen gelet moeten worden op de veiligheid van deze stoffen maar ook op de effectiviteit ervan.

3. Door chemie meten wat we eten suggereert het kwantitatief weten van voedselbestanddelen, maar zegt niets over de beschikbaarheid ervan.

4. Hoewel het magnesiumgehalte in serum (of plasma) de meest gebruikte parameter is voor het vaststellen van de magnesiumstatus, weerspiegelt dit gehalte slechts acute effecten op de magnesiumhuishouding.
   RJ Elin, 1987

5. De vitamine E concentratie in het serum wordt mede beïnvloed door het type voedingsvet.

6. Het hoge fytaatgehalte in vegetarische voedingen heeft waarschijnlijk geen negatieve invloed op de magnesiumstatus vanwege het eveneens hoge magnesiumgehalte in deze voedingen.

7. De vorming van een calcium-magnesium-fosfaat complex is complex.
   Dit proefschrift

8. De opvatting dat natrium de uitscheiding van calcium met de urine verhoogt is waarschijnlijk onjuist.
   JL Greger et al., 1991

9. Het feit dat suppletie van magnesium het optreden van hartaandoeningen vermindert zou erop kunnen wijzen dat de aanbevolen magnesiuminneming is onderschat.
   MS Seelig, 1986; RB Singh 1990

10. "Humane Voeding" en "Moleculaire Fytopathologie" staan dichter bij elkaar dan men op het eerste gezicht zou denken.

11. "Word Perfect" kan ertoe bijdragen dat een proefschrift "Perfect Word(t)"

Stellingen behorende bij het proefschrift van Lisette Brink: "Nutrition and magnesium absorption". *1 april 1992*

Contents

Chapter 1

Scope of this thesis

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Magnesium is an essential mineral and is indispensable for the course of many fundamental biological processes in vertebrate animals, including man (1). Inadequate magnesium intake and/or impaired magnesium absorption might be involved in the pathogenesis of osteoporosis, hypertension, coronary heart disease and cancer (2-5). Thus, it is important to identify dietary constituents that either reduce or improve the intestinal absorption of magnesium.

Intestinal absorption of minerals can be determined by epithelial and luminal characteristics. Although the process of epithelial uptake of magnesium is not yet fully understood (6), it appears that the concentration of soluble magnesium in the intestinal lumen is a major determinant of the amount of magnesium absorbed (7-12).

This thesis attempts to elucidate the influence of various nutrients on the solubility of intestinal magnesium and its impact on magnesium absorption in rats and in humans. Various nutrients present in dairy products and soybean protein-based products, namely casein, soybean protein, phytate, calcium, phosphate and lactose were studied. There is an increasing use of soybean products as substitute for animal protein in the diets of adults and also in infants foods (13), whereas the impact of this dietary change on magnesium absorption is not clear. Although the influence of the above-mentioned nutrients on magnesium absorption has been studied by various authors, there is considerable discrepancy and underlying mechanisms are largely unknown (Chapter 2).

In Chapter 2, literature data on the effects of different nutrients on intestinal absorption of magnesium are reviewed. Chapter 3 deals with the effects of casein, soybean protein, phytate and lactose on apparent magnesium absorption in rats. To see whether the observed effects of the single components are also exerted when present in intact products, the effects of cow's milk and soybean beverage are compared (Chapter 4). Chapter 5 describes the effect of lactose on intestinal pH and intestinal magnesium solubility in rats in an attempt to explain its effect on magnesium absorption. Chapter 6 focusses on the interaction between dietary magnesium, calcium and phosphate in the intestinal lumen of rats and its impact on apparent magnesium absorption. The effects of casein, soybean protein, phytate, lactose and phosphate on true magnesium absorption and endogenous fecal magnesium loss in rats are presented in Chapter 7. Chapter 8 concerns the effect of lactose intake on apparent absorption of magnesium in humans. Finally, in Chapter 9 the results of the presented studies are discussed.

References

1. Ryan M.F. (1991) The role of magnesium in clinical biochemistry: an overview. *Ann. Clin. Biochem.* **28**: 19-26.
2. Altura B.M. & Altura B.T. (1990) Role of magnesium in the pathogenesis of hypertension. In: Hypertension: pathophysiology, diagnosis and management. Laragh J.H. & Brenner B.M., eds. Raven Press, New York, pp 1003-1025.
3. Driessens F.C.M., Steidl L. & Ditmar R. (1990) Magnesium, calcium and zinc status in different forms of osteoporosis. *Magn. Bull.* **12**: 158-160.

4. Seelig M.S. (1979) Magnesium (and trace substance) deficiencies in the pathogenesis of cancer. *Biol. Trace Element Res.* **1**: 273-297.
5. Shills M.E. (1988) Magnesium in health and disease. *Ann. Rev. Nutr.* **8**: 429-460.
6. Hardwick L.L., Jones M.R., Brautbar N. & Lee D.B.N. (1991) Magnesium absorption: Mechanisms and the influence of vitamin D, calcium and phosphate. *J. Nutr.* **121**: 13-23.
7. Walser M. (1967) Magnesium metabolism. *Ergeb. Physiol.* **59**: 185-296.
8. Behar J. (1974) Magnesium absorption by the rat ileum and colon. *Am. J. Physiol.* **227**: 334-340.
9. Ross D.B. (1962) *In vitro* studies on the transport of magnesium across the intestinal wall of the rat. *J. Physiol.* **160**: 417-428.
10. Roth P. & Werner E. (1979) Intestinal magnesium absorption in man. *Int. J. Appl. Radiat. Isot.* **30**: 523-526.
11. Graham L.A., Ceasar J.J. & Burgen A.S. (1960) Gastrointestinal absorption and excretion of Mg^{28} in man. *Metab. Clin. Exp.* **9**: 646-659.
12. Marshall D.H., Nordin B.E.C. & Speed R. (1976) Calcium, phosphorus and magnesium requirement. *Proc. Nutr. Soc.* **35**: 163-173.
13. Erdman J.W. & Fordyce E.J. (1989) Soy products and the human diet. *Am. J. Clin. Nutr.* **49**: 725-737.

Chapter 2

Nutrition and magnesium absorption: a review

Elizabeth J. Brink and Anton C. Beynen

Introduction

Magnesium is an essential mineral for vertebrate lifeforms and plays a key role in many fundamental biological processes such as muscle contraction, enzyme activition, blood coagulation and neural excitability (1). It has been suggested that aging, stress and various disease states may increase magnesium needs (2). Inadequate intake and impaired absorption of magnesium are thought to contribute to the pathogenesis of osteoporosis, hypertension, coronary heart disease and cancer (2-5).

Thus, it is important to identify factors that either reduce or improve the intestinal absorption of magnesium. In this paper, the effects of various dietary components on intestinal magnesium absorption are summarized. Magnesium absorption in humans is emphasized, but dietary effects in rats are also discussed because the rat is frequently used as an experimental model. In the appendix tables, the design and outcome of studies in humans and rats are briefly described. The objective of this paper is to provide an inventory of nutritional influences on magnesium absorption. Differences in magnesium absorption may be reflected in magnesium concentrations of tissues. Thus, dietary effects on magnesium concentrations in various tissues, including blood plasma, are given also. In most cases, there is little information on the mechanisms underlying nutritional influences on magnesium absorption; when opportune, speculations are made.

Magnesium metabolism

Intake. Magnesium is widely distributed in foods. The daily intake of magnesium in adults usually is 250-500 mg (10-20 mmol), which mainly comes from cereals (6). Magnesium requirements as based on supposedly adequate intakes of magnesium and on metabolic studies vary from 100 to 1000 mg per day (7, 8). The National Research Council in the United States recommends 300 and 350 mg/day for young women and men, respectively, with an extra 150 mg/day during pregnancy (9).

Absorption. In a recent review, Hardwick *et al.* (10) showed that despite extensive research on magnesium absorption, there is still uncertainty regarding the site and mechanism of intestinal magnesium transport. Most studies with humans (11-13) as well as with rats (14-18) indicate that the predominant site of magnesium absorption is the distal small intestine. This conclusion could be drawn independently of the experimental methods used, diet, age, and magnesium status of the experimental subjects.

As to the mechanism of magnesium absorption, it appears that passive diffusion (13, 15, 18-21) and active transport (22-26) both participate in intestinal magnesium absorption. For the small intestine, active, or rather saturable transport, is substantiated mainly by the observation that the rate of magnesium absorption plateaus at high luminal magnesium concentrations. The active absorptive mechanism in the descending colon probably is important only under conditions of extremely low dietary magnesium intake or rapid growth.

Hardwick *et al.* (10) conclude that, at usual magnesium intakes, magnesium absorption occurs primarily by diffusional and solvent drag mechanisms. Thus, the concentration of soluble, or at least available magnesium on the luminal site is the major factor controlling the amount of magnesium absorbed.

Intestinal magnesium absorption in humans varies between 35 and 70% of magnesium intake (7, 13, 26, 27).

Excretion. Absorbed magnesium is retained either for tissue growth, including bone formation, or for use in turnover replacement. The kidney is the major excretory pathway for absorbed magnesium and thus tubular reabsorptive processes control magnesium homeostasis (28-30). Supplementing a diet with magnesium raises urinary magnesium excretion without altering serum levels provided that these are in the normal range (31).

Hormonal regulation. Various hormones affect magnesium metabolism, but there is no evidence for a specific magnesium-regulating hormone (32). Numerous hormones may influence magnesium absorption, such as parathyroid hormone (33), calcitonin (34), growth hormone (35), aldosterone (36, 37) and vitamin D (22, 38-40). A stable serum magnesium concentration must be the result of balance between gastrointestinal uptake and renal reabsorption and excretion processes.

Magnesium deficiency. Subclinical magnesium deficiency may occur frequently (7), but there are no solid data as to its incidence and impact. In contrast, severe hypomagnesemia is a well recognized clinical syndrome (2) characterized by muscular symptoms (tetany, muscular tremor), psychic disorders (agitation, confusion and hallucinations) and cardiological signs (disturbed ECG). Among the various clinical conditions associated with magnesium depletion, the most important are prolonged fasting and excessive losses via the gastro-intestinal tract due to absorptive disorders (2).

Nutritional factors affecting magnesium absorption

Amount of protein. It is clear that increasing the amount of dietary protein raises apparent magnesium absorption (ingested minus fecal magnesium) in rats (Appendix table 1). Enhanced magnesium absorption is invariably associated with elevated urinary magnesium excretion. One study with rats showed that increased protein intake lowered magnesium retention, but data on magnesium intake and absorption are not given (41). Other rat experiments demonstrated that high protein intake did not affect whole-body retention of magnesium (49, 51) or raised magnesium concentrations in plasma and carcass (47).

Human studies also indicate that high protein intake results in increased apparent magnesium absorption (Appendix table 1). However, in some studies this may relate to the raised magnesium intake while ingesting the high-protein diet (55, 61, 63). Two studies reported no effect of increased protein intake on magnesium absorption in humans, but the

increase in protein intake was relatively small (57, 64).

The increased apparent magnesium absorption on high protein diets was not associated with systematic changes in magnesium retention and urinary excretion. It would be expected that urinary magnesium excretion rises with increased protein intake. First, under steady state conditions increased magnesium absorption results in increased urinary magnesium excretion (65, 66). Second, sulfur containing amino acids in protein could stimulate urinary magnesium excretion (see below).

Type of protein. Most studies in rats emphasize the effects of soybean protein versus casein, probably because of the increasing use of soybean protein as substitute for animal protein. Soybean products usually contain phytate (67), which lowers magnesium absorption (see below). Thus, soybean protein versus casein may be expected to decrease magnesium absorption which was indeed seen in a well-controlled experiment (42). In this experiment soybean protein also lowered urinary excretion of magnesium.

Various workers reported that magnesium concentrations in serum, femur, kidney and liver of rats were not affected by feeding soybean protein instead of casein (Appendix table 1), but magnesium intakes were not given or were higher with the soybean-protein diets. At marginal, but equal magnesium intakes, feeding soybean protein versus casein resulted in decreased magnesium concentrations in femur and plasma (42). In a recent study (68) it was shown that the impairment of apparent magnesium absorption in rats fed soybean protein versus casein was due to enhanced fecal excretion of endogenous magnesium, whereas true magnesium absorption was unaffected. This increased endogenous loss was compensated for by decreased urinary excretion so that magnesium retention remained unchanged. The observed effects were most likely caused by phytate in the soybean protein. Addition of phytate to a diet with casein, to a concentration identical to that in diets containing soybean protein, caused apparent magnesium absorption to fall to a level seen after feeding soybean protein (42, 68), whereas the effects on fecal excretion of endogenous magnesium were also similar (68).

There are only sparse data on effects of type of protein on magnesium absorption in humans. Soybean protein versus meat protein had no effect on apparent magnesium absorption (59). A lactalbumin-casein preparation raised apparent magnesium absorption when compared with either beef protein or peanut flour (62).

Sulphate and chloride. The increased urinary magnesium excretion seen after ingesting additional protein (Appendix table 1) might be analogous to the protein effect on urinary calcium excretion, which is ascribed to the sulfur containing amino acids in protein (69, 70). Indeed, in a study with rats, Greger *et al.* (71) demonstrated that dietary sulphate or bisulphate significantly raised urinary magnesium excretion, which was associated with slightly increased apparent magnesium absorption. Data on magnesium retention are not available, but magnesium concentration in tibia was decreased after sulphate feeding (71).

The chloride anion, which is often associated with isolated proteins could also be

responsible for the increased urinary magnesium excretion after protein ingestion. Ingestion of chloride from different sources raises urinary magnesium excretion in rats (71, 72). However, apparent magnesium absorption was not significantly influenced by chloride, which explains the observed decreased magnesium concentration in tibia.

Bicarbonate and ammonium. Greger *et al.* (71) observed in studies with rats that feeding sodium bicarbonate increased apparent magnesium absorption in one experiment, but not in another. Urinary magnesium excretion was not influenced by sodium bicarbonate. In contrast, Levin and Winaver (73) showed that bicarbonate infusion, causing metabolic alkalosis, enhanced tubular reabsorption of magnesium associated with decreased urinary magnesium excretion. Metabolic acidosis may decrease tubular reabsorption of magnesium, resulting in increased urinary excretion. McDougal and Koch (74) did show that ammonium chloride directly inhibits renal magnesium reabsorption, but Toothill (50) was unable to demonstrate an effect on apparent magnesium absorption.

Fat. Studies with rats demonstrated that a diet containing equal parts of medium chain triglycerides, sunflower seed oil and olive oil significantly raised apparent magnesium absorption in comparison with a diet containing olive oil as only fat source (75). This might be caused by the lower capacity of medium chain triglycerides to form soaps with magnesium (76, 77). Studies of Behling *et al.* (78) and Kaup *et al.* (79) showed that increasing the dietary fat concentration from 5% (w/w) to 20% (w/w) can improve apparent magnesium absorption. Because of the possible formation of magnesium soaps in the intestine the opposite would be expected. However, the effect depended on dietary magnesium and calcium concentrations. Increasing fat intakes improved apparent magnesium absorption at a dietary magnesium concentration of 0.5% (78), but no effect was seen when dietary magnesium concentration was 0.05% (79). In rats fed diets containing high calcium concentrations, fat seemed to improve magnesium absorption, but it was depressed in rats fed high-fat diets with a low calcium concentration. This suggests that fatty acids preferentially form complexes with calcium rather than with magnesium. Tadayyon and Ludwak (80) showed that supplementation of a fat-free diet with 25 energy % in the form of either tripalmitin or tristearin depressed apparent magnesium absorption in young rats.

Studies with humans could not demonstrate any effect of concentration or kind of dietary fat on magnesium absorption. Results of Van Dokkum *et al.* (81) indicate that decreasing the dietary fat intake from 42 to 22 energy % does not influence apparent absorption, urinary excretion and retention of magnesium in young men. Increasing the amount of linoleic acid at constant fat intakes did not affect magnesium metabolism either (81). In addition, Rickets *et al.* (82) found no effect of kind and amount of dietary fat on magnesium balance in humans.

Carbohydrates. Lactose is the carbohydrate most often studied in relation to magnesium absorption (Appendix table 2). Its influence in rats is clear. Lactose in the diet increases

apparent absorption and urinary excretion of magnesium. This is probably due to the fact that rats become lactase-deficient after weaning (97). Thus, rats are not capable to hydrolyse lactose in the intestine, resulting in microbial fermentation of lactose and lowered luminal pH. This in turn could increase the solubility of intestinal magnesium, leading to increased magnesium absorption (89).

Based on the above mentioned it would be expected that lactose exerts no effect on apparent magnesium absorption in lactose-tolerant subjects. Indeed, in lactose-tolerant adults with constant magnesium intake, lactose did not influence apparent magnesium absorption (66). Studies with infants indicate that lactose increases apparent magnesium absorption (94-96), but in these studies magnesium intake was not reported or was higher in the infants fed lactose. A higher magnesium intake by itself raises apparent absolute magnesium absorption (65, 66) and thus any effect of lactose could not be determined.

One study showed that a solution of glucose polymer increased magnesium absorption (98). However, glucose polymer was given together with magnesium and magnesium absorption was measured by triple-lumen perfusion in the jejunum which is not the main site of magnesium absorption. In any event, these data may not predict the effect of glucose polymer in a diet on magnesium absorption.

Fructose versus glucose has been reported to increase magnesium absorption in a study with rats (85). This was also seen for fructose versus cornstarch in an experiment with humans (93). The metabolic basis for fructose-induced stimulation of magnesium absorption is unknown.

Fiber. Various fiber sources (wheat bran, cellulose, lignin, pectin and guar gum) have been reported to bind magnesium *in vitro* (99). When insoluble fiber-magnesium complexes are formed in the intestine, magnesium absorption may be depressed. Appendix table 3 shows that in many rat and human experiments increased intake of cellulose or cellulose-rich preparations such as wheat bran produced enhanced fecal and urinary magnesium losses. However, absolute magnesium absorption was generally increased because higher fiber intakes were associated with higher magnesium intakes. Therefore, independent effects of fiber on magnesium absorption cannot be estimated. In a rat study in which magnesium intake was controlled, dietary cellulose had no effect on apparent absorption of magnesium (102). Results of the well-controlled studies in man indicate that dietary cellulose might lower magnesium absorption (8, 107, 110). In practical diets, high fiber intake usually is associated with high magnesium intake. Thus, no deleterious effects of high-fiber diets on magnesium balance would be expected. Indeed, Schwartz *et al.* (117) showed that feeding a bran-rich diet to humans for 4 wk did not affect magnesium balance.

Few studies have assessed the effect of pectin on magnesium absorption. In rats, galacturonic acid, which is the basic building block of pectin, significantly raised apparent absorption and urinary excretion of magnesium (102). However, pectin lowered magnesium absorption (102) and magnesium retention (103) in rats, whereas no effect was seen in a study with humans (107).

Oxalic acid. Oxalic acid might bind magnesium ions which could result in decreased magnesium absorption (118). Ingestion of spinach, which is high in oxalic acid, raised fecal magnesium excretion, but this may have been due to an increased magnesium intake (119). Absolute apparent magnesium absorption and urinary magnesium excretion were not significantly influenced by oxalic acid.

Phytate. At neutral pH, phytate can form with magnesium an insoluble complex, which may be a calcium-magnesium-phytate (120) or protein-magnesium-phytate complex (121). Formation of these insoluble complexes in the intestine might decrease magnesium absorption. However, the participation of magnesium in the formation of insoluble mineral-phytate complexes depends on several factors. First, a high calcium:magnesium ratio favors the *in vitro* formation of a calcium-phytate complex without co-precipitation of magnesium. Second, titration curves for calcium and magnesium have indicated that the stability of the magnesium-phytate complex is lower than that of a calcium-phytate complex. In keeping with this, it can be questioned whether the high calcium:magnesium ratio in rat diets allows the formation of an insoluble calcium-magnesium-phytate complex in the intestine. Brink *et al.* (68) found that phytate increased fecal excretion of endogenous magnesium in rats, whereas it exerted no effect on true magnesium absorption, which speaks against the formation of an insoluble magnesium-phytate complex in the intestine. It is not clear why phytate increased fecal excretion of endogenous magnesium. In any event, it has been shown in rats that dietary phytate causes a decreased magnesium solubility in the gut (122) and lowers apparent magnesium absorption (Appendix table 4) and urinary excretion of magnesium (42). At normal and relatively high dietary magnesium concentrations, phytate feeding does not affect magnesium retention and magnesium concentrations in plasma, tibia, femur, carcass, liver and kidney (42, 101, 123). However, at marginal dietary magnesium concentrations, phytate decreases body weight gain (125) and magnesium concentrations in femur (42).

Various studies with humans dealing with the effect of phytate on magnesium absorption are difficult to interpret because high-phytate diets were fortified with fiber-rich foodstuffs, so that magnesium intake was usually elevated (64, 127-129). However, studies with controlled magnesium intake using dephytinized products or supplemental phytate demonstrate that phytate decreases apparent absorption and urinary excretion of magnesium with no effect on magnesium retention (65, 126, 128).

Thus, phytate in the diet can decrease apparent absorption of magnesium. However, with natural diets, high intakes of phytate are generally accompanied with high intakes of magnesium, ensuring sufficient magnesium for absorption.

Ethanol. A single dose of ethanol produces increased urinary excretion of magnesium by human subjects (130, 131). This effect may explain the magnesium depletion observed in many alcoholic patients (132). Data concerning ethanol effects on intestinal magnesium absorption, if any, are not available.

Magnesium. Increased magnesium intakes cause increased rates of absolute magnesium absorption, resulting in enhanced urinary magnesium excretion (39, 42, 45, 46, 60, 65, 104, 133-136). Extra intake of magnesium could raise magnesium retention which may be reflected by higher magnesium concentrations in plasma, bone and muscle. Whether this happens depends on magnesium status, age, habitual magnesium intake and the magnitude of the increase in magnesium absorption.

The source of dietary magnesium may determine magnesium absorption by influencing the solubility of magnesium in the intestine. In *in vitro* incubations, magnesium citrate is more soluble than magnesium oxide (137). In rats, magnesium given either as a chloride, oxide or carbonate salt resulted in higher apparent magnesium absorption than magnesium given in the form of either a sulphate, phosphate or silicate salt (138, 139). However, the differences were small and may not have physiological significance. Bøhmer *et al.* (136) showed in adult females that magnesium supplemented as either magnesium citrate, lactate, hydroxide or chloride did not exert differential effects on urinary magnesium excretion.

Calcium. The effect of dietary calcium on intestinal absorption of magnesium has been subject of much controversy. Studies in rats have clearly demonstrated that apparent absorption of magnesium is decreased by high calcium intake (Appendix table 5). This has often been explained by competition between magnesium and calcium for a common carrier system in the ileum (19, 140, 165). However, Karbach and Rummel (24) could not confirm this. Human studies on magnesium absorption as influenced by dietary calcium have yielded contradictory results (Appendix table 5). Although some studies show decreased apparent magnesium absorption at high calcium intakes, most well-controlled studies indicate that high calcium intake does not affect intestinal magnesium absorption.

Recently, an explanation has been offered for the controversy between the outcome of the rat studies and many human experiments. In rats, Brink *et al.* (143) showed that the inhibitory effect of dietary calcium on apparent magnesium absorption depends on the phosphate:magnesium ratio of the diet. This ratio determines whether or not an insoluble magnesium-calcium-phosphate complex is formed in the intestine. The relatively low phosphate:magnesium ratio and the relatively low calcium concentration in human diets, as compared with rat diets, might result in negligible formation of insoluble complexes in the human intestine after the intake of extra calcium. This would explain why addition of calcium to human diets does not affect apparent magnesium absorption whereas addition of calcium to rat diets inhibits it. This explanation is confirmed by the study of Giles *et al.* (133), who found in infants that high-calcium diets, with a high phosphate:magnesium ratio comparable to that in rat diets caused a decreased apparent magnesium absorption.

Phosphate. The influence of dietary phosphate on magnesium absorption is not fully settled. Generally, increased phosphate concentrations in the diet have been reported to lower apparent magnesium absorption in rats and humans, but lack of effect has also been reported (Appendix table 5). The phosphate-induced decrease of magnesium absorption might be

caused by the formation of an insoluble magnesium-phosphate complex in the intestine (10). However, Brink *et al.* (143) recently presented evidence that the intestinal formation of an insoluble magnesium-calcium-phosphate complex is responsible for the decrease in apparent magnesium absorption. Because the formation of this complex depends on the dietary phosphate:magnesium ratio, controversy of literature data may relate to differences in this ratio.

Potassium. Studies in rats indicate that dietary potassium does not influence magnesium absorption (Appendix table 6). Infusion of a solution containing 0, 20 or 40 mmol/L potassium into a ligated loop of rat colon depressed magnesium uptake by the loop (170). In a study with humans it was demonstrated that potassium supplementation during a 40-day period of total fasting caused an increase in fecal magnesium loss (53). However, this effect may be independent of magnesium absorption.

Sodium. *In vitro* studies using rat gut sacs (171) or rat ileum (19) suggest that sodium ions may optimize magnesium transport. However, balance studies in rats could not demonstrate any systematic effect of sodium on intestinal, apparent magnesium absorption (Appendix table 6). Increasing dietary sodium with either chloride, sulphate or bisulphate as anion raised urinary magnesium excretion which was not consistently associated with an increase in apparent magnesium absorption (71). Urinary anion excretion was found to be responsible for the increase in urinary magnesium excretion (71).

Vitamin D. The vitamin most studied in relation to magnesium absorption is vitamin D, but its role remains uncertain (10). Several studies in man suggest that $1,25(OH)_2D_3$ or vitamin D_3 have little or no effect on apparent absorption of magnesium (172-174), whereas others showed that pharmacological doses of vitamin D_3 may increase apparent magnesium absorption (175-177). Rat experiments demonstrate that large doses of vitamin D_3 can increase apparent magnesium absorption (38, 39, 178, 179), whereas a realistic dose had no effect (180). Magnesium absorption by the colon of vitamin D-repleted rats may be normal (23). Hardwick *et al.* (10) put forward that a significant portion of magnesium absorption must be vitamin D-independent because it persists under conditions of vitamin D-deficiency. However, repletion of vitamin D is associated with increments in magnesium absorption (38).

Pyridoxine. Eisinger and Dagorn (134) showed that extremely high doses of pyridoxine lower apparent magnesium absorption in humans. However, at normal intakes of pyridoxine an effect on magnesium absorption was not seen.

Zinc. Forbes *et al.* (87) reported that dietary zinc does not affect apparent absorption, urinary excretion and retention of magnesium in rats. Likewise, supplemental zinc had no significant effect on apparent magnesium absorption in adolescent females (181).

Tin. In adult men, daily consumption of 50 versus 0.1 mg tin did not affect apparent absorption, urinary excretion and retention of magnesium (182).

Aluminum. *In vitro* studies suggest that aluminum forms an insoluble complex with magnesium (183). However, in a balance study with adult males, ingestion of 125 mg aluminum per day had no significant effect on apparent magnesium absorption and retention (184).

Caffeine. Yeh *et al.* (185) found in rats that subcutanous injection of caffeine raised apparent magnesium absorption and urinary magnesium excretion. The effect of dietary caffeine on magnesium absorption is not known.

Summary

This paper reviews the evidence that certain dietary components can affect intestinal absorption of magnesium. Increased intakes of protein and fructose improve apparent magnesium absorption in humans, whereas a lowering effect occurs with consumption of cellulose and phytate. Although dietary concentrations of lactose, fat, calcium and phosphate have clear effects on magnesium absorption in experiments with rats, the impact of these nutrients on magnesium absorption in humans remains unsettled. Mechanisms underlying the effects of dietary components on magnesium absorption in humans are generally poorly understood.

A change in magnesium absorption not necessarily results in a change in magnesium retention. When consuming practical diets, the fall of apparent magnesium absorption caused by phytate and cellulose is generally compensated by increased magnesium intake due to high magnesium concentrations in phytate- and cellulose-rich products. Furthermore, to maintain homeostasis, urinary magnesium excretion will be raised after stimulation of apparent magnesium absorption and it will be lowered after impairment of apparent magnesium absorption. Thus, the effects of dietary components on magnesium absorption probably are critically important only at low intakes of magnesium. At low magnesium intakes, differences in magnesium absorption may be expected to influence magnesium retention and thus can either induce or abolish magnesium deficiency.

References

1. Ryan, M.F. (1991) The role of magnesium in clinical biochemistry: an overview. *Ann. Clin. Biochem.* **28**: 19-26.
2. Shills, M.E. (1988) Magnesium in health and disease. *Ann. Rev. Nutr.* **8**: 429-460.
3. Altura, B.M. & Altura, B.T. (1990) Role of magnesium in the pathogenesis of hypertension. In: Hypertension: pathophysiology, diagnosis and managment. Laragh, J.H. & Brenner, B.M., eds. Raven Press, New York, pp 1003-1025.
4. Driessens, F.C.M., Steidl, L. & Ditmar, R. (1990) Magnesium, calcium and zinc status in different forms of osteoporosis. *Magnesium Bull.* **12**: 158-160.

5. Seelig, M.S. (1979) Magnesium (and trace substance) deficiencies in the pathogenesis of cancer. *Biol. Trace Elem. Res.* **1**: 273-297.
6. Hazell, T. (1985) Minerals in foods: Dietary sources, chemical forms, interactions, bioavailability. *Wld. Rev. Nutr. Diet.* **46**: 1-123.
7. Seelig, M.S. (1964) The requirements of magnesium by the normal adult. *Am. J. Clin. Nutr.* **14**: 342-390.
8. Kelsay, J.L., Behall, K.M. & Prather, E.S. (1979) Effects of fiber from fruits and vegetables on metabolic responses of human subjects. II. Calcium, magnesium, iron and silicon balances. *Am. J. Clin. Nutr.* **32**: 1876-1880.
9. Committee on Dietary Allowances (1980) Recommended dietary allowances. Magnesium. Ninth revised edition. National Academy of Sciences, Washington DC, pp 134-136.
10. Hardwick, L.L., Jones, M.R., Brautbar, N. & Lee, D.B.N. (1991) Magnesium absorption: Mechanisms and the influence of vitamin D, calcium and phosphate. *J. Nutr.* **121**: 13-23.
11. Aikawa, J.K., Gordon, G.S. & Rhodes, E.L. (1960) Magnesium metabolism in human beings: studies with ^{28}Mg. *J. Appl. Physiol.* **15**: 503-507.
12. Danielson, B.G., Johansson, S., Jung, B., Ljunghall, S., Lundqvist, H. & Malmborg, P. (1979) Gastrointestinal magnesium absorption: kinetic studies with ^{28}Mg and a simple method for determination of fractional absorption. *Min. Electrolyte Metab.* **2**: 116-123.
13. Graham, L.A., Ceasar, J.J. & Burgen, A.S. (1960) Gastrointestinal absorption and excretion of ^{28}Mg in man. *Metab. Clin. Exp.* **9**: 646-659.
14. Aldor, T.A.M. & Moore, E.W. (1970) Magnesium absorption by everted sacs of rat intestine and colon. *Gastroenterology* **59**: 745-753.
15. Behar, J. (1974) Magnesium absorption by the rat ileum and colon. *Am. J. Physiol.* **227**: 334-340.
16. Chutkow, J.G. (1964) Sites of magnesium absorption and excretion in the intestinal tract of the rat. *J. Lab. Clin. Med.* **63**: 71-79.
17. Hendrix, J.Z., Alcock, N.W. & Archibald, R.M. (1963) Competition between calcium, strontium and magnesium for absorption in the isolated rat intestine. *Clin. Chem.* **9**: 734-744.
18. Ross, D.B. (1962) *In vitro* studies on the transport of magnesium across the intestinal wall of the rat. *J. Physiol.* **160**: 417-428.
19. Behar, J. (1975) Effect of calcium on magnesium absorption. *Am. J. Physiol.* **229**: 1590-1595.
20. Marshall, D.H., Nordin, B.E.C. & Speed, R. (1976) Calcium, phosphorus and magnesium requirement. *Proc. Nutr. Soc.* **35**: 163-173.
21. Walser, M. (1967) Magnesium metabolism. *Ergeb. Physiol.* **59**: 185-296.
22. Brannan, P.G., Vergne-Marini, P., Pak, C.Y.C., Hull, A.R. & Fordtran, J.S. (1976) Magnesium absorption in the human small intestine. Results in normal subjects, patients with chronic renal disease, and patients with absorptive hypercalciuria. *J. Clin. Invest.* **57**: 1412-1418.
23. Karbach, U. (1989) Cellular-mediated and diffusive magnesium transport across the colon descendens of the rat. *Gastroenterology* **96**: 1282-1289.
24. Karbach, U. & Rummel, W. (1990) Cellular and paracellular magnesium transport across the terminal ileum of the rat and its interaction with the calcium transport. *Gastroenterology* **98**: 985-992.
25. Roth, P. & Werner, E. (1979) Intestinal magnesium absorption in man. *Int. J. Appl. Radiat. Isotopes* **30**: 523-526.
26. Wilkinson, R. (1976) Absorption of calcium, phosphorus and magnesium. In: Calcium, phosphate and magnesium metabolism. (Nordin, B.E.C., ed.), Churchill Livingstone, Edinburgh, pp 35-112.
27. Spencer, H., Lesniak, M., Gatza, C.A., Osis, D. & Lender, M. (1980) Magnesium absorption and metabolism in patients with chronic renal failure and in patients with normal renal function. *Gastroenterology* **79**: 26-34.
28. Brunette, M., Wen, S.F., Evanson, R.L. & Dirks, J.H. (1969) Micropuncture study of magnesium reabsorption in the proximal tubule of the dog. *Am. J. Physiol.* **216**: 1510-1516.
29. Dibona, G.F. & Sawin, L.L. (1982) Effect on renal arterial magnesium infusion on renal function in the dog. *Magnesium* 1: 104-109.
30. Quamme, G.A. (1986) Renal handling of magnesium: drug and hormone interactions. *Magnesium* **5**: 248-272.
31. Heaton, F.W. (1969) The kidney and magnesium homeostasis. *Ann. NY Acad. Sci.* **162**: 775-785.
32. Anast, C.S. & Gardner, D.W. (1981) Magnesium metabolism. In: Disorders of mineral metabolism, Vol III. Bronner, F. & Coburn, J.W., eds. Academic Press, New York, pp 423-506.

33. MacIntyre, I., Matthews, E.W. & Robinson, C.J. (1966) Parathyroid hormone and magnesium absorption. *J. Physiol.* **184**: 83P-84P.
34. Care, A.D. & Keynes, W.M. (1964) The role of parathyroid hormones in the absorption of calcium and magnesium from the small intestine. *Proc. Royal Soc. Med.* **57**: 867-868.
35. Hanna, S., Harrison, M.T., MacIntyre, I. & Fraser, R. (1961) Effect of growth hormone on calcium and magnesium metabolism. *Br. Med. J.* **2**: 12-15.
36. Hanna, S. & MacIntyre, I. (1960) Influence of aldosterone on magnesium metabolism. *Lancet* **2**: 348-350.
37. Mader, I.J. & Isberi, L.T. (1955) Spontaneous hypomagnesaemia, alkalosis and tetany during hypersecretion of cortison-like mineralocorticoid. *Am. J. Med.* **19**: 976-988.
38. Hanna, S. (1961) Influence of large doses of vitamin D on magnesium metabolism in rats. *Metabolism* **10**: 734-743.
39. Levine, B.S., Brautbar, N., Walling, M.W., Lee, D.B.N. & Coburn, J.W. (1980) Effects of vitamin D and diet magnesium on magnesium metabolism. *Am. J. Physiol.* **239**: E515-E523.
40. Norman, D.A., Fordtran, J.S., Brinkley, L.J., Nicar, M.J., Strowig, S.M. & Pak, C.Y.C. (1981) Jejunal and ileal adaptations to alterations in dietary calcium. *J. Clin. Invest.* **67**: 1599-1603.
41. Azami, S., Hiratzuka, S., Kitano, T. & Esashi, T. (1989) Effects of dietary casein and soy protein isolate on calcium, phosphorus and magnesium balance in female rats. *Jpn. J. Nutr.* **47**: 103-112.
42. Brink, E.J., Dekker, P.R., van Beresteijn, E.C.H. & Beynen, A.C. (1991a) Inhibitory effect of soybean protein vs. casein on magnesium absorption in rats. *J. Nutr.* **121**: 1374-1381.
43. Forbes, R.M. (1964) Mineral utilization in the rat. III. Effects of calcium, phosphorus, lactose and source of protein in zinc-deficient and in zinc-adequate diets. *J. Nutr.* **83**: 225-233.
44. Forbes, R.M., Weingarter, K.E., Parker, H.M., Bell, R.R. & Erdman, J.W. (1979) Bioavailability to rats of zinc, magnesium and calcium in casein-, egg- and soy protein-containing diets. *J. Nutr.* **109**: 1652-1660.
45. Kubena, K.S., Landmann, W.A., Young, C.R. & Carpenter, Z.L. (1985) Influence of magnesium deficiency and soy protein on magnesium and zinc status in rats. *Nutr. Res.* **5**: 317-328.
46. Lo, G.S., Steinke, F.H. & Hopkins, D.T. (1980) Effect of isolated soybean protein on magnesium bioavailability. *J. Nutr.* **110**: 829-836.
47. Schwartz, R., Wang, F.L. & Woodcock, N.A. (1969) Effect of varying dietary protein-magnesium ratios on nitrogen utilization and magnesium retention in growing rats. *J. Nutr.* **97**: 185-193.
48. Shahkhalili, Y. & Mettraux, C. (1991) Relative importance of carbohydrate and protein sources in the differential effects of soy-based vs casein-based formulas on bone minerals in rats. *Am. J. Clin. Nutr.* **53**: 947-953.
49. Sterck, J.G.H., Ritskes-Hoitinga,J. & Beynen, A.C. (1992) Inhibitory effect of high protein intake on nephrocalcinogenesis in female rats. *Br. J. Nutr.*: in press.
50. Toothill, J. (1963) The effect of certain dietary factors on the apparent absorption of magnesium by the rat. *Br. J. Nutr.* **17**: 125-134.
51. Van Camp, I., Ritskes-Hoitinga, J., Lemmens, A.G. & Beynen, A.C. (1990) Diet-induced nephrocalcinosis and urinary excretion of albumin in female rats. *Lab. Anim.* **24**: 137-141.
52. Alcantara, E.N. & Linkswiler, H. (1969) Effect of protein and of magnesium intake on magnesium balance of older adolescent males. *Fed. Proc.* **28**: 562.
53. Fisler, J.S. & Drenick, E.J. (1984) Calcium, magnesium and phosphate balances during very-low calorie diets of soy or collagen protein in obese men: comparison to total fasting, *Am. J. Clin. Nutr.* **40**: 14-25.
54. Hunt, S.M. & Schofield, F.A. (1969) Magnesium balance and protein intake level in adult human females. *Am. J. Clin. Nutr.* **22**: 367-373.
55. Kitano, T., Esashi, T. & Azami, S. (1988) Effect of protein intake on mineral (calcium, magnesium and phosphorus) balance in Japanese males. *J. Nutr. Sci. Vitaminol.* **34**: 387-398.
56. Lakshaman, F.L., Rao, R.B., Kim, W.W. & Kelsay, J.L. (1984) Magnesium intakes, balances and blood levels of adults, consuming self-selected diets. *Am. J. Clin. Nutr.* **40**: 1380-1389.
57. Mahalko, J.R., Sandstead, H.H., Johnson, L.K. & Milne, D.B. (1983) Effect of a moderate increase in dietary protein on the retention and excretion of Ca, Cu, Fe, Mg, P, and Zn by adult males. *Am. J. Clin. Nutr.* **37**: 8-14.
58. McCance, R.A., Widdowson, E.M. & Lehmann, H. (1942) The effect of protein intake on the absorption of calcium and magnesium. *Biochem. J.* **36**: 686-691.
59. Sandström, B., Andersson, H., Kivisto, B. & Sandberg, A-S. (1986) Apparent small intestinal absorption of nitrogen and minerals from soy and meat-protein-based diets. A study on human ileostomy patients. *J. Nutr.* **116**: 2209-2218.

60. Schwartz, R., Walker, G., Linz, M. & MacKellar, I. (1973) Metabolic responses of adolescent boys to two levels of dietary magnesium and protein. I. Magnesium and nitrogen retention. *Am. J. Clin. Nutr.* **26**: 510-518.
61. Shier, N. (1984) The relationship of dietary protein, protein type, and magnesium to urinary magnesium excretion in the human. *Nutr. Rep. Int.* **29**: 1301-1306.
62. Stephenson, M.G., Butler, L.C. & Adams, Y.L. (1970) Effect of protein source on magnesium balance of young women. *Fed. Proc.* **29**: 696.
63. Van Dokkum, W., Wesstra, A., Luyken, R. & Hermus, R.J.J. (1986) The effect of a high-animal and a high-vegetable protein diet on mineral balance and bowel function of young men. *Br. J. Nutr.* **56**: 341-348.
64. Wisker, E., Nagel, R., Tanudjaja, T. & Feldheim, W. (1991) Calcium, magnesium, zinc and iron balances in young women: effects of a low-phytate barley-fiber concentrate. *Am. J. Clin. Nutr.* **54**: 553-559.
65. Morris, E.R., Ellis, R., Steele, P. & Moser, P.B. (1988) Mineral balance of adult men consuming whole or dephytinized wheat bran. *Nutr. Res.* **8**: 445-458.
66. Brink, E.J., van Beresteijn, E.C.H., Dekker, P.R. & Beynen, A.C. (1991b) Lactose intake does not affect the apparent absorption of magnesium and calcium in healthy adults. *Internal report.*
67. Reddy, N.R., Sathe, S.K. & Salunkhe, P.K. (1982) Phytates in legumes and cereals. *Adv. Food Res.* **28**: 1-92.
68. Brink, E.J., van den Berg, G.J., van der Meer, R., Dekker, P.R., Wolterbeek, H.Th. & Beynen, A.C. (1991c) Inhibitory effect of soybean protein versus casein on apparent magnesium absorption in rats due to increased fecal excretion of endogenous magnesium. *Internal report.*
69. Whiting, S.J. & Draper, H.H. (1981) Effect of a chronic load as sulphate of sulphur amino acids on bone metabolism in adult rats. *J. Nutr.* **111**: 1721-1726.
70. Zemel, M.B., Schuette, S.A., Hegsted, M. & Linkswiler, H.M. (1981) Role of sulphur-containing amino acids in protein-induced hypercalciuria in men. *J. Nutr.* **111**: 545-552.
71. Greger, J.L., Kaup, S.M. & Behling, A.R. (1991) Calcium, magnesium and phosphorus utilization by rats fed sodium and potassium salts of various inorganic anions. *J. Nutr.* **121**: 1382-1388.
72. Kaup, S.M. & Greger, J.L. (1990) Effect of various chloride salts on the utilization of phosphorus, calcium, and magnesium. *J. Nutr. Biochem.* **1**: 542-548.
73. Levin, M. & Winaver, J. (1989) Effects of systemic alkalosis on urinary magnesium excretion in the rat. *Min. Electrolyte Metab.* **15**: 214-220.
74. McDougal, W.C. & Koch, M. (1989) Effect of sulphate on calcium and magnesium homeostasis following urinary diversion. *Kidney Int.* **35**: 105-115.
75. Aliaga, I.L., Barrionuevo, M., Campos, M.S., Coves, F. & Lisbona, F. (1991) Influence of intestinal resection and type of diet on digestive utilization and metabolism of magnesium in rats. *Int. J. Vit. Nutr. Res.* **61**: 61-66.
76. Booth, C.C., Babouris, M.B., Hanna, S. & MacIntyre I. (1963) Incidence of hypomagnesaemia in intestinal malabsorption. *Br. Med. J.* **2**: 141-143.
77. Hanna, S., Harrison, M.T., MacIntyre, I. & Fraser, R. (1960) The syndrome of magnesium deficiency in man. *Lancet* **ii**: 172-175.
78. Behling, A.R., Kaup, S.M. & Greger, J.L. (1990) Changes in intestinal function of rats initiated with DMH and fed varying levels of butterfat, calcium and magnesium. *Nutr. Cancer* **13**: 189-199.
79. Kaup, S.M., Behling, A.R., Choquette, L. & Greger, J.L. (1990) Calcium and magnesium utilization in rats: effect of dietary butterfat and calcium and of age. *J. Nutr.* **120**: 266-273.
80. Tadayyon, B. & Ludwak, L. (1969) Interrelationship of triglycerides with calcium, magnesium and phosphorus in the rat. *J. Nutr.* **97**: 246-254.
81. Van Dokkum, W., Cloughley, F.A., Hulshof, K.F.A.M. & Oosterveen, L.A.M. (1983) Effect of variations in fat and linoleic acid intake on the calcium, magnesium and iron balance of young men. *Ann. Nutr. Metab.* **27**: 361-369.
82. Rickets, C., Kies, C., Garcia, P. & Fox, H.M. (1985) Manganese and magnesium utilization of humans as affected by level and kind of dietary fat. *Fed. Proc.* **44**: 1850.
83. Andrieux, C. & Sacquet, E. (1983) Effect of microflora and lactose on the absorption of calcium, phosphorus and magnesium in the hindgut of the rat. *Reprod. Nutr. Develop.* **23**: 259-271.
84. Behling, A.R. & Greger, J.L. (1990) Importance of lactose in yogurt for mineral utilization. *J. Agric. Food Chem.* **38**: 200-204.

85. Bergstra, A.E., Lemmens, A.G. & Beynen, A.C. (1991) Stimulatory effect of dietary fructose versus glucose on nephrocalcinogenesis in female rats. *Internal report.*
86. Brink, E.J., Dekker, P.R., van Beresteijn, E.C.H. & Beynen, A.C. (1992a) Bioavailability of magnesium and calcium from cow's milk and soybean beverage in rats. *Br. J. Nutr.*: in press.
87. Forbes, R.M. (1961) Excretory patterns and bone deposition of zinc, calcium and magnesium in the rat as influenced by zinc deficiency, EDTA and lactose. *J. Nutr.* **74**: 194-200.
88. Greger, J.L., Gutkowski, C.M., Khazen, R.R. (1989) Interactions of lactose with calcium, magnesium and zinc in rats. *J. Nutr.* **119**: 1691-1697.
89. Heijnen, A.M.P., Brink, E.J., Lemmens, A.G. & Beynen, A.C. (1991) Solubility of calcium, magnesium and phosphorus in ileal chyme of rats fed diets containing either lactose or lactulose: Impact on mineral absorption and nephrocalcinogenesis. *Internal report.*
90. Koh, E.T., Reiser, S., Fields, M. (1989) Dietary fructose as compared to glucose and starch increases the calcium content of kidney of magnesium-deficient rats. *J. Nutr.* **119**: 1173-1178.
91. Schaafsma, G., Dekker, P.R. & de Waard, H. (1988) Nutritional aspects of yogurt. 2. Bioavailability of essential minerals and trace elements. *Neth. Milk Dairy J.* **42**: 135-146.
92. Debognie, J.C., Newcomer, A.D., McGill, D.B. & Phillips, S.F. (1979) Absorption of nutrients in lactase-deficiency. *Dig. Dis. Sci.* **24**: 225-231.
93. Holbrook, J.T., Smith, J.C. & Reiser, S. (1989) Dietary fructose or starch: effects on copper, zinc, iron, manganese, calcium and magnesium balance in humans. *Am. J. Clin. Nutr.* **49**: 1290-1294.
94. Kobayashi, A., Kawai, S., Ohbe, Y. & Nagashima, Y. (1975) Effects of dietary lactose and a lactase preparation on the intestinal absorption of calcium and magnesium in normal infants. *Am. J. Clin. Nutr.* **28**: 681-683.
95. Wirth, F.H., Numerof, B., Pleban, P. & Neylan, M.J. (1990) Effect of lactose on mineral absorption in preterm infants. *J. Pediatr.* **117**: 283-287.
96. Ziegler, E.E. & Fomon, S.J. (1983) Lactose enhances mineral absorption in infancy. *J. Pediatr. Gastr. Nutr.* **2**: 288-294.
97. De Groot, A.P. & Hoogendoorn, P. (1957) The detrimental effect of lactose. II. Quantitative lactase determinations in various mammals. *Neth. Milk Dairy J.* **11**: 290-303.
98. Bei, L., Wood, R.J. & Rosenberg, I.H. (1986) Glucose polymer increases jejunal calcium, magnesium and zinc absorption in humans. *Am. J. Clin. Nutr.* **44**: 244-247.
99. Camire, A.L. & Clydesdale, F.M. (1981) Effect of pH and heat treatment on the binding of calcium, magnesium, zinc, and iron to wheat bran and fractions of dietary fiber. *J. Food Sci.* **44**: 548-551.
100. Bagheri, S.M. & Gueguen, L. (1982) Bioavailability to rats of calcium, magnesium, phosphorus and zinc in wheat bran diets containing equal amounts of these minerals. *Nutr. Rep. Int.* **25**: 583-589.
101. Ballam, G.C., Nelson, T.S. & Kirby, L.K. (1984) The effect of phytate and fibre source on phytate hydrolysis and mineral availability in rats. *Nutr. Rep. Int.* **30**: 1089-1100.
102. De Jong, H.J.A.M., Lemmens, A.G. & Beynen, A.C. (1991) Nephrocalcinogenesis in female rats fed diets containing either pectin or cellulose. *Internal report.*
103. Mercurio, K.C. & Behm, P.A. (1981) Effects of fiber type and level on mineral excretion, transit time and intestinal histology. *J. Food Sci.* **46**: 1462-1463.
104. Shah, B.G., Malcolm, S., Belonje, B., Trick, K.D., Brassard, R. & Monmgeau, R. (1990) Effect of dietary cereal brans on the metabolism of calcium, phosphorus and magnesium in a long term rat study. *Nutr. Res.* **10**: 1015-1028.
105. Anderson, H., Navert, B., Bingham, S.A., Englyst, H.N. & Cummings, J.H. (1983) The effect of breads containing similar amounts of phytate but different amounts of wheat bran on calcium, zinc and iron balances. *Br. J. Nutr.* **50**: 503-510.
106. Behall, K.M., Scholfield, D.J., Lee, K., Powell, A.S. & Moser, P.B. (1987) Mineral balance in adult men: effect of four refined fibers. *Am. J. Clin. Nutr.* **46**: 307-314.
107. Drews, L.M., Kies, C. & Fox, H.M. (1979) Effect of dietary fiber on copper, zinc and magnesium utilization by boys. *Am. J. Clin. Nutr.* **32**: 1893-1897.
108. Hallfrisch, J., Powell, A., Carafelli, C., Reiser, S. & Prather, E.S. (1987) Mineral balances of men and women consuming high fiber diets with complex or simple carbohydrates. *J. Nutr.* **117**: 48-55.
109. Ismail-Beigi, F., Reinhold, J.G., Faraji, B. & Abadi, P. (1977) Effects of cellulose added to diets of low and high fiber content upon the metabolism of calcium, magnesium, zinc and phosphorus by man. *J. Nutr.* **107**: 510-518.
110. McHale, M., Kies, C. & Fox, H.M. (1979) Calcium and magnesium nutritional status of adolescent humans fed cellulose or hemicellulose supplements. *J. Food Sci.* **44**: 1412-1417.

111. Reinhold, J.G., Abadi, P., Faradji, B., Ismail-Beigi, F. & Russell, R.M. (1980) Depletion of calcium, magnesium, zinc and phosphorus associated with consumption by humans of wholemeal wheaten bread rich in fiber and phytate. *Baroda J. Nutr.* **7**: 55-62.
112. Reinhold, J.G., Faradji, B., Abadi, P. & Ismail-Beigi, F. (1991) Decreased absorption of calcium, magnesium, zinc and phosphorus by humans due to increased fiber and phosphorus consumption as wheat bread. *Nutr. Rev.* **49**: 204-206.
113. Schweitzer, T.F., Bekhechi, A.R., Koellreutter, B., Reimann, S., Pometta, D. & Bronn, B.A. (1983) Metabolic effect of dietary fiber from dehulled soybeans in humans. *Am. J. Clin. Nutr.* **38**: 1-11.
114. Slavin, J.L. & Marlett, J.A. (1980) Influence of refined cellulose on human bowel function and calcium and magnesium balance, *Am. J. Clin. Nutr.* **33**: 1932-1939.
115. Taper, L.J., Milam, R.S., McCallister, M.S., Bowen, P.E. & Thye, F.W. (1988) Mineral retention in young men consuming soy-fiber-augmented liquid-formula diets. *Am. J. Clin. Nutr.* **48**: 305-311.
116. Van Dokkum, W., Wesstra, A. & Schippers, F.A. (1982) The effect of dietary fibre from bread on the mineral balance of young men. *Br. J. Nutr.* **47**: 451-460.
117. Schwartz, R., Apgar, B.J. & Wien, E.M. (1986) Apparent absorption and retention of Ca, Cu, Mg, Mn, and Zn from a diet containing bran. *Am. J. Clin. Nutr.* **43**: 444-455.
118. Kelsay, J.L. (1987) Effects of fiber, phytic acid, and oxalic acid in the diet on mineral availability. *Am. J. Gastroenterology* **82**: 983-986.
119. Kelsay, J.L. & Prather, E.S. (1983) Mineral balances of humans subjects consuming spinach in a low-fiber diet and in a diet containing fruits and vegetables. *Am. J. Clin. Nutr.* **38**: 12-19.
120. Cheryan, M., Anderson, F.W. & Grynspan, F. (1983) Magnesium-phytate complexes: Effect of pH and molar ratio on solubility characteristics. *Cereal Chem.* **69**: 235-237.
121. Champagne, E.T. (1988) Effect of pH on mineral-phytate, protein-mineral-phytate, and mineral-fiber interactions. Possible consequences of atrophic gastritis on mineral bioavailability from high-fiber foods. *J. Am. Coll. Nutr.* **7**: 499-508.
122. Shinoda, S. & Yoshida, T. (1989) Influence of sodium phytate and gut microflora on the solubility of Ca, Mg and Zn in the rat digestive tract. *Nutr. Rep. Int.* **40**: 909-922.
123. Churella, H.R. & Vivian, V.M. (1989) Effect of phytic acid level in soy protein based infant formulas on mineral availability in the rat. *J. Agric. Food Chem.* **37**: 1352-1357.
124. Miyazawa, E. & Yoshida, T. (1991) Effects of dietary levels of phytate and inorganic phosphate on phytate breakdown and absorption of calcium and magnesium in rats. *Nutr. Res.* **11**: 797-806.
125. Roberts, A.H. & Yudkin, J. (1960) Dietary phytate as a possible cause of magnesium deficiency. *Nature* **185**: 823-825.
126. McCance, R.A. & Widdowson, E.M. (1942b) Mineral metabolism on dephytinized bread. *J. Physiol.* **101**: 304-313.
127. Cullumbine, H., Basnayake, V., Lemottee, J. & Wickramanayake, T.W. (1950) Mineral metabolism on rice diets. *Br. J. Nutr.* **4**: 101-111.
128. McCance, R.A. & Widdowson, E.M. (1942a) Mineral metabolism of healthy adults on white and brown bread dietaries. *J. Physiol.* **101**: 44-85.
129. Sandberg, A-S., Hasselblad, C., Hasselblad, K. & Hulten, L. (1982) The effect of wheat bran on the absorption of minerals in the small intestine. *Br. J. Nutr.* **48**: 185-191.
130. Kalbfleisch, J.M., Lindemann, R.D., Ginn, H.E. & Smith, W.O. (1963) Effects of ethanol administration on urinary excretion of magnesium and other electrolytes in alcoholic and normal subjects. *J. Clin. Inv.* **42**: 1471-1475.
131. McCollister, R.J., Flink, E.B. & Lewis, M.D. (1963) Urinary excretion of magnesium in man following the ingestion of ethanol. *Am. J. Clin. Nutr.* **12**: 415-420.
132. Jones, J.E., Shane, S.R., Jacobs, W.H. & Flink, E.B. (1969) Magnesium balance studies in chronic alcoholism. *Ann. N.Y. Acad. Sci.* **162**: 934-946.
133. Giles, M.M., Laing, I.A., Elton, R.A., Robins, J.B., Sanderson, M. & Hume, R. (1990) Magnesium metabolism in preterm infants: Effect of calcium, magnesium, and phosphorus, and of postnatal gestational age. *J. Pediatr.* **117**: 147-154.
134. Eisinger, J. & Dagorn, J. (1986) Vitamin B_6 and magnesium. *Magnesium* **5**: 27-32.
135. Fine, K.D., Santa Ana, C.A., Porter, J.L. & Fordtran, J.S. (1991) Intestinal absorption of magnesium from food and supplements. *J. Clin. Inv.* **88**: 396-402.
136. Bøhmer, T., Roseth, A., Holm, A., Weberg-Tegen, S. & Wahl, L. (1990) Bioavailability of oral magnesium supplementation in female students evaluated from elimination of magnesium in 24-h urine. *Magnesium Trace Elem.* **9**: 272-278.

137. Lindberg, J.S., Zobitz, M.M., Poindexter, J.R. & Pak, C.Y.C. (1990) Magnesium bioavailability from magnesium citrate and magnesium oxide. *J. Am. Coll. Nutr.* **9**: 48-55.
138. Cook, D.A. (1973) Availability of magnesium: Balance studies in rats with various inorganic magnesium sources. *J. Nutr.* **103**: 1365-1370.
139. Ranhotra, G.S., Loewe, R.J. & Puyat, L.V. (1975) Bioavailability of magnesium from wheat flour and various organic and inorganic salts. *Cer. Chem.* **53**: 770-776.
140. Alcock, N. & MacIntyre, I. (1962) Inter-relation of calcium and magnesium absorption. *Clin. Sci.* **22**: 185-193.
141. Al-Jurf, A.S. & Chapman-Furr, F. (1985) Magnesium balance and distribution during total parenteral nutrition: Effect of calcium additives. *Metabolism* **34**: 658-664.
142. Behling, A.R. & Greger, J.L. (1988) Mineral metabolism of aging female rats fed various commercially available calcium supplements or yoghurt. *Pharmaceut. Res.* **5**: 510-505.
143. Brink, E.J., Beynen, A.C., Dekker, P.R., van Beresteijn, E.C.H. & van der Meer, R. (1992b) Interaction of calcium and phosphate decreases ileal magnesium solubility and apparent magnesium absorption in rats. *J. Nutr.*: in press.
144. Bunce, G.E., Sauberlich, H.E., Reeves, P.G. & Oba, T.S. (1965) Dietary phosphorus and magnesium deficiency in the rat. *J. Nutr.* **86**: 406-414.
145. Ericsson, Y, Luoma, H. & Ekberg, O. (1986) Effects of calcium, fluoride and magnesium supplementations on tissue mineralization in calcium- and magnesium-deficient rats. *J. Nutr.* **116**: 1018-1027.
146. Forbes, R.M. (1963) Mineral utilization in the rat. I. Effects of varying dietary ratios of calcium, magnesium and phosphorus. *J. Nutr.* **80**: 321-326.
147. Hardwick, L.L., Clemens, R.A. & Jones, M.R. (1987) Effect of calcium phosphate supplementation on calcium, phosphorus and magnesium metabolism in the Wistar rat. *Nutr. Res.* **7**: 787-796.
148. Hoek, A.C., Lemmens, A.G., Mullink, J.W.M.A. & Beynen, A.C. (1988) Influence of dietary calcium:phosphorus ratio on mineral excretion and nephrocalcinosis in female rats. *J. Nutr.* **118**: 1210-1216.
149. Mars, Y.W.H.M., Lemmens, A.G. & Beynen, A.C. (1988) Dietary phosphorus and nephrocalcinosis in female rats. *Nutr. Rep. Int.* **38**: 249-258.
150. O'Dell, B.L., Morris, E.R. & Regan, W.O. (1960) Magnesium requirements of guinea pigs and rats: effect of calcium and phosphorus and symptoms of magnesium deficiency. *J. Nutr.* **70**: 103-110.
151. Parker, H.E. (1985) Magnesium and phosphorus interrelationships at deficient to normal dietary levels. *Nutr. Rep. Int.* **32**: 983-990.
152. Ritskes-Hoitinga, J., Lemmens, A.G., Danse, L.H.J.C. & Beynen, A.C. (1989) Phosphorus-induced nephrocalcinosis and kidney function in female rats. *J. Nutr.* **119**: 1423-1431.
153. Sawamura, T. & Goto, S. (1973) The effect of dietary Ca and P levels on magnesium balance. *Jpn. J. Nutr.* **31**: 190-194.
154. Schaafsma, G., Duursma, S.A., Visser, W.J. & Dekker, P.R. (1985) The influence of dietary calcium on kidney calcification and renal function in rats fed high-phosphate diets. *Bone* **6**: 155-163.
155. Wienk, K.J.H., Lemmens, A.G. & Beynen, A.C. (1989) Dietary potassium does not affect magnesium excretion in rats. *Nutr. Rep. Int.* **39**: 415-420.
156. Clarkson, E.M., Warren, R.L., McDonald, S.J. & de Wardener, H.E. (1967) The effect of a high intake of calcium on magnesium metabolism in normal subjects and patients with chronic renal failure. *Clin. Sci.* **32**: 11-18.
157. Heaton, F.W., Hodgkinson, A. & Rose, G.A. (1964) Observation on the relationship between calcium and magnesium metabolism in man. *Clin. Sci.* **27**: 31-40.
158. Kim, Y. & Linkswiler, H. (1979) Effect of level of calcium and of phosphorus intake on calcium, phosphorus and magnesium metabolism in young adult males. *Fed. Proc.* **39**: 895.
159. Leichsenring, J.M., Norris, L.M. & Lamison S.A. (1951) Magnesium metabolism in college women: Observations on the effects of calcium and phosphorus intake levels. *J. Nutr.* **45**: 477-485.
160. Lewis, N.M., Marcus, M.S.K., Behling, A.R. & Greger, J.L. (1989) Calcium supplements and milk: effects on acid-base balance and on retention of calcium, magnesium, and phosphorus. *Am. J. Clin. Nutr.* **49**: 527-533.
161. Moya, M. & Donemech, E. (1982) Role of calcium-phosphate ratio of milk formula on calcium balance in low birth weight infants during the first three days of life. *Pediatr. Res.* **16**: 675-681.
162. Spencer, H. (1980) Calcium and magnesium interactions in man. *Clin. Chem.* **25**: 1043.

163. Storey, M.L., Greger, J.L., Kiratli, B.J. & Smith, E.L. (1988) Urinary calcium and magnesium excretion by women in response to short-term calcium supplementation. *Nutr. Res.* **8**: 617-624.
164. Van der Meer, R., Welberg, J.W.M., Kuipers, F., Kleibeuker, J.H., Mulder, N.H., Termont, D.S.M.L., Vonk, R.J., de Vries, H.T. & de Vries, E.G.E. (1990) Effects of supplemental calcium on the intestinal association of calcium, phosphate and bile acids. *Gastroenterology* **99**: 1653-1659.
165. Care, D.A. & van 't Klooster, A.Th. (1965) In vivo transport of magnesium and other cations across the wall of the gastrointestinal tract of the sheep. *J. Physiol.* **177**: 174-191.
166. Charlton, J.A. & Armstrong, D.G. (1989) The effect of varying the sodium or potassium intake, or both, on magnesium status in the rat. *Br. J. Nutr.* **62**: 399-406.
167. Greene, L.W., Bratton, G.R., Zmudzki, J., Schelling, G.T. & Byers, F.M. (1986) Effect of dietary potassium and sodium on magnesium balance in rats fed different concentrations of magnesium. *Nutr. Rep. Int.* **33**: 235-240.
168. Schricker, B.R. (1985) Effect of dietary potassium sources on apparent absorption and retention of potassium, magnesium and sodium. *Nutr. Rep. Int.* **31**: 615-626.
169. Shortt, C. & Flynn, A. (1991) Effect of dietary lactose on salt-mediated changes in mineral metabolism and bone composition in the rats. *Br. J. Nutr.* **66**: 73-81.
170. Scharrer, E. & Schneider, B. (1988) Inhibitory effect of potassium on magnesium absorption by the rat colon. *Nutr. Rep. Int.* **37**: 197-202.
171. Ross, D.B. (1961) Influence of sodium on the transport of magnesium across the intestinal wall of the rat *in vitro*. *Nature* **189**: 840-841.
172. Brickman, A.S., Hartenbower, D.L., Norman, A.W. & Coburn, J.W. (1975) Action of 1,25 $(OH)_2$- and 1α(OH)vitamin D_3 on magnesium metabolism in man. *Clin. Res.* **23**: 315A.
173. Coburn, J.W., Massry, S.G. & Kleeman, C.R. (1970) The effect of calcium infusion on renal handling of magnesium with normal and reduced glomerular filtration rate. *Nephron* **7**: 131-143.
174. Hodgkinson, A., Marshall, D.H. & Nordin, B.E.C. (1979) Vitamin D and magnesium absorption in man. *Clin. Sci.* **57**: 121-123.
175. Anast, C.S. (1967) Magnesium studies in relation to vitamin D-resistant rickets. *Pediatrics* **40**: 425-435.
176. Eliel, L.P., Smith, W.O. & Thomson, C. (1960) Magnesium and calcium interrelationships. *J. Okla. State Med. Assoc.* **53**: 359-367.
177. McCance, R.A. (1947) Osteomalacia with Looser's notes (Milkman's syndrom) due to a raised resistance to vitamin D acquired about the age of 15 years. *Q. J. Med.* **16**: 33-46.
178. Jacob, M. & Forbes, R.M. (1970) The effect of vitamin D deficiency and the role of citrate in kidney calcification of magnesium deficient rats. *J. Nutr.* **100**: 228-234.
179. Meintzer, R.B. & Steenbock, H. (1955) Vitamin D and magnesium absorption. *J. Nutr.* **56**: 285-294.
180. Marsman, G., Pastoor, F.J.H., Mathott, J.N.J.J., Theuns, H.M. & Beynen, A.C. (1991) Vitamin D, within its range of fluctuation in commercial rat diets, does not influence nephrocalcinogenesis in female rats. *Lab. Anim.* **25**: 330-336.
181. Greger, J.L., Huffman, J., Abernathy, R.P., Bennett, O.A. & Resneck, S.E. (1979) Phosphorus and magnesium balance of adolescent females fed two levels of zinc. *J. Food Sci.* **44**: 1765-1766.
182. Johnson, M.A., Baier, M.J. & Greger, J.L. (1982) Effects of dietary tin on zinc, iron, copper, manganese and magnesium metabolism of adult males. *Am. J. Clin. Nutr.* **35**: 1332-1338.
183. Allen, V.G. & Robinson, D.L. (1980) Occurrence of Al and Mn in grass tetany cases and their effects on the solubility of Ca and Mg. *Agron. J.* **72**: 957-960.
184. Greger, J.L. & Baier, M.J. (1983) Effect of dietary aluminum on mineral metabolism of adult males. *Am. J. Clin. Nutr.* **38**: 411-419.
185. Yeh, J.K., Aloia, J.F., Semla, H.M. & Chen, S.Y. (1986) Influence of injected caffeine on the metabolism of calcium and the retention and excretion of sodium, potassium, phosphorus, magnesium, zinc and copper in rats. *J. Nutr.* **116**: 273-280.

Appendix table 1. *Effect of type and amount of dietary protein on magnesium (Mg) balance and tissue concentrations in rats and humans.*

Reference	Diet variables[1]	Experimental parameters	Observation[2]	Remarks
		Rat experiments		
Azami *et al.* (41)	- soybean protein vs casein - 20 % vs 40 % protein - dietary Mg: not given	- duration expt: 25 wk - balance study - young female rats - number of rats not given	- soybean protein raised Mg retention - high protein raised urinary Mg excretion and lowered Mg retention	- abstract - no data on Mg intake and Mg absorption
Brink *et al.* (42)	- soybean protein vs casein (18 %) - dietary Mg: 0.02, 0.04 and 0.06 %	- duration expt: 4 wk - balance study - Mg concentration of plasma, femur and kidney - young male rats - 7 per treatment	- soybean protein decreased Mg absorption (by 13 %) and urinary Mg excretion (by 40 %) - soybean protein decreased femur Mg (by 7 %) at low dietary Mg - no effect on kidney Mg plasma Mg and Mg retention	
Forbes (43)	- soybean protein vs egg protein (12 %) - dietary Mg: 0.04%	- duration expt: 4 wk - balance study - Mg concentration of femur and kidney - young male rats - 24 per treatment	- soybean protein decreased Mg absorption (by 11 %) - no effect on femur Mg and kidney Mg	- group differences in feed intake
Forbes *et al.* (44)	- soybean protein vs casein (20 %) - dietary Mg: from 0.006 to 0.07 %	- duration expt: not given - body wt gain, Mg concentration of serum and tibia - young male rats - 5 per treatment	- soybean protein did not affect the parameters measured	- group differences in dietary Mg concentration
Kubena *et al.* (45)	- soybean protein vs casein (20 %) - dietary Mg: 0.02 and 0.08 %	- duration expt: 17 wk - Mg concentration of serum, kidney, liver and carcass - young female rats - 17 per treatment	- soybean protein did not affect the parameters measured	
Lo *et al.* (46)	- soybean protein vs casein (12 %) - dietary Mg: 0.02 and 0.04 %	- duration expt: 4 wk - Mg concentration of serum and femur - young male rats - 5 per treatment	- soybean protein did not affect the parameters measured	

Appendix table 1 *(Continued).*

Reference	Diet variables[1]	Experimental parameters	Observation[2]	Remarks
Schwartz *et al.* (47)	- casein: 36 % vs 12 % - dietary Mg: 0.01, 0.05 and 0.1 %	- duration expt: 5 wk - body wt gain, Mg concentration of plasma, liver and carcass - young male rats - 6 per treatment	- high protein increased body wt gain (by 50 %), plasma Mg (by 28 %) and Mg in carcass (by 15 %) - liver Mg was not affected by amount of dietary protein	
Shahkhalili & Mettraux (48)	- soybean protein vs casein (25 %) - dietary Mg: 0.06 %	- duration expt: 2 wk or 6 wk - body wt gain, Mg concentration of femur - young male rats - 8 per treatment	- soybean protein increased Mg intake (by 9 %) and decreased femur Mg (by 6 %) - no effect on body wt gain	- group differences in Mg intake
Sterck *et al.* (49)	- casein (15 %) vs casein (15 %) plus ovalbumin (15 %) - dietary Mg: 0.01 and 0.04 %	- duration expt: 4 wk - balance study - Mg concentration of kidney - adult female rats - 6 or 8 per treatment	- high protein increased absorption (by 46 to 66 %) and urinary excretion (by 36 to 100 %) of Mg at high but not at low dietary Mg - no consistent effects on Mg retention - kidney Mg was decreased (by 17 %) at low but not at high dietary Mg	
Toothill (50)	- casein: 16 % and 8% - dietary Mg: 0.02 %	- duration expt: 3 wk - balance study - adult male rats - 2 or 4 per treatment	- high protein increased body wt gain (by 70%) and retention of Mg (by 38 %) - no effect on absorption and urinary excretion of Mg	- few animals per treatment
Van Camp *et al.* (51)	- casein (15 %) vs casein (15 %) plus ovalbumin (15 %) - dietary Mg: 0.04%	- duration expt: 4 wk - balance study - Mg concentration of kidney - young female rats - 6 per treatment	- high protein increased absorption (by 21 %) and urinary excretion of Mg (by 43 %) - no effect on Mg retention and* kidney Mg	
		Human experiments		
Alcantara & Linkswiler (52)	- mixed protein: 141 and 48 g/d[3,4] - Mg intake: 245, 490 and 735 mg/d	- cross-over: 6 x 15 d - balance study - Mg concentration in red blood cells (RBC) - adult males, n = 6	- high protein increased absorption (by 20 %) and urinary excretion (by 28 %) of Mg - no effect on Mg in RBC	- abstract

Fisler & Drenick (53)	- soybean protein vs collagen protein - 100 g/d - Mg intake: 106 and 23 mg/d	- duration expt: 40 d - balance study - Mg concentration in serum - obese adult males - 7 per treatment	- collagen increased urinary Mg excretion and decreased fecal excretion and retention of Mg - no effect on serum Mg concentration	- soybean protein increased Mg intake
Hunt & Schofield (54)	- mixed protein: 48, 30 and 20 g/d - Mg intake: ± 180 mg/d	- duration expt: 3 x 30 d; parallel - balance study - adult females - 4 or 5 per treatment	- high vs low protein increased absorption (by 140 %) and retention of Mg (-43 to +24 mg/d) - no effect on urinary Mg excretion	
Kitano *et al.* (55)	- mixed protein: 140 and 70 g/d[4] - Mg intake: 357 and 259 mg/d	- cross-over: 2 x 14 d - balance study - adult males, n = 6	- high protein increased Mg intake (by 38 %), Mg absorption (by 30 %) and retention (by -11 to +10 mg/d) - no effect on urinary Mg excretion	- group differences in Mg intake
Lakshaman *et al.* (56)	- mixed protein: high vs low - Mg intake: 235 and 320 mg/d	- self-selected diets - food records during 1 yr - balance study, every season - multiple regression - adult males (16) and females (18)	- high protein increased fecal and urinary Mg excretion - the effect depended on age and sex	- protein effect estimated with multiple regression
Mahalko *et al.* (57)	- mixed protein: 94 and 65 g/d - Mg intake: 250 mg/d	- cross-over: 2 x 28 d - balance study - adult males, n = 10	- no effect on intake, absorption, urinary excretion and retention of Mg	
McCance *et al.* (58)	- protein as amino acid mixtures: 145-200 vs 45-70 g/d - Mg intake: 517 mg/d	- cross-over: 2 x 14 d - balance study - adult males (4) and female (1)	- high protein increased absorption (by 27 %) and urinary excretion (by 18%) of Mg - no effect on retention	- few subjects
Sandström *et al.* (59)	- soybean protein vs meat protein - 60 g/d - Mg intake: 140-250 mg/d	- cross over: 4 x 2 d - Mg concentration in ileal contents - adult male and female ileostomy patients, n = 8	- replacement of animal protein by soybean protein did not affect Mg absorption	- no balance
Schwartz *et al.* (60)	- mixed protein: 93 and 43 g/d[4] - Mg intake: 294 or 980 mg/d	- cross-over: 2 x 30 d - balance study - adolescent boys, n = 12	- high protein increased absorption (by 24-20 %) and retention (by -58 vs +28 mg/d) of Mg at both dietary Mg intakes - no effect on urinary Mg excretion	- adolescent boys not in steady state - cross-over after 1 yr

Appendix table 1 *(Continued).*

References	Diet variables[1]	Experimental parameters	Observations[2]	Remarks
Shier (61)	- mixed protein: high vs low - vegetable vs animal protein	- self-selected diets - 7 d nutrient intake with dietary history - urinary Mg excretion - regression analysis - adult males, n = 5	- high protein increased Mg intake and urinary Mg excretion - vegetable protein increased Mg intake and urinary Mg excretion	- few subjects - effect based on regression - no clear aim of the study
Stephenson *et al.* (62)	- beef, casein-lactalbumin and peanut flour - 23.8 g/d[3] - Mg intake: 400 mg/d	- duration expt: 27 d - balance study - adult females - 3 per treatment	- casein-lactalbumin increased Mg absorption (data not given) and retention (by 32 %)	- abstract - few subjects per treatment
Van Dokkum *et al.* (63)	- mixed protein: 115 and 65 g/d[5] - vegetable vs animal (115 g/d) - Mg intake: 233, 300 and 370 mg/d	- cross-over: 3 x 20 d - balance study - adult males, n = 12	- high protein increased intake (by 29-59 %), fecal (by 58 %) and urinary (by 7%) Mg excretion - vegetable protein increased intake, fecal and urinary excretion of Mg - no effects on Mg absorption and retention	- high protein and vegetable protein increased Mg intake
Wisker *et al.* (64)	- mixed protein: 77 and 55 g/d[4] - Mg intake : 240 mg/d	- cross-over: 3 x 22 d - balance study - adult females, n = 12	- no effect	

[1] Diet composition in % represents weight/weight.

[2] If not stated otherwise, data refer to absolute Mg absorption. Increase or decrease in parameters is expressed as relative change when compared to the control.

[3] Conversion factor used for calculating protein intake from N intake: 6.25.

[4] Body weight used to convert intake/kg body wt/day to intake/day: 70 kg.

[5] Protein intake calculated from energy intake.

Appendix table 2. *Effect of type and amount of dietary carbohydrate on magnesium (Mg) balance and tissue concentrations in rats and humans.*

Reference	Diet variables[1]	Experimental parameters	Observation[2]	Remarks
		Rat experiments		
Andrieux & Sacquet (83)	- lactose vs glucose - lactose: 10 % vs 0 % - dietary Mg: not given	- duration expt: 16 wk - Mg concentrations in ileum, caecum, colon and faeces - adult rats - 4 per treatment	- lactose increased percentage Mg absorption from ileum (by 72 %), colon (by 30 %), caecum (by 28 %) and total Mg absorption (by 31 %)	- Mg intake not given
Behling & Greger (84)	- lactose vs sucrose and galactose/glucose - lactose: 8.5 and 18.5 % vs 0 % - dietary Mg: 0.05 %	- duration expt: 4 wk - balance study - Mg concentration of tibia - adult female rats - 6 per treatment	- lactose increased Mg absorption: 8.5 % lactose by 16 %; 18.5 % lactose by 40 %) - no effect on tibia Mg	
Bergstra *et al.* (85)	- fructose vs glucose - fructose: 70 vs 0 % - dietary Mg: 0.02 and 0.04 %	- duration expt: 4wk - balance study - Mg concentration of plasma and kidney - young female rats - 8 per treatment	- fructose increased absorption (by 6-34 %) and urinary Mg excretion (by 62-100 %) - no effect on Mg in plasma and kidney	- the effect of fructose depended on dietary Mg and P concentrations
Brink *et al.* (42)	- lactose vs glucose - lactose: 10 % - dietary Mg: 0.02 and 0.04 %	- duration expt: 4 wk - balance study - Mg concentration of plasma, femur and kidney - young male rats - 6 per treatment	- lactose increased absorption (by 8-11 %) and urinary Mg excretion (by 16-23 %) - no effect on Mg retention Mg in plasma, femur and kidney	
Brink *et al.* (86)	- milk vs lactase-treated milk - lactose: 12 vs 1 % - dietary Mg: 0.06 %	- duration expt: 4 wk - balance study - Mg concentration of plasma, femur and kidney - young male rats - 6 per treatment	- milk increased Mg absorption (by 18 %) and urinary excretion (by 36 %) - no effect on Mg retention and Mg in plasma, femur and kidney	
Forbes (87)	- lactose vs glucose - lactose: 25 vs 0 % - dietary Mg: 0.04 %	- duration expt: 6 wk - balance study - Mg concentration of femur - young male rats - 5 per treatment	- lactose increased percentage Mg absorption (by 8 %) and decreased Mg retention (by 50 %) - no effect on femur Mg	- urinary excretion not given

Appendix table 2 *(Continued).*

Reference	Diet variable[1]	Experimental parameters	Observation[2]	Remarks
Greger *et al.* (88)	- milk vs lactase-treated milk - lactose: not given - dietary Mg: 0.1 %	- duraton expt: 4 wk - balance study - Mg concentration of tibia and kidney - young male rats - 10 per treatment	- lactose increased percentage Mg absorption (by 14 %), urinary excretion (by 21 %) and tibia Mg (by 5 %) - no effect on Mg retention and kidney Mg	
Heijnen *et al.* (89)	- lactose and lactulose vs glucose - lactose (lactulose): 10 vs 0 % - dietary Mg: 0.04 %	- duration expt: 4 wk - balance study - Mg concentration of kidney and ileal contents - young female rats - 12 per treatment	- lactose and lactulose increased absorption (by 28 and 48 %) and retention of Mg (by 30 and 46 %) - no effect on kidney Mg - effect on solubility of Mg not clear	
Koh *et al.* (90)	- fructose and starch vs glucose (63 %) - dietary Mg: 0.002 and 0.06 %	- duration expt: 4 wk - Mg concentrations of plasma, kidney and liver - young male rats - number of rats not given	- type of carbohydrate did not affect Mg in kidney and liver - starch increased plasma Mg (by 10 %)	- no data on Mg absorption
Schaafsma *et al.* (91)	- milk vs lactase-treated milk - lactose: 19 vs 6 % - dietary Mg: 0.07 %	- duration expt: 6 wk - balance study - Mg concentration of femur - young male rats - 12 per treatment	- milk increased percentage Mg absorption (by 57 %) and urinary excretion (by 52 %) - no effect on femur Mg	- Mg intake was decreased in rats fed milk
Shahkhalili & Mettraux (48)	- lactose vs glucose - lactose: 25 vs 0 % - dietary Mg: 0.06 %	- duration expt: 2 and 6 wk - body wt gain and Mg concentration of femur - young male rats - 8 per treatment	- lactose increased femur Mg (by 2-6 %) - no effect on body wt gain	
		Human experiments		
Brink *et al.* (66)	- milk vs lactase-treated milk - lactose intake: 49 vs 3 g/d - Mg intake: 407 and 420 mg/d	- cross-over: 3 x 7 d - urinary Mg excretion - adult males and females, n = 24	- milk did not affect urinary Mg excretion	

Debognie *et al.* (92)	- milk vs lactase-treated milk - lactose: 10 vs 0 g - Mg intake: 31 mg	- cross-over: 2 x 5 h - ileal perfusion - adult lactose-tolerant males (4) and adult lactase-deficient females (4)	- milk increased Mg concentration in the ileum (by 71 %) in lactase-deficient subjects	
Holbrook *et al.* (93)	- fructose vs cornstarch - fructose: 148 g/d (20 energy %) - cornstarch: 131 g/d (20 energy %) - Mg intake: 455 and 393 mg/d	- cross-over: 2 x 7 wk - balance study - adult males, n = 24	- fructose increased intake (by 13 %), Mg absorption (by 140 %), urinary excretion (by 25 %) and retention (by -27 to 47 mg/d)	- Mg intake differed between diets
Kobayashi *et al.* (94)	- milk vs lactase-treated and lactose-free milk - lactose: not given - Mg intake: not given	- duration expt: 9 d - balance study - infants - 3 per treatment	- milk vs lactose-free milk increased percentage Mg absorption (by 65 %) - milk vs lactase-treated milk decreased percentage Mg absorption (by 16 %)	- Mg intake not given - few subjects per treatment
Wirth *et al.* (95)	- lactose vs glucose - lactose: 50 or 100 % - Mg intake: 25.5 mg/d and 34.5 mg/d	- duration expt: 14 d - balance study - preterm infants - 8 or 10 per treatment	- Mg intake (by 35 %) and absorption (by 50 %) were increased when fed 100 % lactose - urinary excretion increased (by 71 %) - no effect on Mg retention	- Mg intake was increased at high lactose diet
Ziegler & Fomon (96)	- lactose vs polycose/sucrose - lactose: 7 % (w/w) - Mg intake: 54-120 mg/d	- cross-over: 2 × 72 h - balance study - infants, n = 6	- lactose increased absorption (by 30 %) and urinary Mg excretion (by 30 %) - no effect on Mg retention	- diet composition depends on age - age varies from 27 to 424 d

[1] Diet composition in % represents weight/weight.

[2] If not stated otherwise, data refer to absolute Mg absorption. Increase or decrease in parameters is expressed as relative change when compared to the control.

Appendix table 3. *Effect of type and concentration of dietary fiber on magnesium (Mg) balance and tissue concentrations in rats and in humans.*

Reference	Diet variables[1]	Experimental parameters	Observation[2]	Remarks
		Rat experiments		
Bagheri & Gueguen (100)	- wheat bran vs starch and sucrose - 0, 5, 10 and 15 % - dietary Mg: 0.16 %	- duration expt: 6 wk - balance study - young male rats - 5 per treatment	- increased dietary bran decreased Mg absorption (by 50 %) and retention (by 81 %); urinary Mg excretion was increased (by 37 %)	
Ballam *et al.* (101)	- cellulose vs basal diet - fiber: 10.6 vs 5.8% - dietary Mg: 0.2 %	- duration expt: 3 wk - Mg concentration of serum, tibia and liver - young male rats - 4 per treatment	- no effect	- high dietary Mg - no balance study
De Jong *et al.* (102)	- cellulose, pectin, galacturonic acid vs glucose - 10% vs 0 % - dietary Mg: 0,04 %	- duration expt: 4 wk - balance study - Mg concentration of plasma and kidney - young female rats - 8 per treatment	- galacturonic acid increased Mg absorption (by 35 %) and urinary excretion (by 100 %) - cellulose and pectin: no effect on Mg absorption - no effects on Mg in plasma and kidney	
Mercurio & Behm (103)	- cellulose, pectin, soy fiber and wheat bran - 1, 5, 10 and 15 % - dietary Mg: not given	- duration expt: 5 wk - balance study - adult male rats - 6 per treatment	- Mg retention pectin < soy protein < cellulose < wheat bran - high fiber resulted in increased Mg retention (by ± 15 %)	- Mg intake not given - no data on Mg absorption
Shah *et al.* (104)	- oat bran, wheat bran, corn bran, wood cellulose - 14 vs 4 % - dietary Mg: 0.06, 0.11 and 0.21 %	- duration expt: 28 wk - balance study - young male and female rats - 20 per treatment	- Mg absorption was determined by dietary Mg; not by type or amount of dietary fiber	- Mg intake differed
		Human experiments		
Anderson *et al.* (105)	- wheat bran - 3.3, 10.9 and 18.7 g/d - Mg intake: 150-403 mmol/d	- cross-over: 3 x 24 d - balance study - adult males and females, n = 6	- wheat bran increased fecal and urinary Mg excretion due to increased Mg intake - no effect on Mg retention	- Mg intake was increased when fed high fiber

Behall *et al.* (106)	- cellulose, carboxy-methylcellulose, karaya gum, locust bean gum vs basal diet - supplement: 24 g/d - Mg intake: 410-545 mg/d	- cross-over: 5 x 4 wk - balance study - adult males, n = 11	- fiber increased fecal and urinary excretion of Mg due to increased Mg intake - type of fiber changed intake, absorption and urinary excretion of Mg, but not retention	- Mg intake was increased when fed high fiber
Drews *et al.* (107)	- cellulose, hemicellulose, pectin vs basal diet - supplement: 14 g/d - Mg intake: 414-425 mg/d	- cross-over: 4 x 4 d - balance study - Mg concentration of serum - adolescent males, n = 8	- hemicellulose and cellulose decreased Mg absorption (by 64 and 19 %) and retention (by 100 and 250 %) - no effect on urinary Mg excretion and serum Mg - pectine did not affect the parameters measured	
Hallfrisch *et al.* (108)	- high fiber (complex and simple carbohydrates) vs self-selected diet - Mg-intake: 180-375 mg/d	- duration expt: 2 x 12 wk - balance study - adult males and females, n = 52	- high fiber increased intake, absorption, and urinary excretion - no effect on retention	- Mg intake was increased when fed high fiber
Ismail-Beigi *et al.* (109)	- cellulose vs basal diet - supplement: 10 g/d - Mg intake: 248-385 mg/d	- duration expt: 2 x 20 d - balance study - adult males, n = 2	- high fiber increased intake, absorption and urinary excretion of Mg and decreased Mg retention	- Mg intake was increased when fed high fiber - few subjects
Kelsay *et al.* (8)	- high cellulose, hemicellulose and lignin vs low fiber - 23.8 vs 4.6 g/d - Mg intake: 322 and 356 mg/d	- cross-over: 2 x 26 d - balance study - adult males, n = 12	- high fiber decreased Mg absorption (by 34 %) and Mg retention (by 72 to 122 mg/d) - no effect on urinary Mg	
McHale *et al.* (110)	- cellulose and hemicellulose vs basal diet - supplement: 10 and 20 g/d - Mg intake: 330 mg/d	- cross-over: 3 x 6 d - balance study - Mg concentration of serum - adolescent males and females, n = 12	- cellulose (20 g/d) and hemicellulose (10 g/d) decreased Mg absorption (by 50 %) and serum Mg (by 2-5%)	
Reinhold *et al.* (111)	- white bread vs bazari - fiber: 14 vs 34 g/d - Mg intake: 315-635 mg/d	- duration expt: 2 x 14 d - balance study - adult males, n = 3	- bazari increased Mg intake, absorption and urinary excretion and decreased Mg retention	- few subjects - Mg intake was increased when fed high fiber
Reinhold *et al.* (112)	- white bread vs tanok - fiber: 2.6 vs 21.9 g/d - Mg intake: 348-724 mg/d	- duration expt: 2 x 20 d - balance study - adult males, n = 2	- high fiber increased Mg intake as well as fecal and urinary excretion; Mg retention decreased	- few subjects - Mg intake was increased when fed high fiber
Schweitzer *et al.* (113)	- soy fiber vs basal diet - fiber: 34 vs 14 g/d - Mg intake: 260 vs 200 mg/d	- duration expt: 2 x 3 wk - balance study - adult males and females, n = 6	- high fiber increased fecal Mg excretion due to increased intake	- Mg intake was increased when fed high fiber - Mg absorption was very low (6 mg/d)

Appendix table 3 *(Continued).*

Reference	Diet variables[1]	Experimental parameters	Observation[2]	Remarks
Slavin & Marlett (114)	- cellulose vs basal diet - supplement: 16 g/d - Mg intake: 270-300 mg/d	- duration expt: 2 x 30 d - balance study - adult females, n = 7	- high fiber increased fecal and urinary Mg excretion - no effect on Mg retention	- Mg intake was slightly increased when fed high fiber - Probably no effect on Mg absorption
Taper *et al.* (115)	- soy polysaccharide vs basal diet - supplement: 20, 30 or 40 g/d - Mg intake: 688-735 mg/d	- cross-over: 4 x 11 d - balance study - Mg concentration of serum - adult males, n = 22	- high fiber decreased Mg absorption (by 15 %) and retention (by 64 %) - no effect on urinary Mg excretion and Mg concentration in serum	
Van Dokkum *et al.* (116)	- white bread vs white bread + coarse bran - fiber: 9 vs 22 g/d - Mg intake: 213 and 373 mg/d	- duration expt: 2 x 20 d - balance study - Mg concentration serum - adult males, n = 12	- high fiber increased Mg intake as well as fecal and urinary Mg excretion - no effect on Mg concentration of serum	- Mg intake was increased when fed high fiber

[1] Diet composition in % represents weight/weight.

[2] If not stated otherwise, data refer to absolute Mg absorption. Increase or decrease in parameters is expressed as relative change when compared to the control.

Appendix table 4. *Effect of dietary phytate on magnesium (Mg) balance and tissue concentration in rats and in humans.*

Reference	Diet variables[1]	Experimental parameters	Observation[2]	Remarks
		Rat experiments		
Brink *et al.* (42)	- sodium phytate - phytate: 0.25% - dietary Mg: 0.02 and 0.04 %	- duration expt: 4 wk - balance study - Mg concentration of plasma, kidney and femur - young male rats - 6 per treatment	- phytate decreased Mg absorption (by 15 %), urinary excretion (by 22-30 %) and at low Mg intake femur Mg (by 8 %) - no effect on Mg retention and Mg in plasma and kidney	
Churella & Vivian (123)	- soy protein vs dephytinized soy protein - phytate: 0.33 vs 0.04 % - dietary Mg: 0.04 %	- duration expt: 3 wk - Mg concentration of femur and carcass - young male rats - 13 per treatment	- no effect	- no balance study
Miyazawa & Yoshida (124)	- sodium phytate - phytate: 0.08-0.26 % as P - dietary Mg: not given	- duration expt: 4 wk - balance study - young male rats - 5 or 6 per treatment	- positive correlation between fecal phytate and Mg excretion ($r=0.702$)	
Roberts & Yudkin (125)	- sodium phytate - supplement: 0, 1 and 10 % (w/w) - dietary Mg: not given (low) and supplement of 0.04 %	- duration expt: 8 wk - body wt gain - young rats - number of rats not given	- phytate decreased body wt gain; addition of Mg prevented this decrease	- no balance study
Shinoda & Yoshida (122)	- sodium phytate - supplement: 1 % (w/w) - dietary Mg: 0.04 %	- duration expt: 2 wk - solubility of Mg in gut - young male rats - 3 per treatment	- phytate decreased Mg solubility in gut: by 18 % in ileum, by 13 % in caecum and by 40 % in colon	
		Human experiments		
McCance & Widdowson (126)	- brown bread vs dephytinized brown bread - phytate: 1.3 vs 0.09 g/d - Mg intake: 750 mg/d	- duration expt: 2 x 3 wk - balance study - adult males and females, n = 6	- brown bread decreased absorption (by 30 %)	

Appendix table 4 *(Continued).*

Reference	Diet variables[1]	Experimental parameters	Observations[2]	Remarks
Morris *et al.* (65)	- whole wheat bran vs dephytinized wheat bran - phytate: 2.1 vs 0.12 g/d - Mg intake: 550 mg/d	- cross-over: 2 x 15 d - balance study - adult males, n = 10	- whole wheat bran decreased absorption (by 25 %) and urinary excretion (by 26 %) - Mg retention was not affected	
Morris *et al.*, personal communication	- sodium phytate - supplement: 0.7, 2.4 or 4.1 g/d - Mg intake: 250 mg/d	- cross-over: 3 x 15 d - balance study - adult males, n = 10	- phytate decreased Mg absorption (by 44 %) and urinary Mg excretion (by 40 %)	

[1] Diet composition in % represents weight/weight.

[2] If not stated otherwise, data refer to absolute Mg absorption. Increase or decrease in parameters is expressed as relative change when compared to the control.

Appendix table 5. *Effect of dietary calcium and phosphate on magnesium (Mg) balance and tissue concentrations in rats and in humans.*

Reference	Diet variables[1]	Experimental parameters	Observation[2]	Remarks
		Rat experiments		
Alcock & MacIntyre (140)	- Ca: 0.006 vs 0.6 % - P: 0.31 % - Mg: 0.04 %	- duration expt: 2 and 3 wk - balance study - young male and female rats - number of rats not given	- Ca decreased Mg absorption (by 40 %)	
Al-Jurf & Chapman-Furr (141)	- Ca: 0, 37 and 90 mg/d - P: 46 mg/d - Mg: 2 mg/d	- duration expt: 7 d - balance study - Mg concentration of plasma, femur and muscle - adult male rats - 6 or 9 per treatment	- Ca decreased Mg concentration in plasma, femur and muscle - no effect on fecal Mg excretion	- parenteral feeding
Behling & Greger (142)	- Ca: 0.4 and 0.8 % - P: 0.6-1.2 % - Mg: 0.04-0.6 %	- duration expt: 4 wk - balance study - Mg concentration of tibia and kidney - adult female rats - 6 per treatment	- Ca decreased percentage Mg absorption (by 11-30%) - no effect on tibia and kidney Mg	- differences in Mg intake - increased dietary Ca often accompanied by increased P
Behling *et al.* (78)	- Ca: 0.25 and 1 % - P: 0.57-0.67 % - Mg: 0.05 and 0.06 %	- duration expt: 7 wk - balance study - young male rats - 20 per treatment	- Ca decreased percentage Mg absorption (by 30 %)	- no data on urinary excretion and retention
Brink *et al.* (143)	- Ca: 0.1, 0.4 and 0.7 % - P: 0.13, 0.32 and 0.51 % - Mg: 0.04 %	- duration expt: 4 wk - balance study - adult male rats - 8 per treatment	- Ca decreased Mg absorption (by 24 %) and urinary Mg excretion (by 24 %) at high but not at low dietary P	
Bunce *et al.* (144)	- Ca: 0.65 % - P: 0.3, 0.5 and 1.0 % - Mg: 0.013, 0.026 and 0.1 %	- duration expt: 4 wk - balance study - young male rats - 12 per treatment	- P decreased Mg absorption (by 21 %) and urinary Mg excretion (by 60 %) - no effects on Mg retention	- no consistent effects due to variable Mg intakes
Ericsson *et al.* (145)	- Ca: 0.12 and 0.32 % - P: 0.82 % - Mg: 0.01 %	- duration expt: 8 wk - Mg concentration of plasma, femur, kidney, heart - young male rats - 15 per treatment	- Ca decreased Mg in plasma (by 60 %), femur (by 65 %) and kidney (by 10 %)	- no balance study - very low dietary Mg

Appendix table 5 *(Continued).*

Reference	Diet variables[1]	Experimental parameters	Observation[2]	Remarks
Forbes (146)	- Ca: 0.4 and 0.8 % - P: 0.19 and 0.5 % - Mg: 0.01 and 0.04 %	- duration expt: 4 wk - balance study - Mg concentration of femur - young male rats - 5 per treatment	- Ca decreased percentage Mg absorption (by 45 %) and retention (by 35 %) - no effects on femur Mg concentration	
Hardwick *et al.* (147)	- Ca: 0.6, 1.0, 1.4 and 2.2 % - P: 0.6, 1.0, 1.5 and 2.2 % - Mg: 0.06 %	- duration expt: 12 wk - balance study - Mg concentration of femur - young female rats - 5 or 6 per treatment	- increase of Ca and P (as CaP_i) decreased Mg absorption (by 75 %), urinary excretion (by 45 %), retention (by 85 %) and femur Mg (by 10 %)	- no independent effects of Ca and P
Hoek *et al.* (148)	- Ca: 0.13, 0.25, 0.50 and 0.75 % - P: 0.25, 0.40 and 0.50 % - Mg: 0.03 %	- duration expt: 4 wk - balance study - young female rats - 6 per treatment	- Ca decreased Mg absorption (by 28 %) and urinary Mg excretion (by 40 %) at constant P - no consistent effect of dietary P	
Kaup *et al.* (79)	- Ca: 0.25 and 1 % - P: not given - Mg: not given	- duration expt: 8 months - balance study - young rats - 20 per treatment	- Ca decreased Mg absorption (by 9-16 %)	- Mg intake not given
Mars *et al.* (149)	- Ca: 0.5 % - P: 0.2, 0.4, 0.6 and 0.8 % - Mg: 0.02 and 0.04 %	- duration expt: 4 wk - balance study - Mg concentration of kidney - young female rats - 6 per treatment	- P decreased Mg absorption (by 14 %) and urinary Mg excretion (by 40-50 %) at high but not at low Mg intake; kidney Mg was increased (by 67 %) - no clear effects on Mg retention	- results not consistent among experiments
O'Dell *et al.* (150)	- Ca: 0.9 and 1.7 % - P: 0.4, 0.8 and 1.7 % - Mg: 0.005 and 0.3 %	- duration expt: 4 wk - body wt gain - young male rats - 4 per treatment	- P decreased body wt gain (by 79 %) at low but not at high dietary Mg	- no balance study - high dietary mineral concentration

Parker (151)	- Ca: not given - P: 0.16, 0.26 and 0.55 % - Mg: 0.007 and 0.06 %	- duration expt: 2 wk - Mg concentration of plasma, red blood cells femur, kidney, liver, muscle, heart, testes - young male rats - 6 per treatment	- P increased Mg in red blood cells (by 19 %), femur (by 20 %), kidney (by 3%) - no effects on other parameters	- no data on Mg intake and absorption
Ritskes-Hoitinga *et al.* (152)	- Ca: 0.50 % - P: 0.40 and 0.60 % - Mg: 0.04 %	- duration expt: 4 wk - balance study - Mg concentration of kidney - young female rats - 16 per treatment	- P increased kidney Mg (by ± 100 %) - no effect on balance	
Sawamura & Goto (153)	- Ca: 0.5 and 2.0 % - P: 0.54 and 0.2 % - Mg: not given	- duration expt: 4 wk - balance study - Mg concentration of femur and kidney - young and adult male rats - number of rats not given	- Ca decreased Mg absorption and retention - P decreased Mg absorption and retention - high Ca/high P decreased Mg in femur and kidney	- abstract - no data on Mg intake
Schaafsma *et al.* (154)	- Ca: 0.3 and 1.2 % - P: 0.15 and 1.2 % - Mg: 0.05 %	- duration expt: 4 wk - balance study - Mg concentration of plasma and femur - adult male rats - 7 or 8 per treatment	- Ca decreased percentage Mg absorption (by 50 %), urinary excretion (by 64 %), plasma Mg (by 30 %) and femur Mg (by 8 %) at high but not at low dietary P	
Toothill (50)	- Ca: 0.34-0.68 % - P: 0.39-0.79 % - Mg: 0.02 %	- duration expt: 3 wk - balance study - young and adult male rats - 4 per treatment	- Ca decreased Mg absorption (by 27% at low P and by 48 % at high P) - P decreased Mg absorption (by 32% at low Ca and by 43% at high Ca)	
Wienk *et al.* (155)	- Ca: 0.25 and 0.5 % - P: 0.4 % - Mg: 0.02 and 0.04 %	- duration expt: 4 wk - balance study - young female rats - 6 per treatment	- Ca decreased Mg absorption (by 17-40 %) and urinary excretion (by 50-60 %) - no effect on Mg retention	
		Human experiments		
Clarkson *et al.* (156)	- Ca: 767, 2767 and 8767 mg/d - P: not given - Mg: 146-292 mg/d	- cross-over: 2 x 8 to 30 d - balance study - adult males and females - 3 normal subjects and 8 patients	- no effect	- patients with renal failure

Appendix table 5 *(Continued).*

Reference	Diet variables[1]	Experimental parameters	Observations[2]	Remarks
Giles *et al.* (133)	- Ca: 128, 227 and 350 mg/d - P: 90, 144 and 180 mg/d - Mg: 15 and 30 mg/d	- duration expt: 47 d - balance study - infants - 7 to 11 per treatment	- Ca decreased Mg absorption (by 60 %) and retention (by 69 %) - P decreased Mg absorption (by 21 %) and retention (by 80 %)	- P:Mg ratio as in rat diets
Heaton *et al.* (157)	- Ca: 1490 and 3771 mg/d - P: 833-5423 mg/d - Mg: 260 mg/d	- duration expt: 11 d - balance study - adult patients - 1 to 8 per treatment	- Ca decreased Mg absorption and urinary excretion (1 patient) - P decreased Mg absorption (by 40 %) and urinary Mg excretion (by 32 %)	- patients with disordered Ca metabolism - data on Ca effect derived from only 1 patient
Kim & Linkswiler (158)	- Ca: 800 and 2400 mg/d - P: 900 and 2400 mg/d - Mg: not given	- duration expt: 60 d - balance study - adult males - number of subjects not given	- Ca decreased Mg absorption and retention; no effect on urinary excretion - P decreased Mg absorption and urinary excretion no effect on retention - Ca + P decreased Mg absorption and urinary excretion	- abstract - Mg intake not given
Leichsenring *et al.* (159)	- Ca: 300 and 1500 mg/d - P: 800 and 1400 mg/d - Mg: 260 mg/d	- duration expt: 2 x 4 wk - balance study - adult females - 3 per treatment	- Ca and P increased urinary Mg excretion (by 30 %) - no effect on Mg absorption and retention	- few subjects per treatment
Lewis *et al.* (160)	- Ca: 697 and 1597 mg/d - P: 1240 mg/d - Mg: 300 mg/d	- cross-over: 4 x 14 d - balance study - adult males, n = 8	- no effect on Mg absorption; Ca (as $CaCl_2$) increased urinary Mg excretion (by 9 %)	
Moya & Donemech (161)	- Ca: 154, 198 and 345 mg/d - P: 88 mg/d - Mg: 22 mg/d	- duration expt: 3 d - balance study - infants - 1 to 10 per treatment	- no effect	
Norman *et al.* (40)	- Ca: 300 and 2000 mg/d - P: not given - Mg: 200 mg/d	- cross-over: 2 x 4 wk - intestinal perfusion study - Mg concentration of plasma - adult males and females, n = 13	- Ca decreased Mg absorption from ileum (by 9 %), not from jejunum; - no effect on Mg in plasma	- no balance study

Spencer (162)	- Ca: 200 and 2000 mg/d - P: 800 and 2000 mg/d - Mg: 250 mg/d	- duration expt: not given - balance study - ^{28}Mg experiments - adult males - number of subjects not given	- no effect	- abstract
Storey *et al.* (163)	- Ca: 900 and 1900 mg/d - P: not given - Mg: 260 mg/d	- duration expt: 14 d - urinary Mg excretion - adult females - 216 per treatment	- no effect	
Van der Meer *et al.* (164)	- Ca: 1400 and 2400 mg/d - P: 1860 mg/d - Mg: 413 mg/d	- cross-over 2 x 1 wk - balance study - adult males, n = 12	- no effect	

[1] Diet composition in % represents weight/weight.

[2] If not stated otherwise, data refer to absolute Mg absorption. Increase or decrease in parameters is expressed as relative change when compared to the control.

Appendix table 6. *Effect of dietary sodium and potassium on magnesium (Mg) balance and tissue concentrations in rats.*

Reference	Diet variables[1]	Experimental parameters	Observation[2]	Remarks
Charlton & Armstrong (166)	- dietary Na: 0.1-0.3 % - dietary K: 0.1 and 0.9 % - dietary Mg: 0.05 %	- duration expt: 6 wk - balance study - Mg concentration of plasma, heart, liver, muscle and femur - young male rats - 5 per treatment	- no linear effects of Na - increasing Na increased and then decreased urinary Mg	
Greene *et al.* (167)	- dietary Na: 0.3 and 0.6 % - dietary K: 0.4 and 1.8 % - dietary Mg: 0.02 and 0.1%	- duration expt: 3 wk - balance study - young male rats - 12 per treatment	- K was associated with increased intake (by 21 %), absorption (by 40 %), urinary excretion (by 88 %) and retention (by 39 %) of Mg - Na: no effect	- differences in Mg intake
Greger *et al.* (71)	- dietary Na: 0.14 and 1 % - dietary K: 0.32 and 1.88 % - dietary Mg: not given	- duration expt: 8 wk - balance study - Mg concentration of serum, tibia and kidney - young male rats - 8 per treatment	- no effect of Na and K; anions were responsible for differences in urinary excretion	
Schricker (168)	- dietary K: 0.1, 0.4 and 1.2 % - dietary Mg: not given	- duration expt: 3 wk - balance study - Mg concentration of plasma, tibia, heart and kidney - young male rats - 6 per treatment	- no effect	- no data on Mg intake
Shortt & Flynn (169)	- dietary Na: 3.1 % as NaCl - dietary Mg: not given	- duration expt: 2 wk - balance study - Mg concentration of serum and tibia - young male rats - 8 per treatment	- high NaCl increased urinary excretion (by 30-62 %) and decreased body weight gain (by 13 %) and tibia Mg (by 7 %)	- no data on Mg intake and absorption - effect of Na independent of Cl cannot be estimated
Wienk *et al.* (155)	- dietary K : 0.4 and 0.8 % - dietary Mg: 0.02 and 0.04 %	- duration expt: 4 wk - balance study - young female rats - 6 per treatment	- no effect	

[1] Diet composition in % represents weitht/weight.
[2] If not stated otherwise, data refer to absolute Mg absorption. Increase or decrease in parameters is expressed as relative change when compared to the control.

Chapter 3

Inhibitory effect of dietary soybean protein versus casein on magnesium absorption in rats

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Abstract. The effects of casein and soybean protein on magnesium absorption and magnesium concentration in femur were investigated in rats. Purified diets containing either casein or soybean protein and three concentrations of added magnesium (0.82, 1.64 or 2.46 mmol/100 g diet) were used. The isonitrogenous diets were carefully balanced for the different mineral concentrations in the protein preparations. Absolute and percentage magnesium absorption and urinary magnesium excretion were significantly decreased in rats fed soybean protein when compared with casein, irrespective of the dietary concentration of added magnesium. The magnesium content of femur was significantly lower in rats fed soybean protein, but this effect was seen only when the diet contained 0.82 mmol magnesium/100 g diet. The addition of sodium phytate to the casein diets, to a concentration identical to that in the diets containing soybean protein as provided by the soybean protein preparation, produced similar effects on magnesium absorption as did the diets containing soybean protein. These results indicate that soybean protein, when compared with casein, decreases magnesium absorption through its phytate component.

Introduction

There is an increasing use of soybean products as substitutes for animal protein in the diets of adults and in infant foods. The impact of this diet change on magnesium absorption is not known. Magnesium absorption from a diet containing milk might be superior to that from a diet containing soybean products. Phytate, which is present in substantial amounts in soybean products, has been reported to suppress the absorption of magnesium (1), while the lactose component of milk improves it (2). The effects of the protein source of milk versus that of soybean products on magnesium absorption are not clear. Magnesium bioavailability, assessed as the amount of magnesium in plasma and bone, was not differently influenced by dietary casein and soybean isolate (3, 4). In these studies however, magnesium intake and absorption by rats fed the different experimental diets were not reported. Thus, it cannot be excluded that differential magnesium intakes had masked protein effects on efficiency of magnesium absorption.

The lack of conclusive information about the effects of dietary casein and soybean protein on magnesium absorption prompted us to perform two experiments with rats. Apparent magnesium absorption and magnesium concentrations of plasma, femur and kidneys were measured. In the first experiment, the effects of dietary casein and soybean protein were studied at different dietary concentrations of added magnesium. The second experiment was conducted in an attempt to determine whether the decreased apparent magnesium absorption resulting from soybean protein was related to its phytate component.

Materials and Methods

General procedures. The experimental protocols were approved by the animal ethical committee of the Agricultural University, Wageningen, The Netherlands. Young, male

Wistar rats (Cpb:WU) from a local breeding colony (Agricultural University, Wageningen, The Netherlands), were housed individually in metabolic cages in a room with controlled temperature (20-24°C), relative humidity (40-70%) and lighting (light, 06.00-18.00 h). For four wk, the animals were fed the experimental diets *ad libitum* with demineralized water freely available. Feed consumption was recorded daily and body weights were measured weekly. During the last four d of the experimental period, feces and urine of each animal were collected separately. In this period the diets were offered as a porridge (diet : demineralized water = 7 : 3, w/w) to prevent feed spillage and contamination of excreta. Feed intake data were corrected for the addition of water. At the end of the experimental period, between 08.30 and 12.30 h, fasting blood samples were drawn under light diethyl ether anesthesia by orbital puncture, which was immediately followed by decapitation. Femur and kidneys were removed.

Diets, feces and kidneys were freeze-dried and homogenized. Diets and feces were wet ashed with nitric acid and perchloric acid. Femora were cleaned of adhering tissue, defatted in diethyl ether for 50 h, dried for 16 h at 105°C, and weighed. Kidneys and fat free femora were dry ashed in a muffle furnace for 16 h at 550°C. The ash was dissolved in 5 mL of 4 mol/L HCl and diluted with distilled water. After appropriate dilution with a solution containing 48 mmol/L $SrCl_2$ and 3 mmol/L CsCl, magnesium was analyzed by atomic absorption spectrophotometry (Perkin Elmer 1100, Bodenseewerk Perkin Elmer & Co GmbH, Uberlingen, Germany). Samples of the diets were also analyzed for calcium, zinc, copper and iron by atomic absorption spectrophotometry, for sodium and potassium by atomic emission spectrophotometry and for phosphorus by the Fiske Subbarow method (5). Nitrogen was determined by the micro-Kjeldahl method (6) and lactose and dextrose by high performance liquid chromatography (7). The phytate content of the diets was analyzed as described by Slump *et al.* (8).

Apparent intestinal absorption, urinary excretion and retention of magnesium as well as magnesium content in plasma, femur and kidneys were determined. Apparent intestinal absorption was calculated as magnesium intake minus fecal excretion, and was expressed as such and as percentage of intake. Magnesium retention was calculated as apparent absorption minus urinary excretion of magnesium.

Experiment 1. This experiment was designed to investigate the effects of casein and soybean protein at different dietary concentrations of added magnesium. It was hypothesized that there might be an interaction of protein source and magnesium content of the diet on magnesium metabolism. When using diets containing either casein or soybean protein, but with different concentrations of added magnesium, the risk of not detecting an effect of the type of protein would be minimized.

Forty-two rats, aged six wk, with mean body weight of 136 g, were randomly divided into six groups of seven and were fed diets containing either casein or soybean protein and three concentrations of added magnesium. Dietary concentrations of added magnesium were 0.82, 1.64 or 2.46 mmol/100 g diet. The composition of the diets is shown in Table 1. The

Table 1. *Composition of the diets used in Experiment 1.*

	Total magnesium content (mmol/100 g)					
	Casein			Soybean protein		
	0.82	1.64	2.46	0.82	1.64	2.46
	g/100 g					
Ingredient						
Casein	18	18	18	-	-	-
Soybean protein	-	-	-	20	20	20
Dextrose	40.67	40.46	40.16	39.47	39.26	39.08
$MgSO_4.7H_2O$	0.16	0.37	0.67	0.08	0.29	0.47
$CaCO_3$	0.07	0.07	0.07	-	-	-
KCl	0.28	0.28	0.28	-	-	-
NaCl	0.15	0.15	0.15	-	-	-
$C_6H_5Na_3O_7.2H_2O$	0.22	0.22	0.22	-	-	-
Constant components[1]	40.45	40.45	40.45	40.45	40.45	40.45
	mmol/100 g					
Chemical analysis						
Nitrogen	184	184	185	196	199	197
Magnesium	1.03	1.77	3.01	0.91	1.80	2.67
Calcium	14.5	14.3	14.3	14.8	14.3	14.4
Phosphorus	10.0	10.1	10.1	10.3	10.0	10.1
Sodium	9.6	9.6	9.7	11.5	11.3	11.3
Potassium	12.6	12.8	12.7	12.8	12.6	12.6
Zinc	0.18	0.19	0.19	0.19	0.19	0.20
Copper	0.05	0.05	0.05	0.05	0.05	0.05
Iron	0.09	0.08	0.08	0.13	0.13	0.11

[1] The constant components consisted of (g/100 g diet): DL-methionine, 0.3; wheat starch, 15; cellulose, 5; palm oil, 15; mineral premix, 3.5; vitamin premix, 1.0; retinyl acetate, 0.025 (400 IU); cholecalciferol, 0.025 (100 IU); choline chloride, 0.2. The mineral premix consisted of the following (mg): $CaCO_3$, 1000; $CaHPO_4.2H_2O$, 333; KH_2PO_4, 443; KCl, 441; $C_6H_5Na_3O_7.2H_2O$, 432; $FeSO_4.7H_2O$, 21; $MnSO_4.H_2O$, 24; $ZnSO_4.7H_2O$, 48; $CuSO_4.5H_2O$, 11; KIO_3, 0.04; $Na_2SeO_3.5H_2O$, 0.04; $CrK(SO_4)_2.12H_2O$, 2; dextrose, 744.92. The vitamin premix consisted of the following (mg): thiamin, 0.6; riboflavin, 0.6; pyridoxin, 0.7; nicotinamid, 3; DL-calcium pantothenate, 1.6; folic acid, 0.2; D-biotin, 0.02; cyanocobalamin, 0.001; DL-α-tocopherylacetate, 10; menadione, 0.005; dextrose, 983.3.

analyzed composition of the casein (acid casein, DMV, Veghel, The Netherlands) and soybean protein (soy isolate, Purina protein, Hofhuis Pentacon, Bunschoten, The Netherlands), respectively, was as follows (g/100 g): nitrogen, 14.3 and 13.8, magnesium,

Table 2. *Composition of the diets used in Experiment 2.*

	Total magnesium content (mmol/100 g)							
	Casein		Soybean protein		Casein + phytate		Casein + lactose	
	0.82	1.64	0.82	1.64	0.82	1.64	0.82	1.64
	g/100 g							
Ingredient								
Casein	18	18	-	-	18	18	18	18
Soybean protein	-	-	20	20	-	-	-	-
Dextrose	40.20	39.99	38.89	38.68	40.44	40.23	30.20	29.99
Lactose	-	-	-	-	-	-	10	10
Sodium phytate	-	-	-	-	0.42	0.42	-	-
$MgSO_4.7H_2O$	0.16	0.37	0.08	0.29	0.16	0.37	0.16	0.37
$CaCO_3$	0.13	0.13	-	-	0.13	0.13	0.13	0.13
KH_2PO_4	0.20	0.20	0.20	0.20	-	-	0.20	0.20
KCl	0.29	0.29	-	-	0.40	0.40	0.29	0.29
NaCl	0.19	0.19	-	-	-	-	0.19	0.19
$C_6H_5Na_3O_7.2H_2O$	0.38	0.38	0.38	0.38	-	-	0.38	0.38
Constant components[1]	40.45	40.45	40.45	40.45	40.45	40.45	40.45	40.45
	mmol/100 g							
Chemical analysis								
Nitrogen	180	186	195	199	181	176	183	187
Lactose	0	0	0	0	0	0	29	29
Phytate	0.00	0.00	0.40	0.37	0.38	0.38	0.00	0.00
Magnesium	0.82	1.56	0.82	1.69	0.82	1.64	0.82	1.64
Calcium	14.8	14.5	14.3	14.0	14.8	14.7	13.9	14.1
Phosphorus	11.0	11.3	11.9	11.9	12.2	12.3	11.3	11.4
Sodium	12.6	12.2	13.0	12.2	14.3	14.2	13.5	13.5
Potassium	15.4	15.1	15.6	15.0	15.2	15.5	15.9	15.4
Zinc	0.20	0.19	0.21	0.20	0.19	0.20	0.21	0.20
Copper	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Iron	0.09	0.08	0.13	0.13	0.09	0.09	0.08	0.08

[1] The constant components consisted of (g/100 g diet): DL-methionine, 0.3; wheat starch, 15; cellulose, 5; palm oil, 15; mineral premix, 3.5; vitamin premix, 1.0; retinyl acetate, 0.025 (400 IU); cholecalciferol, 0.025 (100 IU); choline chloride, 0.2. The mineral premix consisted of the following (mg): $CaCO_3$, 1000; $CaHPO_4.2H_2O$, 333; KH_2PO_4, 443; KCl, 441; $C_6H_5Na_3O_7.2H_2O$, 432; $FeSO_4.7H_2O$, 21; $MnSO_4.H_2O$, 24; $ZnSO_4.7H_2O$, 48; $CuSO_4.5H_2O$, 11; KIO_3, 0.04; $Na_2SeO_3.5H_2O$, 0.04; $CrK(SO_4)_2.12H_2O$, 2; dextrose, 744.92. The vitamin premix consisted of the following (mg): thiamin, 0.6; riboflavin, 0.6; pyridoxin, 0.7; nicotinamid, 3; DL-calcium pantothenate, 1.6; folic acid, 0.2; D-biotin, 0.02; cyanocobalamin, 0.001; DL-α-tocopherylacetate, 10; menadione, 0.005; dextrose, 983.3.

0.005 and 0.073, calcium, 0.056 and 0.384, phosphorus, 0.682 and 0.688, sodium, 0000 and 0.672, potassium, 0.072 and 0.579. As indicated in Table 1, the diets were balanced for the differential composition of the protein preparations. Analyzed concentrations of magnesium were on average 14% higher than the calculated values. Although we did not balance for zinc, iron and copper, only the iron concentrations appeared to be somewhat higher in the soybean protein than in the casein diets.

Experiment 2. This experiment was designed to elucidate whether the protein effect on magnesium absorption as found in experiment 1, was caused by the protein itself or by phytate in the soybean protein. For this purpose, casein diets without or with added sodium phytate were compared. To check the reproducibility of experiment 1, diets containing soybean protein were included. As a positive control, the effect of lactose versus dextrose was studied. Lactose has been shown to increase magnesium absorption (2, 9, 10). All dietary comparisons were studied at two concentrations of added magnesium.

Forty-eight rats, aged four wk, with mean body weight of 109 g, were randomly divided into eight groups of six and were fed the diets given in Table 2. The soybean protein preparation was found to contain 1.93 mmol phytate/100 g, resulting in a dietary concentration of 0.39 mmol/100 g in the soybean protein based diets. Thus, the effect of added phytate in a casein diet was studied at a dietary concentration of 0.39 mmol/100 g to simulate the amount in the diet containing soybean protein. Lactose was added to the casein diet at a concentration of 29 mmol/100 g at the expense of dextrose. Calculated concentrations of added magnesium were either 0.82 or 1.64 mmol/100 g; these concentrations agreed well with those analyzed. The diets were balanced for the differential calcium, magnesium, sodium and potassium concentrations in the protein preparations.

Statistics. Analysis of variance was applied to test the significance of main and interaction effects. If F-values reached statistical significance, group means were compared with Tukey's test (11) or Student's t-test. The level of significance was preset at $P < 0.05$.

Results

Body weight gain and feed intake. In experiments 1 and 2, body weight gain and feed consumption did not differ significantly between the dietary groups (results not shown). In experiment 1, body weight gain and feed intake were averaged 140 $\pm$ 8 and 480 $\pm$ 11 g/28 days (mean $\pm$ SEM). In experiment 2, these values were 129 $\pm$ 7 and 368 $\pm$ 10 g/28 days.

Dietary magnesium and magnesium balance. Tables 3 and 4 show that increasing magnesium concentrations in the diet caused an increase of absolute intestinal magnesium absorption and lowered the relative efficiency of this process. Increased intakes of magnesium produced increased rates of urinary magnesium excretion and increased magnesium contents of femur. Increased magnesium intake caused increased group mean

Tabel 3. *Effect on magnesium metabolism of dietary casein vs. soybean protein at different concentrations of added magnesium, Experiment 1*[1].

	Total magnesium content (mmol/100 g)						
	Casein			Soybean protein			Significant effects[2]
	0.82	1.64	2.46	0.82	1.64	2.46	
Mg absorption, μmol/d	134 ± 3^{a}	212 ± 6^{b}	332 ± 11^{c}	$97\pm3^{A,*}$	$188\pm6^{B,*}$	$265\pm11^{C,*}$	Mg, P, MgxP
Mg absorption, % of intake	70 ± 1^{b}	66 ± 2^{ab}	60 ± 2^{a}	$63\pm2^{B,*}$	$58\pm2^{AB,*}$	$55\pm2^{A,*}$	Mg, P
Fecal Mg, μmol/d	59 ± 3^{a}	111 ± 10^{b}	217 ± 8^{c}	58 ± 4^{A}	137 ± 8^{B}	215 ± 6^{C}	Mg, P
Urinary Mg, μmol/d	58 ± 6^{a}	133 ± 4^{b}	228 ± 6^{c}	$29\pm4^{A,*}$	$92\pm4^{B,*}$	$136\pm8^{C,*}$	Mg, P, MgxP
Mg retention, μmol/d	76 ± 3^{a}	79 ± 3^{ab}	104 ± 12^{b}	68 ± 2^{A}	96 ± 7^{B}	129 ± 8^{C}	Mg
Plasma Mg, mmol/L	0.76 ± 0.04^{a}	0.82 ± 0.03^{ab}	0.90 ± 0.06^{b}	0.67 ± 0.04^{A}	0.81 ± 0.04^{B}	0.87 ± 0.06^{B}	Mg
Femur Mg, μmol/g ash	285 ± 6^{a}	303 ± 3^{b}	314 ± 3^{c}	$256\pm6^{A,*}$	295 ± 5^{B}	313 ± 3^{C}	Mg, P, MgxP
Kidney Mg, μmol/g dry matter	33 ± 1	33 ± 1	35 ± 1	34 ± 1	33 ± 2	34 ± 1	

[1] Values are means ± SEM for seven rats per dietary group. Means within a row for a particular dietary protein not sharing the same superscript are significantly different (Tukey's test, $P < 0.05$). Lower case letters refer to comparisons of groups fed diets containing casein; upper case letters refer to comparisons of groups fed diets containing soybean protein. *Significantly different from corresponding magnesium group fed diet containing casein. Student's t test, $P < 0.05$.

[2] Significance ($P < 0.05$) was calculated by ANOVA: Mg, magnesium effect; P, protein effect; MgxP, interaction.

values of magnesium retention and increased magnesium contents in plasma but these effects did not reach statistical significance in all comparisons. The magnesium content of kidney was not significantly influenced by the dietary magnesium concentration.

Protein source and magnesium balance. Rats fed casein versus soybean protein based diets had significantly increased apparent magnesium absorption and urinary magnesium excretion (Tables 3 and 4). Casein produced a significant increase of magnesium content in femur when the diets contained 0.82 mmol/100 g magnesium; at higher concentrations of added magnesium no such effect was seen. Magnesium retention as well as plasma and kidney concentrations of magnesium were not significantly influenced by protein source.

Phytate and magnesium balance. Magnesium absorption and urinary magnesium excretion were significantly decreased in rats fed the casein diets supplemented with sodium phytate when compared with rats fed the casein diets without phytate (Table 4). These phytate effects were seen irrespective of the added magnesium concentration of the diet. Phytate produced a decrease in femur magnesium when the diet contained 0.82 mmol/100 g added magnesium,

Table 4. *Effect of dietary protein source, magnesium, phytate and lactose on magnesium metabolism, Experiment 2*[1].

	Total magnesium content (mmol/100 g)							
	Casein		Soybean protein		Casein + phytate		Casein + lactose	
	0.82	1.64	0.82	1.64	0.82	1.64	0.82	1.64
Mg absorption, μmol/d	$99 \pm 4^{b,*}$	189 ± 8^{B}	$86 \pm 4^{a,*}$	165 ± 7^{A}	$86 \pm 4^{a,*}$	160 ± 7^{A}	$107 \pm 5^{c,*}$	210 ± 5^{C}
Mg absorption, % of intake	78 ± 2^{b}	74 ± 2^{B}	$73 \pm 1^{a,*}$	65 ± 1^{A}	69 ± 2^{a}	67 ± 2^{A}	83 ± 2^{c}	85 ± 1^{C}
Fecal Mg, μmol/d	$29 \pm 3^{b,*}$	70 ± 4^{B}	$33 \pm 2^{b,*}$	86 ± 5^{C}	$37 \pm 3^{b,*}$	78 ± 4^{C}	$21 \pm 2^{a,*}$	36 ± 3^{A}
Urinary Mg, μmol/d	$35 \pm 2^{a,*}$	111 ± 4^{B}	$20 \pm 1^{b,*}$	78 ± 2^{A}	$17 \pm 1^{b,*}$	83 ± 8^{A}	$43 \pm 1^{c,*}$	129 ± 7^{C}
Mg retention, μmol/d	64 ± 3	78 ± 4	$66 \pm 3^{*}$	87 ± 6	69 ± 4	77 ± 3	64 ± 3	81 ± 4
Plasma Mg, mmol/L	0.78 ± 0.04	0.82 ± 0.03	$0.74 \pm 0.03^{*}$	0.82 ± 0.03	$0.79 \pm 0.04^{*}$	0.86 ± 0.05	0.79 ± 0.03	0.83 ± 0.03
Femur Mg, μmol/g ash	$300 \pm 4^{b,*}$	321 ± 5	$284 \pm 8^{a,*}$	317 ± 7	$276 \pm 7^{a,*}$	320 ± 6	$305 \pm 7^{b,*}$	321 ± 6
Kidney Mg, μmol/g dry matter	36 ± 1	37 ± 1	38 ± 1	37 ± 1	37 ± 1	37 ± 1	37 ± 1	37 ± 1

[1] Values means ± SEM for six rats per dietary group. Means within a row for a particular magnesium concentration not sharing the same superscript are significantly different (Tukey's test, $P < 0.01$). Lower case letters refer to comparisons of groups fed diets containing 0.82 mmol magnesium/100 g diet; upper case letters refer to comparisons of groups fed diets containing 1.64 mmol magnesium/100 g diet. *Significantly different from corresponding groups fed diets containing 1.64 mmol magnesium/100 g diet. Student's t test, $P < 0.05$.

but not when it contained 1.64 mmol/100 g diet. Phytate did not cause significant differences in magnesium retention and magnesium concentrations in plasma and kidneys.

Lactose and magnesium balance. Lactose, when compared with dextrose, significantly increased intestinal magnesium absorption and urinary magnesium excretion (Table 4). Magnesium retention and magnesium concentrations in plasma, femur and kidney were not significantly affected by lactose.

Discussion

This study confirms the findings of Wacker and Parisi (12) that the amount of magnesium in the diet influences various aspects of magnesium metabolism. Increased intakes of magnesium consistently produced a decreased percentage and increased apparent absolute magnesium absorption and magnesium retention as well as magnesium concentrations in urine, feces, plasma and femur. Kidney concentrations of magnesium were not significantly affected by magnesium intake.

This study also confirms (2, 9, 10) that lactose improves magnesium absorption. Lactose did not alter magnesium retention and magnesium concentration in plasma, femur or kidney. Forbes (10) also found that appparent magnesium absorption, but not magnesium retention, was increased in young rats fed lactose in comparison to dextrose. However, in that study (10) kidney magnesium concentrations were also increased in rats fed lactose. This might be caused by the fact that Forbes (10) used a higher lactose concentration than we did (25% versus 10%). The mechanism by which lactose stimulates magnesium absorption is not clear. Rats become lactase-deficient after the weanling period (13), and thus are not capable of hydrolyzing lactose in the intestine. Possibly, microbial fermentation of lactose in the intestine lowers luminal pH. Shiga *et al.* (14) showed that a decreased pH increases solubility of magnesium in the gastrointestinal tract. Because magnesium is absorbed by a passive absorption mechanism (15), we hypothesize that lactose-induced luminal solubility of magnesium increases its intestinal absorption.

The new finding that emerged from this study is that rats fed soybean protein had reduced apparent absorption of magnesium when compared to rats fed casein. In rats fed diets containing soybean protein the percentage and absolute apparent absorption of magnesium was consistently decreased. This effect of casein versus soybean protein did not depend on the amount of added magnesium in the diet. Figure 1, in which the data from the two experiments are pooled, shows that irrespective of the intake of magnesium, the absorption of magnesium in rats fed casein was higher than that in rats fed soybean protein. The slopes of the regression lines for soybean protein and casein shown in Figure 1, did not differ significantly. However, the intercepts of the two regression lines were significantly different ($P < 0.05$, Student's t test). Guenter and Sell (16) reported that in chickens magnesium from soybean meal was as available as that from $MgSO_4$. However, they did not compare the effects of casein and soybean protein on absorption of added magnesium.

The type of dietary protein did not significantly affect magnesium concentrations in plasma and kidney, but it did influence magnesium concentration of femur. However, only at the low dietary concentration of added magnesium (0.82 mmol/100 g diet), the concentrations of femur magnesium were higher in rats fed casein diets compared with rats fed soybean protein diets. This dietary magnesium concentration, is below the requirement (1.64 mmol/100 g diet) for rats (17) and appeared to be marginal in this study and in other studies (3, 18). Apparently, a decrease in magnesium absorption only leads to decreased magnesium accumulation in femur when dietary magnesium concentrations are below the requirement.

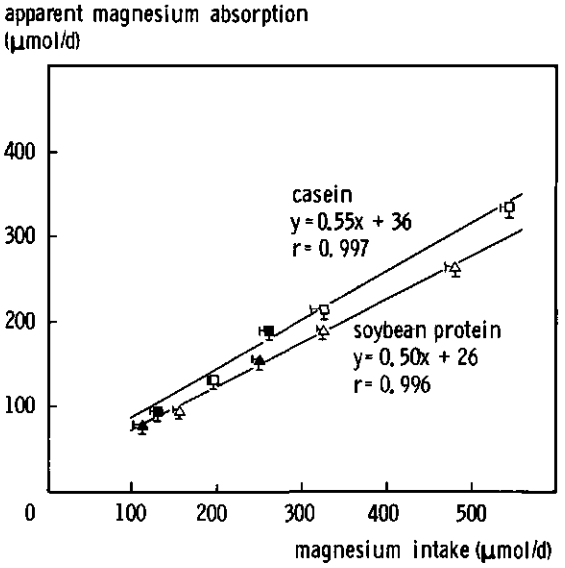

Figure 1. Relationship between magnesium intake and apparent magnesium absorption in rats fed purified diets containing either casein or soybean protein. Symbols: □, casein, Experiment 1; ■, casein, Experiment 2; △, soybean protein, Experiment 1; ▲, soybean protein, Experiment 2. The values are means ± SEM for seven rats in Experiment 1 and for six rats in Experiment 2. The slopes of the regression lines did not differ, but the intercepts were significantly different ($P < 0.05$, Student's t test).

Lo *et al.* (3) and Forbes *et al.* (4) reported that casein and soybean protein do not differently affect magnesium content of femur. Lo *et al.* (3) used diets with added magnesium concentration of only 0.82 mmol/100 g, and thus the lack of protein effect on femur magnesium cannot be readily explained. Forbes *et al.* (4) studied availabilty of endogenous magnesium from different soybean protein preparations in comparison to that from inorganic magnesium supplemented to casein, whereas we studied the effects of protein source on magnesium absorption of added magnesium. It is not clear whether this difference in study design is responsible for the different findings concerning femur magnesium concentration. However, in both studies (3, 4) magnesium intakes of rats fed the different experimental diets were not reported, and thus it cannot be excluded that magnesium intake of rats fed the diets containing soybean protein were higher than those of rats fed the casein diets. Such a difference might obscure a protein effect on magnesium content of femur.

Roberts and Yudkin (19) showed that dietary sodium phytate concentrations of 1 to 10% (w/w) cause magnesium deficiency in rats. Phytate decreases the absorption of magnesium, which is probably due to the formation of intestinal magnesium-calcium-phytate complexes (20, 21), resulting in a decrease of soluble magnesium in the intestine. Thus it could be suggested that phytate, which is known to be present in soybean protein preparations

(22), is responsible for the observed differential effect of casein and soybean protein on magnesium absorption. This suggestion is supported by two lines of evidence in our study. First, Figure 1 illustrates that soybean protein versus casein reduced magnesium absorption by a constant amount independent of magnesium intake: not the slopes but the intercepts of the regression lines for magnesium absorption as function of magnesium intake were significantly different. Second, the diet containing soybean protein and the addition of phytate to the casein diet, to a concentration identical to that in the soybean protein diet, influenced magnesium metabolism similarly (Table 4). However, naturally occurring phytate in soybean protein might be less effective in binding magnesium than added sodium phytate, because the phosphate groups can be bound to other components in the protein preparation. Thus, less phosphate groups may be available for binding with magnesium, which in turn diminishes the phytate effect on magnesium absorption. Moreover, it cannot be excluded that part of the effect of protein type on magnesium absorption is due to a difference of amino acid profile in the protein preparations.

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References

1. Davies, N.T. (1979) Anti-nutrient factors affecting mineral utilization. *Proc. Nutr. Soc.* **38**: 121-128.
2. Schaafsma G., Dekker, P.R. & de Waard, H. (1988) Nutritional aspects of yogurt. 2. Bioavailability of essential minerals and trace elements. *Neth. Milk Dairy J.* **42**: 135-146.
3. Lo, G.S., Steinke, F.H. & Hopkins, D,T. (1980) Effect of isolated soybean protein on magnesium bioavailability. *J. Nutr.* **110**: 829-836.
4. Forbes, R.M., Weingarter, K.E., Parker, H.M., Bell, R.R. & Erdman, J.W. (1979) Bioavailability to rats of zinc, magnesium and calcium in casein-, egg- and soy protein containing diets. *J. Nutr.* **109**: 1652-1660.
5. Fiske, C.H. & Subbarow, Y. (1925) The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**: 375-400.
6. Koops, J., Klomp, H. & Elgersma, R.H.C. (1975) Rapid determination of nitrogen in milk and dairy products by coloric estimation of ammonia following an accelerated digestion procedure. *Neth. Milk Dairy J.* **29**: 169-180.
7. Brons, C. & Olieman, C. (1983) Study of high performance liquid chromatographic separation of reducing sugars applied to the determination of lactose in milk. *J. Chromatography* **259**: 79-86.
8. Slump, P., Bos, K.D. & Wolters, M.G.E. (1987) Improved determination of phytate by HPLC. In: Euro Food Chem IV. Rapid analysis in food processing and food control, vol. 1, pp. 151-155, Norwegian Food Research Institute, Loen, Norway.
9. Andrieux, C. & Sacquet, E. (1983) Effect of microflora and lactose on the absorption of calcium, phosphorus and magnesium in the hindgut of the rat. *Reprod. Nutr. Develop.* **23**: 258-271.
10. Forbes, R.M. (1964) Mineral utilization in the rat. III. Effects of calcium, phosphorus, lactose and source of protein in zinc-deficient and zinc-adequate diets. *J. Nutr.* **83**: 225-233.
11. Tukey, J.W. (1953) The problem of mutiple comparisons. Princeton University, Princeton, N.J.
12. Wacker, W.E.C. & Parisi, A.F. (1968) Magnesium metabolism. *New Engl. J. Med.* **278**: 658-663.
13. De Groot, A.P. & Hoogendoorn, P. (1957) The detrimental effect of lactose. II. Quantitative lactose determinations in various mammals. *Neth. Milk Dairy J.* **11**: 290-303.

14. Shiga, A., Sasaki, T. & Horii, N. (1987) Correlations among pH and Mg, Ca, P, Na, K, Cl^- and HCO_3^- contents of digesta in the gastro-intestinal tract of rats. *Jpn. J. Vet. Sci.* **49**: 973-979.
15. Aldor, T.A.M. & Moore, E.W. (1970) Magnesium absorption by everted sacs of rat intestine and colon. *Gastroenterology* **59**: 745-753.
16. Guenter, W. & Sell, J.L. (1974) A method for determining "true" availability of magnesium from foodstuffs using chickens. *J. Nutr.* **104**: 1446-1457.
17. National Research Council (1978) Nutrient requirements of laboratory animals, 3rd rev. ed., pp 7-37, National Academy of Sciences, Washington D.C.
18. Fisher, P.W.F. & Giroux, A. (1987) Effects of dietary magnesium on sodium-potassium pump action in the heart of rats. *J. Nutr.* **117**: 2091-2095.
19. Roberts, A.H. & Yudkin, J. (1960) Dietary phytate as a possible cause of magnesium deficiency. *Nature* **185**: 823-825.
20. Champagne, E.T. (1988) Effects of pH on mineral-phytate, protein-mineral-phytate, and mineral-fiber interactions. Possible consequences of atrophic gastritis on mineral bioavailability from high-fiber foods. *J. Am. Coll. Nutr.* **7**: 499-508.
21. Cheryan, M., Anderson, F.W. & Grynspan, F. (1983) Magnesium-phytate complexes: effect of pH and molar ratio on solubility characteristics. *Cereal Chem.* **60**: 235-237.
22. Reddy, N.R., Sathe, S.K. & Salunkhe, P.K. (1982) Phytates in legumes and cereals. *Adv. Food Res.* **28**: 1-92.

Chapter 4

Bioavailability of magnesium and calcium from cow's milk and soybean beverage in rats

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Abstract. The milk components, lactose and casein, enhance the apparent absorption of magnesium and possibly also of calcium, whereas phytate, which occurs in soybean products, has an inhibitory effect. This implies that soybean beverage versus cow's milk could lower bioavailability of magnesium and calcium. In two experiments with growing rats, this hypothesis was tested. Feeding soybean beverage versus cow's milk consistently lowered body weight gain, enhanced bone turnover, as measured by increased plasma alkaline phosphatase (EC 3.1.3.1) activities and increased urinary hydroxyproline excretion, and decreased magnesium and calcium concentrations in femur. Because the mineral composition of soybean beverage and cow's milk was different, the intake of magnesium was higher in rats fed soybean beverage, whereas that of calcium was higher in rats fed cows's milk. Supplementation of soybean beverage either with phosphorus and calcium or with phosphorus, calcium and methionine, to concentrations identical to those in milk, restored growth and bone mineralization. When using diets carefully balanced for magnesium, calcium, phosphorus, sodium, potassium and methionine, soybean beverage versus cow's milk in the diets decreased apparent absorption and urinary excretion of magnesium and calcium. Hydrolysis of lactose in milk decreased absorption and urinary excretion of magnesium; it did not significantly affect calcium absorption, but lowered urinary calcium excretion. This study shows that soybean beverage versus cow's milk depresses magnesium and calcium bioavailability as would be predicted on the basis of reported effects of their purified components.

Introduction

Constituents of cow's milk products, when compared with those of soybean products, increase the apparent absorption of magnesium and possibly also of calcium. Casein versus soybean protein improves magnesium absorption (1), but does not affect calcium absorption (2). Lactose, a constituent of milk, stimulates the absorption of magnesium (1, 3-5). The effect of lactose on calcium absorption is controversial, with improved calcium absorption (3-5) and no effect (6-8) being reported. Phytate, which is present in soybean products in significant amounts (9), suppresses the absorption of magnesium and calcium (1, 10-12).

Based on the effects of constituents of milk products versus those of soybean products, it follows that substitution of soybean beverage for cow's milk in the diet lowers bioavailability, especially apparent absorption, of magnesium and calcium. However, it could be suggested that the constituents do not exhibit the anticipated effects when present in the matrix of the intact products. In order to test this suggestion, we fed cow's milk and soybean beverage to young growing rats and determined mineral bioavailability. The effect of lactose in intact milk was ascertained by comparing intact and lactase-treated milk. The soybean beverage used was deficient in methionine and calcium but contained more magnesium than cow's milk. To find out whether differences in mineral absorption from the intact products could be caused by differences in concentrations of these nutrients, we also used diets that were balanced for methionine, calcium and magnesium. Magnesium and calcium bioavailability were assessed by measurement of their apparent absorption, urinary excretion and

concentrations in plasma and femur. Plasma alkaline phosphatase (EC 3.1.3.1) activity and urinary hydroxyproline excretion were determined as indices of bone turnover.

Materials and Methods

General procedures. Male Wistar (Cpb:WU) rats, aged four wk, were housed individually in polycarbonate-topped metabolic cages with stainless steel wire bottoms (314 cm^2 x 12 cm) in a room with controlled temperature (20-22°C), relative humidity (50-60%) and lighting (light, 06.00-18.00 h). For four wk, the animals were fed the experimental diets containing cow's milk, lactase-treated cow's milk or soybean beverage. Diets and demineralized water were freely available.

Sterilized cow's milk was prepared in The Netherlands Institute for Dairy Research. Lactase-treated milk was obtained by addition of 200 mg lactase (EC 3.2.3.23, Maxilact-a 40.000, Gist Brocades, Delft, The Netherlands) to one litre of the sterilized milk and incubation for 24 h at 10°C. This led to hydrolysis of 80% of the lactose. The soybean beverage was obtained from Protevit, Fijnaart, The Netherlands. Table 1 shows the analyzed composition of the cow's milk, lactase-treated cow's milk and soybean beverage. The milk, lactase-treated milk and soybean beverage were fed in the form of diets shown in Tables 2 and 3. Basically, either a protein-free mixture without minerals or a protein-rich mixture with minerals were added to make a porridge. The experimental diets were freshly prepared twice a week.

Feed consumption was recorded daily and body weights were measured weekly. During the last four d of the experimental period, feces and urine of each animal were collected. At the end of the experimental period, between 08.30 and 12.30 h, fasting blood samples were drawn under light diethyl ether anaesthesia by orbital puncture, which was immediately followed by decapitation. Femora were removed.

For analysis of minerals, diets and feces were freeze-dried, homogenized and wet ashed with nitric acid and perchloric acid. Femora were cleaned of adhering tissue, defatted in diethyl ether for 50 h, weighed in air as well as under water for volume measurement. Fat free femora were dried for 16 h at 105°C and dry ashed in a muffle furnace for 16 h at 550°C. After appropiate dilution, magnesium and calcium were analyzed by atomic absorption spectrophotometry (Perkin Elmer 1100, Bodenseewerk Perkin Elmer & Co. GmbH, Germany). Samples of the diets were also analyzed for sodium and potassium by atomic emission spectrophotometry and for phosphorus by the Fiske Subbarow method (14). Nitrogen in the diets was analyzed by the macro-Kjeldahl method (15) and lactose by high-performance liquid chromatography (16). Methionine was determined according to the method described by Moore (17). The phytate content of the base products and the complete diets was analyzed as described by Slump *et al.* (18).

Bioavailability of magnesium and calcium was evaluated by determination of their apparent intestinal absorption, urinary excretion and contents in plasma and femur. Bone turnover was assessed by measurement of plasma alkaline phosphatase and urinary

Table 1. *Analyzed composition of the cow's milk (M), lactase-treated cow's milk (L) and soybean beverage (S).*

	M	L	S
Energetic value (kJ/kg)[1]	2180	2180	2180
Nitrogen (g/kg)	5.3	5.3	6.1
Fat (g/kg)	23	23	26
Lactose (g/kg)	45	9	0
Other carbohydrates (g/kg)	0	36	39
Phytate (mmol/kg)	nd[2]	nd	2.6
Magnesium (mmol/kg)	4.9	4.9	11.5
Calcium (mmol/kg)	30	30	5
Phosphorus (mmol/kg)	31	31	23
Potassium (mmol/kg)	40	40	47
Sodium (mmol/kg)	21	21	28
Water (mol/kg)	64	64	64

[1] Calculated on the basis of chemical analysis; energetic values used (kJ/g): protein, 16.8; carbohydrates, 16.8; fat, 37.8. For conversion of nitrogen into protein concentrations a factor of 6.35 was used for cow's milk and lactase-treated cow's milk, and a factor of 5.91 was used for soybean beverage.

[2] nd = not determined.

hydroxyproline using commercial kits purchased from Boehringer, Mannheim, Germany and Hypronosticon, Organon Teknica, Oss, The Netherlands. Apparent intestinal absorption was calculated as mineral intake minus fecal excretion, and was expressed as such and as percentage of intake.

Experiment 1. This experiment was designed to compare bioavailability of magnesium and calcium from cow's milk, lactase-treated cow's milk and soybean beverage. These products were compared in either a protein/mineral-free or protein/mineral-rich (magnesium-free) diet. The use of two dietary backgrounds was anticipated to provide clues as to whether the differential effects of cow's milk and soybean beverage become less pronounced when incorporated into more nutritionally adequate diets. Since we were especially interested in magnesium bioavailability from intact cow's milk and soybean beverage, no magnesium was added to the protein/mineral-rich dietary background.

Forty-two rats, with a mean body weight of 83 g, were randomly divided into six groups of equal size and were fed diets containing either cow's milk, lactase-treated cow's milk or soybean beverage. To 2800 g of these products, which corresponded to 300 g of dry matter, 700 g of either a protein/mineral-free or protein/mineral-rich ingredient mixture was added as indicated in detail in Table 2. In essence, the protein/mineral-free mixture consisted of rice flour (Molenaar kindermeel, Milupa, Amersfoort, The Netherlands), supplemented

Table 2. *Composition of the diets containing cow's milk (M), lactase-treated cow's milk (L) or soybean beverage (S), Experiment 1.*

	Experimental diets					
	Protein/mineral-free background			Protein/mineral-rich background		
	M	L	S	M	L	S
	g dry weight					
Ingredient						
Cow's milk[1]	300	-	-	300	-	-
Lactase-treated milk[1]	-	300	-	-	300	-
Soybean beverage[1]	-	-	300	-	-	300
Protein/mineral-free mix[2]	700	700	700	-	-	-
Protein/mineral-rich mix[3]	-	-	-	700	700	700
	mmol/kg dry weight					
Chemical analysis						
Nitrogen	1543	1536	1729	2286	2264	2464
Phytate	nd[4]	nd	6.8	nd	nd	7.0
Lactose	339	35	0	348	35	0
Magnesium	20.6	20.6	37.0	24.7	24.7	41.2
Calcium	92.5	92.5	15.0	135.0	135.0	67.5
Phosphorus	109.7	109.7	90.3	154.8	154.8	135.5
Sodium	60.9	60.9	82.6	156.5	156.5	178.3
Potassium	169.2	169.2	189.7	130.8	130.8	151.3

[1] Used as such: to 2800 g of these products, which corresponded with 300 g of dry matter, 700 g of either the protein/mineral-free or protein/mineral-rich mixture was added.

[2] The protein/mineral-free mixture consisted of the following (g/kg dry diet): rice flour, 620.91; cellulose, 57; sunflower oil, 8; vitamin premix, 10; $FeSO_4$, 0.09; chromium trioxide, 4. The composition of the vitamin premix (AIN^{-76}) was as described (13).

[3] The protein/mineral-rich mixture consisted of the following components (g/kg dry diet): ovalbumin, 105; cellulose, 56; rice flour, 396.2; palm oil, 105; mineral premix, 24.5; vitamin premix, 9.1; choline chloride, 1.4; chromium trioxide, 2.8. The mineral premix consisted of the following (mg): $CaCO_3$, 1926; $CaHPO_4.2H_2O$, 4667; KH_2PO_4, 3418; $FeSO_4.7H_2O$, 176; $MnSO_4.H_2O$, 167; $ZnSO_4.7H_2O$, 98; $CuSO_4.5H_2O$, 78; KIO_3, 0.3; $Na_2SeO_3.5H_2O$, 0.3; $CrK(SO_4)_2.12H_2O$, 13; dextrose, 13,956. The composition of the vitamin premix (AIN^{-76}) was as described (13).

[4] nd = not determined.

with cellulose, iron and vitamins. The protein/mineral-rich mixture contained rice flour, cellulose, ovalbumin (NIVE-WRC, Amersfoort, The Netherlands), mineral and vitamin premix. The protein/mineral-rich mixture did not contain magnesium.

Experiment 2. The aim of this experiment was to compare mineral availability from the different diets under conditions of equal growth and mineral intake by the rats. The soybean

Table 3. *Composition of the diets containing cow's milk (M) or soybean beverage (S), Experiment 2.*

	Experimental diets with protein/mineral-free background					
	M		S			
Supplements	None	Mg+Na+K	None	Met	P+Ca	P+Ca+Met
	g dry weight					
Ingredient						
Cow's milk[1]	300	300	-	-	-	-
Soybean beverage[1]	-	-	300	300	300	300
DL-Methionine (Met)	-	-	-	0.95	-	0.95
$CaHPO_4.2H_2O$	-	-	-	-	4.4	4.4
$CaCO_3$	-	-	-	-	6.6	6.6
$MgCO_3$	-	1.2	-	-	-	-
$NaHCO_3$	-	1.8	-	-	-	-
$KHCO_3$	-	5.8	-	-	-	-
Protein/mineral-free mix[2]	700	700	700	700	700	700
	mmol/kg dry weight					
Chemical analysis						
Nitrogen	1678	1664	1793	1771	1764	1786
Methionine	27.5	27.5	22.1	28.1	20.8	28.2
Phytate	nd[3]	nd	6.8	6.8	6.9	7.0
Magnesium	20.6	34.6	37.0	36.8	36.9	37.1
Calcium	100.0	100.0	12.5	12.7	102.5	103.8
Phosphorus	106.5	106.1	80.6	77.4	104.8	105.2
Sodium	52.2	82.6	82.6	81.5	80.3	80.8
Potassium	125.6	164.1	164.8	163.7	163.9	164.2

[1] Used as such: to 2800 g of these products, which corresponded with 300 g of dry matter, 700 g of either the protein/mineral-free or protein/mineral-rich mixture was added.

[2] The protein/mineral-free mixture consisted of the following (g/kg dry diet): rice flour, 620.91; cellulose, 57; sunflower oil, 8; vitamin premix, 10; $FeSO_4$, 0.09; chromium trioxide, 4. The composition of the vitamin premix (AIN^{-76}) was as described (13).

[3] nd = not determined.

beverage is deficient in phosphorus, calcium and methionine; thus these nutrients were added, either alone or in combination, to concentrations identical to those in cow's milk. The soybean beverage contained more magnesium, sodium and potassium than cow's milk. In order to balance the diets for these components as well they were added to cow's milk to concentrations identical to those in soybean beverage. The composition of the diets used in experiment 2 is shown in Table 3. All diets contained the protein/mineral-free mixture.

Forty-two rats, with a mean body weight of 79 g, were randomly divided into six dietary groups of equal size and fed the diets.

Statistics. *A priori* defined contrasts were evaluated using Bonferroni's test for multiple comparisons (19). The level of statistical significance adjusted for multiple comparisons was preset at $P < 0.05$. The following contrasts were tested. Experiment 1: lactase-treated cow's milk versus cow's milk against both dietary backgrounds; soybean beverage versus cow's milk against both dietary backgrounds; protein/mineral-rich diets versus protein/mineral-free diets with identical product. Experiment 2: non-supplemented soybean beverage versus non-supplemented cow's milk; supplemented cow's milk versus non-supplemented cow's milk; soybean beverage supplemented with phosphorus, calcium and methionine versus supplemented cow's milk; soybean beverage supplemented with methionine versus non-supplemented soybean beverage; soybean beverage supplemented with phosphorus and calcium versus non-supplemented soybean beverage; soybean beverage supplemented with phosphorus, calcium and methionine versus non-supplemented soybean beverage; soybean beverage supplemented with phosphorus, calcium and methionine versus soybean beverage supplemented with methionine; soybean beverage supplemented with phosphorus, calcium and methionine versus soybean beverage supplemented with phosphorus and calcium. Only those contrasts that reached statistical significance are indicated in Tables 4-7.

Results

Experiment 1 (Tables 4 and 5).

Body weight gain and feed intake. Body weight gain and feed intake of rats fed soybean beverage against the protein/mineral-free background were significantly decreased when compared with rats fed cow's milk (Table 4). There were no significant differences between groups fed the diets containing the protein/mineral-rich mixture. Replacement of the protein/mineral-free mixture by the protein/mineral-rich mixture in the diet with soybean beverage significantly increased body weight gain. Such replacement did not influence body weight gain when the diet contained either cow's milk or lactase-treated cow's milk, while it significantly decreased feed intake.

Magnesium balance. Irrespective of the composition of the dietary background, which in either case did not contain added magnesium, magnesium intake was significantly increased in rats fed soybean beverage (Table 4). This was associated with an increased absolute magnesium absorption and urinary and fecal magnesium excretion, while percentage magnesium absorption was decreased. Lactase-treated versus normal cow's milk significantly decreased intestinal magnesium absorption and lowered urinary magnesium excretion. Magnesium concentration in plasma was increased by soybean beverage versus cow's milk against a protein/mineral-free background.

Table 4. *Body weight gain, feed intake, magnesium and calcium balance of rats given diets containing cow's milk (M), lactase-treated cow's milk (L) and soybean beverage (S) in a protein/mineral-free or protein/mineral-rich background, Experiment 1*[1].

	Experimental diets						
	Protein/mineral-free background			Protein/mineral-rich background			
	M	L	S	M	L	S	SED[2]
Body-weight gain (g/28 d)	137	144	88[B]	140	150	148[D]	6
Feed intake (g/28 d)[3]	455	478	391[B]	397[E]	421[F]	407	8
Magnesium balance							
Mg intake (μmol/d)	358	366	556[B]	325	329	551[D]	22
Fecal Mg excretion (μmol/d)	90	144[A]	226[B]	70	95[C]	222[D]	14
Mg absorption (μmol/d)	267	226[A]	329[B]	255	235[C]	329[D]	9
Mg absorption (%)	75	62[A]	59[B]	79	71[C,F]	60[D]	1
Urinary Mg excretion (μmol/d)	185	136[A]	206	177	152	222[D]	8
Plasma Mg (mmol/L)	0.82	0.82	0.90[B]	0.86	0.83	0.88	0.01
Calcium balance							
Ca intake (μmol/d)	1525	1575	225[B]	1950[E]	1973[F]	937[D,G]	94
Fecal Ca excretion (μmol/d)	351	419	20[B]	810[E]	788[F]	44[D,G]	37
Ca absorption (μmol/d)	1174	1156	205[B]	1139	1185	893[D,G]	80
Ca absorption (%)	77	73	94[B]	59[E]	60[F]	95[D]	2
Urinary Ca excretion (μmol/d)	33	9[A]	3[B]	26	17	4[D]	2
Plasma Ca (mmol/L)	2.90	2.93	2.85	2.93	2.95	2.91	0.01

[1] Values are expressed as group means for seven rats per dietary group. Superscripts refer to *a priori* selected contrast which are significantly different (Bonferroni's test, $P < 0.05$). A = lactase-treated cow's milk versus cow's milk in the protein/mineral-free background; B = soybean beverage versus cow's milk in the protein/mineral-free background; C = lactase-treated cow's milk versus cow's milk in the protein/mineral-rich background; D = soybean beverage versus cow's milk in the protein/mineral-rich background; E = cow's milk in the protein/mineral-rich background versus cow's milk in the protein/mineral-free background; F = lactase-treated cow's milk in the protein/mineral-rich background versus lactase-treated cow's milk in the protein/mineral-free background; G = soybean beverage in the protein/mineral-rich background versus soybean beverage in the protein/mineral-free background.

[2] SED = Standard Error of Difference.

[3] On a dry weight basis.

Calcium balance. Calcium intake was significantly lower in rats fed soybean beverage instead of cow's milk, which was associated with decreased absolute calcium absorption, decreased urinary and fecal calcium excretion and an increased percentage calcium absorption (Table 4). Absolute calcium absorption in rats fed soybean beverage was significantly higher

Table 5. *Bone parameters in rats given diets containing cow's milk (M), lactase-treated cow's milk (L) or soybean beverage (S) in a protein/mineral-free or protein/mineral-rich background, Experiment 1*[1].

	Experimental diets						
	Protein/mineral-free background			Protein/mineral-rich background			
	M	L	S	M	L	S	SED[2]
Plasma alkaline phosphatase activity (U/L)	91	96	119[B]	110	97	84[D,G]	4
Urinary hydroxyproline excretion (μmol/d)	7.6	8.0	9.4[B]	8.0	8.3	9.0[D]	0.3
Femur Mg (mmol/cm³)	0.16	0.15	0.11[B]	0.16	0.16	0.14[D,G]	0.006
Femur Ca (mmol/cm³)	4.85	4.68	2.72[B]	4.84	4.67	4.20[D,G]	0.16

[1] Values are expressed as group means for seven rats per dietary group. Superscripts refer to *a priori* selected contrast which are significantly different ($P < 0.05$). B = soybean beverage versus cow's milk in the protein/mineral-free background; D = soybean beverage versus cow's milk in the protein/mineral-rich background; G = soybean beverage in the protein/mineral-rich background versus soybean beverage in the protein/mineral-free background.
[2] SED = Standard Error of Difference.

with the diets containing the protein/mineral-rich mixture instead of the protein/mineral-free mixture. Hydrolysis of lactose in milk did not significantly affect calcium absorption, but it decreased urinary calcium excretion. The latter effect just failed to reach statistical significance in rats fed diets with the protein/mineral-rich background. Plasma concentrations of calcium were not significantly influenced by the experimental diets.

Bone metabolism. When the rats were fed the diets containing the protein/mineral-free mixture, urinary hydroxyproline excretion and plasma alkaline phosphatase activity were significantly increased by soybean beverage versus cow's milk (Table 5). When soybean beverage and cow's milk were compared against a protein/mineral-rich (magnesium-free) background, urinary hydroxyproline excretion was still increased, but plasma alkaline phosphatase activity was now significantly decreased in rats fed soybean beverage. Magnesium and calcium concentrations in femur were significantly lower in rats fed soybean beverage instead of cow's milk, irrespective of the background composition of the diet. Hydrolysis of lactose in milk did not significantly affect bone parameters.

Experiment 2 (Tables 6 and 7).

Body weight gain and feed intake. Body weight gain was significantly lower in rats fed non-supplemented soybean beverage than in rats given non-supplemented milk (Table 6). Growth retardation in the rats fed soybean beverage had disappeared after supplementation of the diet with either phosphorus plus calcium or with the combination of phosphorus, calcium and methionine. Feed intakes of rats fed the experimental diets were not significantly different.

Table 6. *Body weight gain, feed intake, magnesium and calcium balance in rats given diets containing cow's milk (M) or soybean beverage (S) in a protein/mineral-free background either with or without supplements, Experiment 2*[1].

	Experimental diets with protein/mineral-free background						
	M		S				
Supplements	None	Mg+Na+K	None	Met	P+Ca	P+Ca +Met	SED[2]
Body-weight gain (g/28 d)	172	165	138[B]	140	158	165[E]	4
Feed intake (g/28 d)[3]	447	446	446	428	472	467	8
Magnesium balance							
Mg intake (μmol/d)	356	625[A]	623[B]	623	705	661	25
Fecal Mg excretion (μmol/d)	98	228[A]	245[B]	258	384[D]	355[C,E,F]	22
Mg absorption (μmol/d)	258	397[A]	378[B]	364	321[D]	305[C,E,F]	12
Mg absorption (%)	72	65[A]	61[B]	59	46[D]	46[C,E,F]	3
Urinary Mg excretion (μmol/d)	133	271[A]	252[B]	239	146[D]	199[C]	15
Plasma Mg (mmol/L)	0.88	0.88	0.98	0.97	0.91	0.91	0.03
Calcium balance							
Ca intake (μmol/d)	1900	2035	213[B]	210	2104[D]	1973[E,F]	130
Fecal Ca excretion (μmol/d)	500	610	8[B]	7	826[D]	753[C,E,F]	71
Ca absorption (μmol/d)	1400	1424	205[B]	203	1278[D]	1220[C,E,F]	98
Ca absorption (%)	74	70	96[B]	97	61[D]	62[C,E,F]	3
Urinary Ca excretion (μmol/d)	70.3	67.5	3.3[B]	3.4	37.1[D]	31.3[C,E,F]	7
Plasma Ca (mmol/L)	2.85	2.84	2.74[B]	2.70	2.80	2.84[F]	0.01

[1] Values are expressed as group means for seven rats per dietary group. Superscripts refer to *a priori* selected contrast which are significantly different (Bonferroni's test, $P < 0.05$): A = cow's milk supplemented with Mg, Na and K versus cow's milk without supplements; B = soybean beverage without supplements versus cow's milk without supplements; C = soybean beverage supplemented with P, Ca and methionine (Met) versus cow's milk supplemented with Mg, Na and K; D = soybean beverage supplemented with P and Ca versus soybean beverage without supplements; E = soybean beverage supplemented with P, Ca and methionine versus soybean beverage without supplements; F = soybean beverage supplemented with P, Ca and methionine versus soybean beverage supplemented with methionine.

[2] SED = standard error of difference.

[3] On a dry weight basis.

Magnesium balance. Intake, absolute absorption, and urinary and fecal excretion of magnesium were significantly higher in rats fed non-supplemented soybean beverage compared with cow's milk (Table 6). Percentage magnesium absorption was decreased in rats fed soybean beverage. Supplementation of soybean beverage with methionine alone did not affect magnesium balance. Adding phosphorus plus calcium to the soybean beverage diet

caused a significant increase in fecal magnesium excretion and reduced urinary excretion as well as intestinal absorption. Further supplementation with methionine did not significantly influence urinary and fecal excretion of magnesium. The addition of magnesium, sodium and potassium to the cow's milk diet caused an increased intake of magnesium, associated with increased urinary and fecal excretion, and absolute absorption of magnesium. As a consequence, percentage magnesium absorption was decreased. Although magnesium intake did not differ between the rats fed the diet containing either soybean beverage supplemented with phosphorus, calcium and methionine or cow's milk supplemented with magnesium, sodium and potassium, the former showed significantly lower absolute and percentage magnesium absorption. Plasma magnesium concentrations were not significantly affected by the dietary treatments.

Calcium balance. Intake, absolute absorption and urinary excretion of calcium were significantly lower in rats fed non-supplemented soybean beverage than in rats fed non-supplemented cow's milk (Table 6). Percentage calcium absorption was significantly higher in rats fed soy beverage. Calcium balance was not affected after supplementation of the soybean beverage with methionine. The addition of phosphorus plus calcium or the combination of phosphorus, calcium and methionine caused similar increases in calcium intake, absolute absorption and urinary and fecal excretion of calcium, while percentage calcium absorption was similarly reduced. Fortification of milk with magnesium, sodium and potassium did not significantly affect calcium balance. Although calcium intake was not different between the two groups, percentage and absolute calcium absorption in rats fed the soybean beverage diet supplemented with phosphorus, calcium and methionine were significantly lower than in rats fed the cow's milk diet either without or with added magnesium, sodium and potassium. Plasma calcium concentrations were decreased in rats fed non-supplemented soybean beverage in comparison with those fed non-supplemented cow's milk.

Bone metabolism (Table 7). Urinary hydroxyproline excretion and plasma alkaline phosphatase activity were significantly increased in rats fed non-supplemented soybean beverage compared with cow's milk. The addition of methionine to the soybean beverage diet did not affect these parameters, but addition of either phosphorus plus calcium or the combination of phosphorus, calcium and methionine decreased both parameters significantly. In rats fed the soybean beverage diet supplemented with phosphorus, calcium and methionine, plasma alkaline phosphatase activity was significantly lower than in rats fed the cow's milk diet supplemented with magnesium, sodium and potassium. However, urinary hydroxyproline excretion did not differ significantly between the two dietary groups.

Magnesium and calcium concentrations in femur were significantly lower in rats fed non-supplemented soybean beverage than in rats fed cow's milk. Femur magnesium and calcium were not significantly affected by the addition of methionine to the soybean beverage diet. Supplementation of the soybean beverage diet with either phosphorus plus calcium or

Table 7. *Bone parameters in rats given diets containing cow's milk (M) or soybean beverage (S) in protein/mineral-free background either with or without supplements, Experiment 2*[1].

Supplements	Experimental diets in protein/mineral-free background						
	M		S				
	None	Mg+Na+K	None	Met	P+Ca	P+Ca +Met	SED[2]
Plasma alkaline phosphatase activity (U/L)	77	83	117[B]	103	60[D]	58[C,E,F]	5
Urinary hydroxyproline excretion (μmol/d)	8.1	8.3	13.4[B]	13.8	8.2[D]	9.2[E,F]	0.5
Femur Mg (mmol/cm³)	0.16	0.16	0.09[B]	0.08	0.15[D]	0.15[E,F]	0.007
Femur Ca (mmol/cm³)	4.78	4.83	2.45[B]	2.18	4.68[D]	4.65[E,F]	0.25

[1] Values are expressed as group means for seven rats per dietary group. Superscripts refer to *a priori* selected contrast which are significantly different (Bonferroni's test, $P < 0.05$): B = soybean beverage without supplements versus cow's milk without supplements; C = soybean beverage supplemented with P, Ca and methionine (Met) versus cow's milk supplemented with Mg, Na and K; D = soybean beverage supplemented with P and Ca versus soybean beverage without supplements; E = soybean beverage supplemented with P, Ca and methionine versus soybean beverage without supplements; F = soybean beverage supplemented with P, Ca and methionine versus soybean beverage supplemented with methionine.

[2] SED = standard error of difference.

phosphorus, calcium and methionine significantly increased magnesium and calcium concentrations in femur. The addition of magnesium, sodium and potassium to cow's milk did not affect bone parameters. Magnesium and calcium concentrations in femur were similar in rats fed either soybean beverage supplemented with phosphorus, calcium and methionine or cow's milk supplemented with magnesium, sodium and potassium.

Discussion

When soybean beverage and cow's milk were incorporated into diets containing a protein/mineral-free mixture, soybean beverage compared with cow's milk consistently depressed body weight gain and caused decreased femur concentrations of magnesium and calcium in both experiments. Bone turnover in rats given soybean beverage was increased, as indicated by increased plasma alkaline phosphatase activity (indicator of bone formation) and increased urinary hydroxyproline excretion (indicator of bone resorption). These effects of soybean beverage were most likely caused by the low concentration of calcium in this product (Table 1) as they were reversed after the addition of calcium (Tables 5 and 7). Calcium deficiency in young rats is known to cause growth retardation and increased bone turnover (20).

Rats fed soybean beverage supplemented with phosphorus, calcium and methionine had lower plasma alkaline phosphatase activities than rats fed cow's milk supplemented with

magnesium, sodium, and potassium (Table 7). This most likely relates to the protein source. With the use of carefully balanced diets, soybean protein isolate decreased plasma alkaline phosphatase activities in rats when compared with casein (unpublished observation). However, the effect of protein on plasma alkaline phosphatase was not associated with decreased magnesium and calcium concentrations in femur (Table 7).

Under conditions of equal growth rates and the feeding of diets with identical magnesium and calcium concentrations, intestinal absorption and urinary excretion of magnesium and calcium were significantly higher in rats fed cow's milk than in those given soybean beverage (Table 6). This was not reflected by a difference in femur concentrations of magnesium and calcium (Table 7). Apparently, the absorbed amounts of these minerals in rats fed soybean beverage sufficed to sustain normal bone mineralization. In rats fed soybean beverage diets not supplemented with phosphorus plus calcium, absolute calcium absorption did not suffice (Tables 6 and 7).

Hydrolysis of lactose in milk significantly decreased magnesium absorption, but did not affect calcium absorption (Table 4). This agrees with the fact that lactose versus other carbohydrates increases magnesium absorption (3-5, 21). The effect of lactose on calcium absorption is controversial. Lactose has been reported to enhance calcium absorption (22-25). However no effect of lactose has also been reported (6-8, 21). The reason why lactose stimulates magnesium absorption, but perhaps not that of calcium, is not known.

Apart from the absence of lactose in soybean beverage the presence of phytate may also explain why soybean beverage versus cow's milk lowers mineral absorption. Phytate decreases magnesium and calcium absorption (10-12). Addition of phytate to diets with casein, to a concentration identical to that in diets containing soybean protein, significantly decreased apparent absorption of magnesium (1) and calcium (unpublished observation) in rats to a level seen after feeding soybean protein. Thus phytate in soybean protein appears responsible for the reduction in magnesium and calcium absorption induced by soybean protein when compared with casein.

In conclusion, the results of this study indicate that lactose and phytate when present in the matrix of intact milk and soybean beverage, respectively, still exert their influence on the bioavailability of magnesium and calcium. Soybean beverage versus cow's milk lowers apparent absorption of magnesium and calcium.

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References

1. Brink, E.J., Dekker, P.R., Van Beresteijn, E.C.H. & Beynen, A.C. (1991) Inhibitory effect of dietary soybean protein versus casein on magnesium absorption in rats. *J. Nutr.* **121**: 1374-1381.

2. Van der Meer, R., De Vries, H.T. & Van Tintelen, G. (1988) The phosphorylation state of casein and the species-dependency of its hypercholesterolaemic effect. *Br. J. Nutr.* **59**: 467-473.
3. Andrieux, C. & Sacquet, E. (1983) Effect of microflora and lactose on the absorption of calcium, phosphorus and magnesium in the hindgut of the rat. *Reprod. Nutr. Develop.* **23**: 258-271.
4. Greger, J.L., Gutkowski, C.M. & Khazen, R.R. (1989) Interactions of lactose with calcium, magnesium and zinc in rats. *J. Nutr.* **119**: 1691-1697.
5. Schaafsma, G., Dekker, P.R. & De Waard, H. (1988) Nutritional aspects of yogurt. 2. Bioavailability of essential minerals and trace elements. *Neth. Milk Dairy J.* **42**: 135-146.
6. Greger, J.L., Krzykowski, C.E., Khazen, R.R. & Krashoc, C. (1987) Mineral utilization by rats fed commercially available calcium supplements or milk. *J. Nutr.* **117**: 717-724.
7. Recker, R.R., Bammi, A., Barger-Lux, J. & Heaney, R. (1988) Calcium absorbability from milk products, an imitation milk and calcium carbonate. *Am. J. Clin. Nutr.* **47**: 93-95.
8. Sheikh, M.S., Santa Ana, C.A., Nicar, M.J., Schiller, L.R. & Fordtran, J.S. (1987) Gastrointestinal absorption of calcium salts. *New Engl. J. Med.* **317**: 532-536.
9. Reddy, N.R., Sathe, S.K. & Salunkhe, P.K. (1982) Phytates in legumes and cereals. *Adv. Food Res.* **28**: 1-92.
10. Davies, N.T. (1979) Anti-nutrient factors affecting mineral utilization. *Proc. Nutr. Soc.* **38**: 121-126.
11. Lonnerdal, B., Sandberg, A.-S., Sandstrom, B. & Kunz, C. (1989) Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. *J. Nutr.* **119**: 211-214.
12. Nwokolo, E.N. & Bragg, D.B. (1977) Influence of phytic acid and crude fibre on the availability of minerals from four protein supplements in growing chicks. *Can. J. Animal Sci.* **57**: 475-477.
13. American Institute of Nutrition (1977) Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. *J. Nutr.* **117**: 1340-1348.
14. Fiske, C.H. & Subbarow, Y. (1925) The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**: 375-400.
15. International Dairy Federation (1986) Determination of nitrogen content (Kjeldahl method) and calculation of crude protein content. International IDF standard no 20A. International Dairy Federation. Brussel. FIL-IDF 20.
16. Brons, C. & Olieman, C. (1983) Study of high performance liquid chromatographic separation of reducing sugars applied to the determination of lactose in milk. *J. Chromatography* **259**: 79-86.
17. Moore, S. (1963) On the determination of cystine as cysteic acid. *J. Biol. Chem.* **238**: 235-237.
18. Slump, P., Bos, K.D. & Wolters, M.G.E. (1987) Improved determination of phytate by HPLC. In: Euro Food Chem. IV. Rapid analysis in food processing and food control, Vol. 1. Loen, Norway: Norwegian Food Research Institute: pp. 151-155.
19. Fleiss, J.L. (1986) The design and analysis of clinical experiments. New York: John Wiley and Sons: pp. 103-107.
20. Irving, J.T. (1973) Calcium and phosphorus metabolism. New York: Academic Press.
21. Behling, A.R. & Greger, J.L. (1990) Importance of lactose in yogurt for mineral utilization. *J. Agr. Food Chem.* **38**: 200-204.
22. Armbrecht, H.J. & Wasserman, R.H. (1976). Enhancement of Ca^{++} uptake by lactose in the rat small intestine. *J. Nutr.* **106**: 1265-1271.
23. Favus, M.J. & Angeid-Backmann, E. (1984) Effects of lactose on calcium absorption and secretion by rat ileum. *Am. J. Physiol.* **246**: G281-G285.
24. Fournier, P., Dupuis, Y. & Fournier, A. (1971) Effect of lactose on the absorption of alkaline earth metals and intestinal lactase activity. *Isr. J. Med. Sci.* 7: 389-391.
25. Leichter, J. & Tolensky, A.F. (1975) Effect of dietary lactose on the absorption of protein, fat and calcium in the postweanling rat. *Am. J. Clin. Nutr.* **28**: 238-241.

Chapter 5

Ileal pH and apparent absorption of magnesium in rats fed diets containing lactose or lactulose

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Abstract. The hypothesis was tested that dietary lactose versus glucose stimulates magnesium absorption in rats because lactose lowers pH of the intestinal lumen. The pH lowering may improve magnesium solubility which in turn elevates magnesium availability for transport across the intestinal epithelium. For comparison, the effects of lactulose were studied because it shares with lactose the characteristic of being poorly digestible. Replacement of glucose by lactose (10%, wt/wt) significantly stimulated apparent absorption of magnesium. Apart from magnesium absorption, lactulose also significantly enhanced apparent absorption of calcium and phosphate. Lactose versus glucose lowered the pH of the intestinal lumen from 7.5 to 7.2, whereas lactulose significantly reduced it to 7.0. In *in vitro* incubations, a decrease in pH, within the range of fluctuation *in vivo*, was found to cause an improved solubility of magnesium, and to a lesser extent also of calcium and phosphate. The smaller fall of ileal pH as induced by feeding lactose instead of lactulose, may explain why lactose improved magnesium absorption only. For individual rats there were negative relationships between ileal pH and apparent absorption of minerals, the relationship being strongest for magnesium. Neither lactose nor lactulose raised ileal solubility of minerals, which could relate to raised flow of chyme. It is suggested that lactose-induced stimulation of magnesium absorption in rats is caused by a lowering of ileal pH.

Introduction

Dietary lactose (ß-1,4-galactosyl-glucose) versus various other carbohydrates influences mineral absorption in rats. Lactose has been consistently shown to improve apparent magnesium absorption (1-5), whereas apparent calcium absorption was enhanced in some studies (6-9), but not in others (4, 10-12). Lactose was found to accelerate the appearance of label in blood when rats were administered lactose and radioactive phosphate together by gavage (13). However, in feeding trials no effect of lactose on apparent phosphate absorption was found (2).

The mechanism by which lactose stimulates magnesium absorption is not known. Rats become lactase-deficient after weaning (14), and thus lactose is poorly digested. This may induce microbial fermentation of lactose in the intestine which in turn may lower the pH in the intestinal lumen. If insoluble calcium-magnesium-phosphate complexes are formed in the intestine, as has been shown *in vitro* (15), then a lowered pH should improve solubility of these minerals in the intestinal lumen (16). Only soluble minerals may cross the intestinal epithelium (17, 18). Thus, it could be hypothesized that lactose enhances ileal solubility of magnesium and thereby stimulates its absorption, whereas lactose has no major impact on ileal solubility of calcium and phosphate and consequently has no effect on calcium and phosphate absorption either. This hypothesis was tested in an experiment with weanling female rats. To see whether the hypothesis can be generalized, the effects of another poorly digestible carbohydrate, lactulose (ß-1,4-galactosyl-fructose) (19) were studied as well.

Materials and Methods

The experimental protocol was approved by the animal experiment committee of the Department of Laboratory Animal Science, Utrecht State University.

Animals, housing and diets. We used female, outbred Wistar rats (Hsd/Cpb:WU, Harlan, Zeist, The Netherlands), aged about 3 wk. On arrival, the rats were fed *ad libitum* a commercial, pelleted diet (RMH-B, Hope Farms, Woerden, The Netherlands) and tap water. They were housed in wire-topped polycarbonate cages (37.5x22.5x15.0 cm) with a layer of sawdust as bedding. Four d after arrival, all rats were fed the purified, control diet (Table 1) and demineralized water *ad libitum*. After another 8 d (d 0 of the experiment), the rats were divided into 3 groups of 12 animals each, so that group mean body weights were similar. As from d 0, the rats were housed individually in metabolic cages (314 cm^2x12 cm). The cages were placed in a room with controlled temperature (20-22°C), lighting (light: 06.00-18.00 h) and relative humidity (50-65%).

Table 1. *Composition of the experimental diets.*

	Control[1]	Lactose	Lactulose
		g	
Ingredient			
Glucose[2]	709.4	609.4	609.4
Lactose[3]	-	100.0	-
Lactulose[4]	-	-	149.9
Constant components[5]	290.6	290.6	290.6
Chemical analysis			
Dry matter (g/kg)	941	941	893
Calcium (mmol/kg)	112.5	115.0	107.5
Magnesium (mmol/kg)	16.4	16.4	16.4
Phosphate (mmol/kg)	125.8	129.0	135.4

[1] This diet also served as pre-experimental diet.
[2] Morsweet 01934, Cerestar, Haubourdin, France.
[3] Whey Products, Borculo, The Netherlands.
[4] Duphulac, Duphar BV, Amsterdam, The Netherlands. Lactulose preparation contained 33.3% (wt/wt) water.
[5] The constant components consisted of (g): casein, 151; corn oil, 25; coconut fat, 25; cellulose, 30; $CaCO_3$, 12.4; $MgCO_3$, 1.4; $NaH_2PO_4.2H_2O$, 15.1; KCl, 1.0; $KHCO_3$, 7.7; mineral premix, 10; vitamin premix, 12. The mineral premix consisted of (mg): $FeSO_4.7H_2O$, 174; MnO_2, 79; $ZnSO_4.H_2O$, 33; $NiSO_4.6H_2O$, 13; NaF, 2; KI, 0.2; $CuSO_4.5H_2O$, 15.7; $Na_2SeO_3.5H_2O$, 0.3; $CrCl_3.6H_2O$, 1.5; $SnCl_2.H_2O$, 1.9; NH_4VO_3, 0.2; corn meal, 9679.2. The vitamin premix consisted of (mg): thiamin, 4; riboflavin, 3; niacinamide, 20; DL-calcium pantothenate, 17.8; pyridoxine, 6; cyanocobalamin, 50; choline chloride, 2000; folic acid, 1; biotin, 2; menadione, 0.05; DL-α-tocopheryl acetate, 60; retinyl acetate and retinyl palmitate, 8 (4000 IU); cholecalciferol, 2 (1000 IU); corn meal, 9826.15.

During the experimental period (d 0-21, 0-22 or 0-23), one group of rats remained on the control diet. The other groups were fed diets with either 10% (wt/wt) lactose or lactulose. These diets were formulated by adding the carbohydrates to the control diet at the expense of the glucose component. Analysis indicated that the carbohydrates contained negligible amounts (<0.001%, wt/wt) of magnesium, calcium and phosphate.

As from d 0, the rats were transferred gradually to the test diets: one fifth of the control diet was replaced daily by the test diets until the transfer was complete after 5 d. The purified diets, which were in powdered form, were stored at 4 °C until feeding. The rats had free access to food and demineralized water. Feed consumption and body weight were recorded at regular intervals. The experiment lasted 21-23 d.

Collection of samples. From d 17 to 19, feces and urine of each rat were collected quantitatively. The cages and tubes for collection of feces and urine were washed with a phosphate-free detergent (Briljant Rose Biosept, Rogier Bosman Chemie, Heijningen, The Netherlands) and rinsed thoroughly with 0.1 mol/L HCl and demineralized water.

On d 21, between 8.30 and 11.00 h, 4 rats of each dietary group were anesthetized by exposure to diethyl ether. Blood was obtained by orbital puncture and the rats immediately killed by cervical dislocation. The entire small intestine, between stomach and cecum, was removed. The contents in the distal third of the intestine (ileum) was collected in pre-weighed tubes by gently squeezing the intestine between finger and thumb. The intestinal contents was immediately centrifuged (10 min, 10,000xg), and supernatant and pellet were separated. Weight of pellet and supernatant was determined. pH of the supernatant was measured directly (Russell combination pH electrode, Type RS-53, Auchtermuchty Fife, Great Britain). Trichloro acetic acid (TCA) was added to the supernatant to a final concentration of 5.4% (w/v) and the TCA-soluble fraction obtained by centrifugation (2 min, 10,000xg). Pellet and TCA-soluble fractions were analyzed for minerals. Cecum (including its contents), kidney and liver were excised and weighed. On d 22 and 23 the entire procedure was repeated with the remaining rats.

In vitro experiments. To check whether the pH, within the range of observed fluctuation in the intestinal fluid, affects mineral solubility, *in vitro* experiments were carried out. The molar ratio of calcium:phosphate:magnesium in the incubations was chosen to be 6:6:1, which is similar to that in the diet. Incubations had a final volume of 1 ml and contained in distilled water either 50 mmol/L MOPS (pH, 6.4-7.4) or 50 mmol/L HEPES (pH, 7.6-8.2), 3.5 mmol/L $MgCl_2$, 20 mmol/L $CaCl_2$, 20 mmol/L Na_2HPO_4 and variable amounts of NaCl to maintain the ionic strength at 150 mmol/L. The mixture (0.9 ml) without Na_2HPO_4 was pre-incubated for 10 min at 37 °C in a shaking waterbath. Then, 0.1 ml of 200 mmol/L Na_2HPO_4 was added and the mixture further incubated for another 15 min. The tubes were then centrifuged (2 min, 10,000xg), supernatants collected and the pH measured. After dilution of the supernatant with TCA (final concentration 5%, wt/v), magnesium, calcium and phosphate were analyzed.

Chemical analyses. Magnesium and calcium in fractions of intestinal contents, feces, urine, feed samples and supernatants of *in vitro* incubations were analyzed as described elsewhere (20, 21). Phosphate was determined in ashed feed samples dissolved in 6 N HCl with the use of a commercial test combination (Phosphate, MA-KIT 10 ROCHE, Roche Diagnostics, Basel, Switzerland) and a COBAS-BIO auto-analyser (Hoffmann-La Roche BV, Mijdrecht, The Netherlands). For complete recovery of phosphate from the ashed samples, analysis was performed at least 1 wk after dissolution.

Calculations. The distribution of minerals between the solid and liquid phase of ileal contents was calculated. The pellet obtained after centrifugation of the ileal contents comprises the solid phase contaminated with liquid phase. Weight of the solid phase was obtained after freeze-drying the pellet. Weight of the liquid phase was calculated as the sum of weight of liquid phase in the pellet (= total pellet weight minus solid phase) and that of supernatant. The concentration of minerals in the supernatant was assumed to be identical to that in the liquid phase. The amount of minerals in the solid phase was calculated as that in the total pellet minus that in the liquid phase of the pellet. Multiplying mineral concentration (mmol/L) in the supernatant with the weight of the liquid phase gave the amount of minerals in the liquid phase. The fraction of mineral in the liquid phase was computed as percentage of the total amount in the ileal contents.

Apparent absorption of minerals was calculated as mineral intake minus fecal excretion and expressed as percentage of intake. Retention of minerals was calculated as mineral intake minus fecal plus urinary excretion and expressed as percentage of intake.

Statistical analyses. Differences between group means were evaluated with Tukey's test. The level of significance was preset at $P < 0.05$.

Results

Growth and organ weights. Final body weight and feed intake of rats fed the lactulose diet were significantly lower than those of the control group (Table 2). Relative kidney and liver weights were similar for all groups. Weight of cecum with contents was significantly elevated in rats fed lactose or lactulose. Lactulose produced a more than twofold higher cecum weight than did lactose.

Excreta production. Feces production of rats fed the lactulose diet was significantly higher than that of rats fed the control diet containing glucose (Table 2). This was due to the elevated water content of feces in the rats fed lactulose. Lactose also produced an increment in the amount of water in feces but this effect was less pronounced. Group mean urine production and urinary pH were highest in the lactulose group.

Table 2. *Growth performance, selected organ weights and excreta production*[1,2].

	Control	Lactose	Lactulose
Body weight			
Initial (g)	94±2	94±2	94±2
Final (g)	160±4[b]	156±3[ab]	148±2[a]
Feed intake (g/d)	12.6±0.4[b]	12.1±0.1[ab]	11.4±0.2[a]
Relative organ weight (% of body weight)			
Kidney	0.39±0.01	0.38±0.01	0.36±0.01
Cecum	0.83±0.05[a]	1.44±0.09[b]	3.09±0.16[c]
Liver	4.29±0.08	4.31±0.08	4.12±0.07
Feces			
Production (g/d)	0.9±0.1[a]	1.0±0.1[ab]	1.2±0.1[b]
Dry matter (%)	69.3±1.0[c]	60.0±1.6[b]	53.4±1.1[a]
Urine			
Production (mL/d)	4.1±0.3[a]	3.8±0.3[a]	5.8±0.4[b]
pH	8.4±0.3[a]	9.0±0.2[ab]	9.2±0.1[b]

[1] Values are means ± SEM for 12 rats per dietary group.
[2] Group means within a row not sharing a common superscript are significantly different (Tukey's test, $P < 0.05$).

Mineral balance. Table 3 shows that intakes of minerals during the balance period (d 17-19) were similar in the dietary groups. Apparent absorption and retention of magnesium and phosphate were significantly higher in the rats fed lactulose than in those fed glucose. Lactose significantly raised apparent magnesium absorption, but did not affect that of phosphate.

Apparent absorption of calcium was not significantly affected by lactose, whereas percentage calcium absorption was significantly increased in rats fed lactulose.

In the rats fed the lactulose diet, urinary calcium excretion was significantly elevated. Lactose did not significantly affect urinary mineral excretion.

Minerals in ileal lumen. Rats fed lactulose had significantly more liquid phase in their ileal lumen than rats fed the control diet (Table 4). Lactose and lactulose produced a lower group mean pH of the liquid phase than did glucose, but the lowering was statistically significant only for lactulose. The amount of solid phase in the ileal lumen was similar for all dietary groups.

Calcium concentration in liquid phase did not differ significantly between the dietary groups. However, lactose and lactulose raised the amount of calcium in the liquid phase.

Table 3. *Balance of calcium and magnesium*[1,2].

	Control	Lactose	Lactulose
Calcium			
Intake (μmol/d)	1418±61	1402±21	1330±27
Fecal excretion (μmol/d)	708±55[b]	587±23[ab]	503±38[a]
Absorption (μmol/d)	708±46	815±35	826±44
Absorption (% of intake)	50±3[a]	58±2[ab]	62±3[b]
Urinary excretion (μmol/d)	10±1[a]	10±2[a]	30±3[b]
Retention (μmol/d)	699±46	804±35	796±43
Magnesium			
Intake (μmol/d)	198±8	189±2	189±3
Fecal excretion (μmol/d)	95±7[c]	58±2[b]	37±2[a]
Absorption (μmol/d)	103±7[a]	134±4[b]	145±4[b]
Absorption (% of intake)	52±3[a]	70±1[b]	79±1[c]
Urinary excretion (μmol/d)	33±5	37±3	41±3
Retention (μmol/d)	71±5[a]	95±5[b]	102±5[b]
Phosphate			
Intake (μmol/d)	1584±69	1574±24	1675±33
Fecal excretion (μmol/d)	561±42	481±17	474±33
Absorption (μmol/d)	1023±48[a]	1094±34[ab]	1201±43[b]
Absorption (% of intake)	65±2[a]	69±1[ab]	72±2[b]
Urinary excretion (μmol/d)	452±48	448±17	355±14
Retention (μmol/d)	568±45[a]	645±34[b]	847±51[c]

[1] Values are means ± SEM for 12 rats per dietary group.
[2] Group means within a row not sharing a common superscript are significantly different (Tukey's test, $P < 0.05$).

The amount of calcium in solid phase was similar for the three groups. The percentage of total calcium in the liquid phase in the ileal lumen was significantly elevated in the lactulose group and tended to be elevated in the rats fed lactose when compared with the control group.

Group mean magnesium amounts in the liquid and solid phase in rats fed the lactulose diet were lower than in rats fed the other diets. The fraction of soluble magnesium did not differ significantly between the dietary groups.

Concentrations of phosphate in the liquid phase and the amount of phosphate in the solid phase were not significantly affected by the experimental diets. The absolute amount and percentage of phosphate in the liquid phase tended to be elevated in the lactulose group.

Table 4. *Distribution of calcium and magnesium between the liquid and solid phase in the ileal lumen*[1,2].

	Control	Lactose	Lactulose
Liquid phase weight (g)	0.30±0.02[a]	0.36±0.03[ab]	0.42±0.03[b]
Liquid phase (pH)	7.5±0.1[b]	7.2±0.1[ab]	7.0±0.1[a]
Solid phase weight (g)	0.10±0.01	0.09±0.01	0.10±0.01
Calcium			
Amount in liquid phase (μmol)	3.5±0.3[a]	5.8±0.6[b]	6.0±0.8[b]
Amount in solid phase (μmol)	90±12	88±10	62±7
Fraction in liquid phase (%)	4.0±0.5[a]	6.5±0.7[ab]	9.0±1.0[b]
Concentration in liquid phase (mmol/L)	12±2	16±1	14±2
Magnesium			
Amount in liquid phase (μmol)	4.5±0.3[b]	4.5±0.3[b]	2.9±0.3[a]
Amount in solid phase (μmol)	9±1[b]	8±1[b]	5±1[a]
Fraction in liquid phase (%)	35±3	38±3	40±4
Concentration in liquid phase (mmol/L)	16±1[b]	13±1[b]	7±1[a]
Phosphate			
Amount in liquid phase (μmol)	1.3±0.2	1.3±0.2	1.9±0.2
Amount in solid phase (μmol)	67±8	69±7	55±6
Fraction in liquid phase (%)	1.7±0.3	1.5±0.2	3.8±1.8
Concentration in liquid phase (mmol/L)	3.9±0.5	2.6±0.3	4.5±0.7

[1] Values are means ± SEM for 12 rats per dietary group.
[2] Group means within a row not sharing a common superscript are significantly different (Tukey's test, $P < 0.05$).

Ileal pH and mineral absorption. There was a negative relationship between the pH in the ileal lumen and percentage apparent magnesium absorption (Figure 1). Similar relationships, although less strong, were calculated for calcium ($r=-0.40$, $n=36$) and phosphate ($r=-0.44$, $n=36$).

In vitro solubility of minerals. Within the pH range of 6.8 to 7.7, the pH and the percentages of magnesium, calcium and phosphate in the supernatant of the incubations were negatively associated (Figure 2). The increase in solubility with decreasing pH was most pronounced for magnesium.

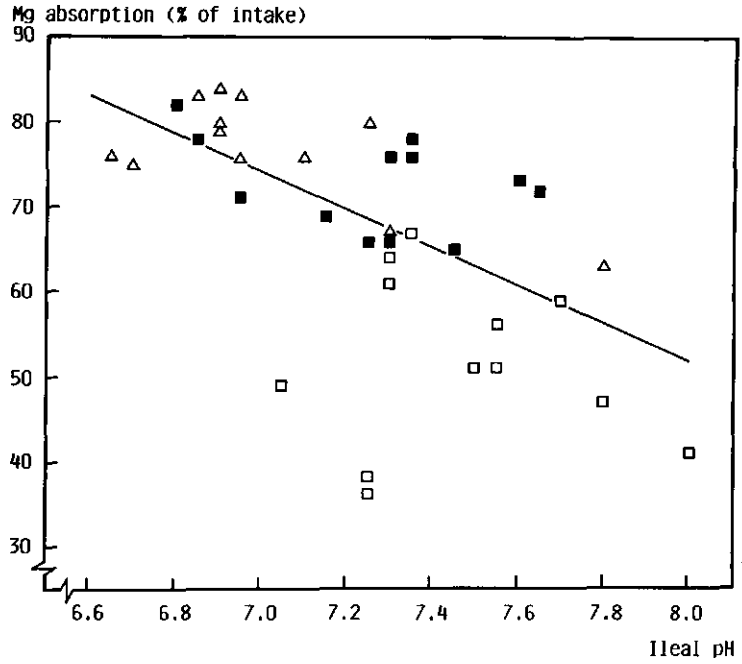

Figure 1. Relationship between pH in the ileal lumen and percentage apparent magnesium absorption in rats fed the experimental diets (y = -22x + 229, r = -0.69, n = 36). □ = control; ■ = lactose; ▲ = lactulose.

Discussion

As would be expected (22, 23), the feeding of either lactose or lactulose produced markedly elevated cecum weights. This substantiates the low digestibility of these carbohydrates (19, 24) and their suitability as substrates for intestinal bacterial fermentation. Lactulose caused an enlargement of intestinal bulk as evidenced by the elevated weight of the ileal liquid phase and raised fecal weight due to enrichment with moisture. Lactose tended to have similar effects, but those of lactulose were much more pronounced. Lactulose also stimulated urine production, which may partly be caused by the higher water content of the lactulose diet.

This study shows that dietary lactose versus glucose significantly enhances the apparent absorption of magnesium, which corroborates other reports (1-5). Lactose versus glucose caused an increment in group mean calcium absorption, but this effect failed to reach statistical significance. Similar results have been published by others (6-9). Lactose in the diet did not significantly affect apparent phosphate absorption, which agrees with

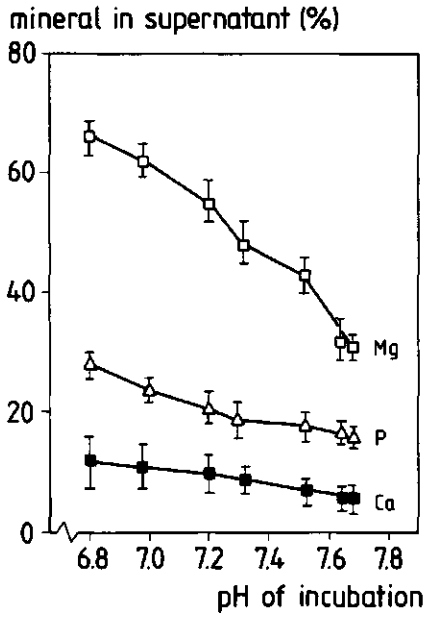

Figure 2. Magnesium, calcium and phosphate in supernatants of incubations with varying pH (means ± SD, n=6). Recovery in supernatant is expressed as percentage of total amount of mineral in the incubation.

other work (2). In this study, marked effects of lactulose on mineral absorption emerged. Lactulose versus glucose significantly stimulated the apparent absorption of magnesium, calcium and phosphate. Another poorly digestible carbohydrate which is structurally related to lactulose, lactitol (ß-D-galacto-pyranosyl-1,4-D-sorbitol), has also been shown to enhance calcium absorption (25).

The major objective of this study was to find out why lactose stimulates magnesium absorption. This can be extended by the question why lactulose enhances the absorption of magnesium, calcium and phosphate. We had hypothesized that lactose lowers the pH in the intestinal lumen, thereby improving the solubility of magnesium which in turn elevates the amount of magnesium that can cross the intestinal epithelium. Indeed, we found that lactose versus glucose tended to lower the pH of the intestinal digesta. With the use of *in vitro* incubations, we demonstrated that a lowering of pH from 7.5 to 7.2, which was found *in vivo* when dietary glucose was replaced by lactose, caused an improved solubility of magnesium. Similar effects, although much less pronounced, were seen for calcium and phosphate. This would explain why lactose stimulated magnesium absorption but not that of calcium and phosphate.

Dietary lactulose significantly lowered the ileal pH from 7.5 to 7.0. In keeping with the effects seen *in vitro*, dietary lactulose versus glucose caused a significant increment in the percentage of calcium in the intestinal liquid phase. Lactulose also raised the fractions of soluble magnesium and phosphate. Due to the large within-group variation, these effects did not reach statistical significance. Thus, the effect of lactulose on group mean ileal solubility of magnesium, calcium and phosphate is consistent with the hypothesis, but the evidence is not solid. Although the pH in the ileal lumen was decreased, the stimulatory effect of lactulose on mineral absorption cannot be explained by a lactulose-induced increment in mineral solubility in the ileal lumen. Nevertheless, for individual rats negative relationships between pH in the ileal lumen and intestinal apparent mineral absorption could be demonstrated. As would be expected on the basis of *in vitro* observations (Figure 2), the relationship was strongest for magnesium (Figure 1).

The concentration of minerals in the liquid phase of the ileal contents may determine mineral absorption rather than the fraction of minerals in the liquid phase. Within this concept, lactulose should elevate the concentration of minerals in the liquid phase. However, as mentioned above this was not seen. As to magnesium, the concentration in the liquid phase was even significantly lowered after feeding lactulose. This may relate to differences in intestinal transit time between rats fed either glucose or lactulose. The intestine of rats fed the lactulose diet tended to contain less minerals (Table 4), possibly due to a raised flow of chyme. It should also be noted that magnesium (18), calcium (26, 27) and phosphate (27) can be absorbed in the cecum and/or colon. Given the marked influence of lactose and lactulose on cecum weight (Table 2), it is likely that these disaccharides alter the milieu of the cecum and/or colon. Whether this influences overall mineral absorption is not known.

In conclusion, under the experimental conditions, the stimulatory effect of lactose on magnesium absorption cannot be explained by increased solubility of magnesium in the ileal lumen. However, the observed negative relationship between pH in the ileal lumen and intestinal magnesium absorption indicates that the decrease in ileal pH after lactose feeding is responsible for the improved magnesium absorption.

References

1. Andrieux, C. & Sacquet, E. (1983) Effects of microflora and lactose on the absorption of calcium, phosphorus and magnesium in the hindgut of the rat. Repr. Nutr. Develop. **23**: 258-271.
2. Schaafsma, G., Dekker, P.R. & De Waard, H. (1988) Nutritional aspects of yoghurt. 2. Bioavailibility of essential minerals and trace elements. Neth. Milk. Dairy J. **42**: 135-146.
3. Brink, E.J., Dekker, P.R., van Beresteijn, E.C.H., Beynen, A.C. (1991) Inhibitory effect of dietary soybean protein vs. casein on magnesium absorption in rats. J. Nutr. **121**: 1374-1381.
4. Behling, A.R. & Greger, J.L. (1990) Importance of lactose in yoghurt for mineral utilization. J. Agr. Food Chem. **38**: 200-204.
5. Greger, J.L., Gutkowski, C.M. & Khazen, R.R. (1989) Interactions of lactose with calcium, magnesium and zinc in rats. *J. Nutr.* **119**: 1691-1697.
6. Armbrecht, H.J. & Wasserman, R.H. (1976) Enhancement of Ca^{++} uptake by lactose in the rat small intestine. *J. Nutr.* **106**: 1265-1271.

7. Favus, M.J. & Angeid-Backmann, E. (1984) Effects of lactose on calcium absorption and secretion by rat ileum. *Am. J. Physiol.* **246**: G281-G285.
8. Fournier, P., Dupuis, Y & Fournier, A. (1971) Effect of lactose on the absorption of alkaline earth metals and intestinal lactase activity. *Isr. J. Med. Sci.* **7**: 389-391.
9. Leichter, J. & Tolensky, A.F. (1975) Effect of dietary lactose on the absorption of protein, fat and calcium in the postweanling rat. *Am. J. Clin. Nutr.* **28**: 238-241.
10. Greger, J.L., Krzykowski, C.E., Khazen, R.R. & Krashoc, C. (1987) Mineral utilization by rats fed commercially available calcium supplements or milk. *J. Nutr.* **117**: 717-724.
11. Recker, R.R., Bammi, A., Barger-Lux, J. & Heaney, R. (1988) Calcium absorbability from milk products, an imitation milk and calcium carbonate. *Am. J. Clin. Nutr.* **47**: 93-95.
12. Sheikh, M.S., Santa Ana, C.A., Nicar, M.J., Schiller, L.R. & Fordtran, J.S. (1987) Gastrointestinal absorption of calcium salts. *New Engl. J. Med.* **317**: 532-536.
13. Debiec, H. & Lorenc, R. (1988) Influence of lactose on phosphate metabolism in rats. *Br. J. Nutr.* **59**: 87-92.
14. De Groot, A.P. & Hoogendoorn, P. (1957) The detrimental effect of lactose. II. Quantitative lactase determinations in various mammals. *Neth. Milk. Dairy J.* **11**: 290-303.
15. Boskey, A.L. & Posner, A.S. (1974) Magnesium stabilization of amorphous calcium phosphate. *Mat. Res. Bull.* **9**: 907-916.
16. Greenwald, I., Redish, J. & Kibrick, A.C. (1940) The dissociation of calcium and magnesium phosphates. *J. Biol. Chem.* **135**: 65-76.
17. Bronner, F. (1987) Intestinal calcium absorption, mechanisms and applications. *J. Nutr.* **117**: 1347-1352.
18. Hardwick, L.L., Jones, M.R, Brautbar, N. & Lee, D.B.N. (1991) Magnesium absorption: mechanisms and the influence of vitamin D, calcium and phosphate. *J. Nutr.* **121**: 13-23.
19. Pomare, E.W., Branch, W.J. & Cummings, J.H. (1985) Carbohydrate fermentation in the human colon and its relation to acetate concentrations in venous blood. *J. Clin. Invest.* **75**: 1448-1454.
20. Hoek, A.C., Lemmens, A.G., Mullink J.W.M.A & Beynen A.C. (1988) Influence of dietary calcium:phosphorus ratio on mineral excretion and nephrocalcinosis in female rats. *J. Nutr.* **118**: 1210-1216.
21. Mars, Y.W.H.M., Lemmens, A.G., & Beynen A.C. (1988) Dietary phosphorus and nephrocalcinosis in female rats. *Nutr. Rep. Int.* **38**: 249-258.
22. Henskens, Y.M.C., Ritskes-Hoitinga, J., Mathot, J.N.J.J., van Camp, I. & Beynen, A.C. (1991) The influence of dietary lactose on phosphorus-induced nephrocalcinosis in female rats. *Int. J. Vit. Nutr. Res.* **61**: 77-86.
23. Beynen, A.C (1989) Increased concentration of liver cholesterol in rats fed lactulose. *Die Nahrung* **33**: 89-90.
24. Andrieux, C., Gadelle, D., Leprince, C. & Sacquet, E. (1989) Effects of some poorly digestible carbohydrates on bile acid bacterial transformations in the rat. *Br. J. Nutr.* **62**: 103-119.
25. Ammann, P., Rizzoli, R. & Fleisch, H. (1988) Influence of the disaccharide lactitol on intestinal absorption and body retention of calcium in rats. *J. Nutr.* **118**: 793-795.
26. Petith, M.M. & Schedl, H.P. (1976) Intestinal adaptation to dietary calcium restriction: *in vivo* cecal and colonic calcium transport in the rat. *Gastroenterology* **71**: 1039-1042.
27. Beynen, A.C., West, C.E., Spaaij, C.J.K., Huisman, J., van Leeuwen, P., Schutte, J.B. & Hackeng, W.H.L. (1990) Cholesterol metabolism, digestion rates and postprandial changes in serum of swine fed purified diets containing either casein or soybean protein. *J. Nutr.* **120**: 422-430.

Chapter 6

Interaction of calcium and phosphate decreases ileal magnesium solubility and apparent magnesium absorption in rats

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Abstract. We have tested the hypothesis that increased intakes of calcium and phosphate lower magnesium solubility in the intestinal lumen, causing a decreased magnesium absorption. In *in vitro* experiments at a constant magnesium concentration, increasing calcium concentrations reduced magnesium solubility. This effect did not occur in the absence of phosphate. Increasing phosphate concentrations decreased the solubility of magnesium in the presence, but not in the absence of calcium. These results suggest that the formation of an insoluble calcium-magnesium-phosphate complex determines magnesium solubility. To extend this concept to *in vivo* conditions, rats were fed purified diets containing a constant concentration of magnesium (16.4 μmol/g) but different concentrations of calcium (25, 100 or 175 μmol/g) and phosphate (58, 103 or 161 μmol/g). Increased intakes of calcium decreased magnesium solubility in the ileal lumen and lowered magnesium absorption. The latter occured only if the dietary phosphate concentration was at least 103 μmol/g. Increasing dietary phosphate concentrations reduced both magnesium solubility in the intestine and magnesium absorption, but only if the dietary calcium concentration was at least 100 μmol/g. These results support those obtained *in vitro*. It is concluded that increased intakes of calcium and phosphate decrease magnesium absorption by the formation of an insoluble calcium-magnesium-phosphate complex in the intestinal lumen.

Introduction

Increased intakes of calcium have been reported to impair apparent intestinal absorption of magnesium in rats (1-5), but not in humans (6-10). No explanation has been offered for this species-dependent effect of dietary calcium (see 11 for review). Because it has been shown *in vitro* that magnesium can form an insoluble complex with calcium and phosphate (12), we hypothesize that this also might occur in the intestine. Magnesium absorption likely depends on the concentration of soluble magnesium in the intestine (13,14). Therefore the intestinal formation of an insoluble calcium-magnesium-phosphate complex may affect magnesium absorption. The low calcium concentrations in human diets, as compared with rat diets, might result in less supersaturation of the human intestinal lumen, and consequently in less formation of insoluble complexes. Furthermore, human diets have a lower molar phosphate:magnesium ratio than rat diets, the ratios being about three (10) and six (15), respectively. This relatively low phosphate:magnesium ratio in human diets, as compared with rat diets, might result in less formation of insoluble complexes in the human intestine in the presence of extra calcium. This would explain why addition of calcium to human diets does not affect magnesium absorption whereas addition of calcium to rat diets inhibits it.

In the present studies it was tested whether the inhibitory effect of calcium on magnesium absorption observed in rats but not in humans is caused by decreased intestinal magnesium solubility due to the formation of an insoluble calcium-magnesium-phosphate complex. In *in vitro* experiments the effects of various calcium concentrations on solubility of magnesium were measured at different phosphate, but constant magnesium concentrations. Further, in a feeding trial with rats using diets with different phosphate but constant

magnesium concentrations, the effects of various dietary calcium concentrations on magnesium solubility in the ileal lumen and on apparent magnesium absorption were determined. The ileal site is predominant in magnesium absorption (11).

Materials and Methods

In vitro experiments. We investigated the effects of calcium on magnesium solubility at different phosphate but constant magnesium concentrations. The molar phosphate:magnesium ratio in the incubations ranged from 0 to 10. Ratios of 3 and 6 were included, which are similar to those in human (10) and rat diets (15), respectively. The incubations in Eppendorf tubes (Greiner BV, Alphen a/d Rijn, The Netherlands) had a final volume of 1 mL, and contained di-sodium hydrogen phosphate (0-50 mmol/L), calcium chloride (0-30 mmol/L), magnesium chloride (5 mmol/L) and N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) buffer (50 mmol/L) in distilled water. Various amounts of sodium chloride were added to maintain a constant ionic strength of 150 mM. After 15 min at 37°C, the tubes were centrifuged for 2 min at 10,000*g. The supernatants were collected and the pH was measured at 37°C; it ranged from 6.96 to 7.04, which was comparable to the pH of the ileal contents (see below). After acidification with trichloroacetic acid (TCA) to a final concentration of 50 g/L, magnesium in the supernatant was analyzed by atomic absorption spectrophotometry (AAS) (Perkin Elmer 1100, Bodenseewerk Perkin Elmer & Co GmbH, Uberlingen, Germany). Solubility of magnesium was measured as absolute amount of magnesium in the supernatant.

In vivo experiment. Using rats, we investigated whether the amount of dietary calcium affects magnesium solubility in the intestinal lumen and whether this parameter is associated with the efficiency of magnesium absorption. Specifically, we addressed the question whether the effect of dietary calcium depends on the concentration of phosphate in the diet. The experimental protocol was approved by the animal ethical committee of the Agricultural University, Wageningen, The Netherlands and its execution supervised by the animal welfare officer.

We used 56 male Wistar rats (Cpb:WU) from a local breeding colony (Laboratory Animals Center, Agricultural University, Wageningen, The Netherlands). They were 10 wk of age and had a mean body weight of 293 g. The rats were housed individually in metabolic cages in a room with controlled temperature (20-22°C), relative humidity (50-60%) and lighting (light, 06.00-18.00 h). They were randomly divided into 7 dietary groups of 8 animals each and were fed experimental diets containing different calculated concentrations of calcium (25, 100 or 175 μmol/g) and phosphate (58, 103 or 161 μmol/g) and a constant magnesium concentration (16.4 μmol/g) for 28 d. The duration of the experimental period is identical to that used in our other rat studies on magnesium absorption (16, 17). The composition of the diets is shown in Table 1; calculated mineral concentrations agreed well with those analyzed (Table 1). The phosphate concentrations were chosen in such a way that

Table 1. *Composition of the experimental diets.*

	Dietary treatment[1]						
	LCaLP	MCaLP	HCaLP	LCaMP	MCaMP	HCaMP	HCaHP
				g/kg			
Ingredient							
Constant components[2]	604.4	604.4	604.4	604.4	604.4	604.4	604.4
$CaCO_3$	2.1	9.6	17.1	2.1	9.6	17.1	17.1
$NaH_2PO_4.H_2O$	1.6	1.6	1.6	7.9	7.9	7.9	15.9
Na_2CO_3	5.5	5.5	5.5	3.1	3.1	3.1	0.0
Dextrose	386.4	378.9	371.4	382.5	375.0	367.5	362.6
Total	1000	1000	1000	1000	1000	1000	1000
				mmol/kg			
Chemical analysis							
Calcium	25	103	175	25	103	180	178
Magnesium	16.3	16.5	16.4	16.4	16.3	16.4	16.4
Phosphorus	58	59	58	106	112	106	165
Sodium	119	113	116	116	120	119	116

[1] LCaLP = low calcium, low phosphate, phosphate:magnesium 3.5; MCaLP = moderate calcium, low phosphate, phosphate:magnesium 3.5; HCaLP = high calcium, low phosphate, phosphate:magnesium 3.5; LCaMP = low calcium, moderate phosphate, phosphate:magnesium 6.3; MCaMP = moderate calcium, moderate phosphate, phosphate:magnesium 6.3; HCaMP = high calcium, moderate phosphate, phosphate:magnesium 6.3; HCaHP = high calcium, high phosphate, phosphate:magnesium 9.8.

[2] The constant components consisted of (g/kg): casein, 200; DL-methionine, 3; palm oil, 150; wheat starch, 150; cellulose, 50; magnesium sulphate, 3.9; mineral premix, 35; vitamin premix, 10; cholecalciferol, 0.25 (1000 IU); retinyl-acetate, 0.25 (4000 IU); choline chloride, 2. The mineral premix consisted of the following (mg): $KHCO_3$, 3240; KCl, 4410; sodium citrate ($C_6H_5Na_3O_7.2H_2O$), 4320; $FeSO_4.7H_2O$, 175; $MnSO_4.H_2O$, 238; $ZnSO_4.7H_2O$, 483; $CuSO_4.5H_2O$, 112; KIO_3, 0.4; $Na_2SeO_3.5H_2O$, 0.4; $CrK(SO_4)_2.12H_2O$, 19; dextrose, 22002.2. The vitamin premix consisted of the following (mg): thiamin, 6; riboflavin, 6; pyridoxine, 7; nicotinamide, 30; DL-calciumpantothenate, 16; folic acid, 2; D-biotin, 0.2; cyanocobalamine, 0.01; DL-α-tocopherylacetate, 100; menadione, 0.05; dextrose, 9832.74.

the phosphate:magnesium ratios were comparable with those used in the *in vitro* experiments. At the phosphate:magnesium ratios of 3.5 and 6.3, three different calcium concentrations were introduced. At the phosphate:magnesium ratio of 9.8, one calcium concentration (175 μmol/g) was used.

The diets were in powdered form and were stored at 4°C until feeding. Diets and demineralized water were freely available. Feed consumption was recorded daily and body weights were measured weekly. During the last four days of the experimental period (d 25-28), feces and urine of each animal were collected separately and measured quantitatively. At the end of the experimental period (d 28), between 08.30 and 10.30 h, the animals were killed by decapitation. Total ileal contents were collected in Eppendorf tubes by gently

squeezing the contents of the distal one-third of the intestine (ileum) between finger and thumb. The ileum was chosen for the *in vivo* solubility studies because this intestinal segment is most prominent in magnesium absorption (11). The ileal contents were centrifuged for 10 min at 10,000×g and the pellet and supernatant were separated. The pH of the supernatant was measured. After acidification with TCA to a final concentration of 50 g/L and the magnesium concentration in the supernatant was analyzed by AAS.

Diet samples, feces and pellets of ileal contents were freeze-dried, homogenized and wet ashed with nitric acid (14 mol/L) and perchloric acid (12 mol/L). After appropriate dilution, magnesium was analyzed in ashed samples as described above. Diet samples were also analyzed for sodium by atomic emission spectrophotometry (Perkin Elmer 1100, Bodenseewerk Perkin Elmer & Co GmbH, Uberlingen, Germany), for calcium by AAS and for phosphate by the Fiske Subbarow method (18).

Solubility of magnesium was estimated as magnesium present in the liquid phase of the ileal contents and expressed as percentage of the sum of magnesium in the supernatant and pellet. Weight of the liquid phase was calculated as the sum of weights of supernatant and liquid phase in the pellet (=total pellet weight minus weight of pellet after freeze-drying). Apparent intestinal absorption of magnesium was calculated as magnesium intake minus fecal excretion, and was expressed as percentage of intake.

Statistics. Analysis of variance was applied to test the significance of main and interaction effects. Tukey's test (19) was used to compare group means. The level of significance was preset at $P < 0.05$.

Results

In vitro experiments.

Effect of calcium on solubility of magnesium. Figure 1 shows the amount of soluble magnesium, that is magnesium in the supernatant, as function of varying calcium and phosphate concentrations in incubations with a constant concentration of magnesium (5 mmol/L). When phosphate was absent (phosphate:magnesium ratio = 0), calcium did not affect the solubility of magnesium. At phosphate:magnesium ratios of either 6 or 10, the decrease of magnesium solubility with increasing calcium concentration was more pronounced than at the phosphate:magnesium ratio of 3. Figure 1 also illustrates that at every calcium concentration an increase in phosphate:magnesium ratio decreased soluble magnesium. Analogous effects were found at a lower (2.5 mmol/L) or higher (10 mmol/L) constant magnesium concentration (results not shown).

Effect of phosphate on solubility of magnesium. Figure 2 shows that in the absence of calcium increasing the phosphate concentration in the incubation did not affect the amount of soluble magnesium. However, in the presence of 30 mmol/L calcium, there was a drastic

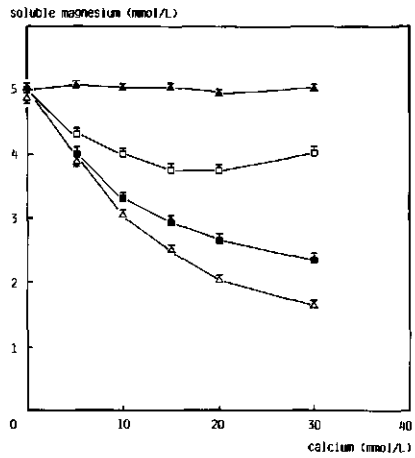

Figure 1. In vitro effects of calcium on solubility of magnesium at different phosphate:magnesium ratios (means ± SEM, n=6). The magnesium concentration was maintained at 5 mmol/L. The molar phosphate:magnesium ratios used were: 0 (▲), 3 (□), 6 (■) and 10 (△). Analysis of variance: calcium effect, $P < 0.001$; phosphate effect, $P < 0.001$; interaction (calcium x phosphate), $P < 0.001$.

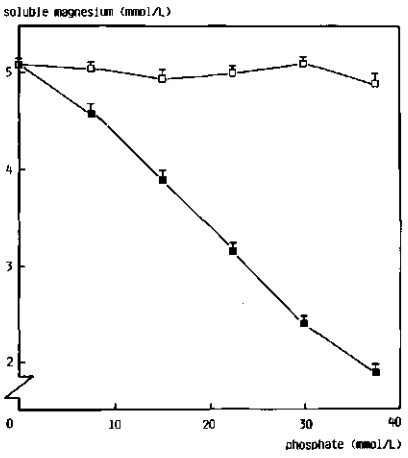

Figure 2. In vitro effects of phosphate on solubility of magnesium at two different concentrations of calcium (means ± SEM, n=6). The magnesium concentration was kept constant at 5 mmol/L. Calcium concentrations were 0 (□) and 30 (■) mmol/L. Analysis of variance: phosphate effect, $P < 0.001$; calcium effect, $P < 0.001$; interaction (calcium x phosphate), $P < 0.001$.

reduction of the amount of magnesium in the supernatant with increasing phosphate concentration.

In vivo experiment.

Body weight and feed intake. Body weight gain and feed consumption did not differ significantly between the dietary groups (results not shown); final body weight was on average 363 ± 5 g and feed consumption 18.6 ± 0.3 g/d (mean ± SEM, n = 56).

Dietary calcium and solubility of magnesium in the ileal lumen. There were no significant differences in pH of the ileal contents between the dietary groups (Table 2). The amounts of magnesium in total ileal contents (Table 2) and the volumes of liquid phases of the ileal contents (results not shown) did not differ significantly between the dietary groups. Therefore, ileal solubility of magnesium was expressed as percentage of the total amount of magnesium in the ileal contents (Figures 3 and 4). Increasing dietary calcium concentrations caused a decrease in the amount of soluble magnesium in the ileal lumen (Figure 3A). This effect was greater at a dietary phosphate:magnesium ratio of 6.3 than of 3.5.

Dietary calcium and magnesium balance. Because magnesium intake was almost identical in rats fed the experimental diets (Table 2), apparent magnesium absorption is expressed as

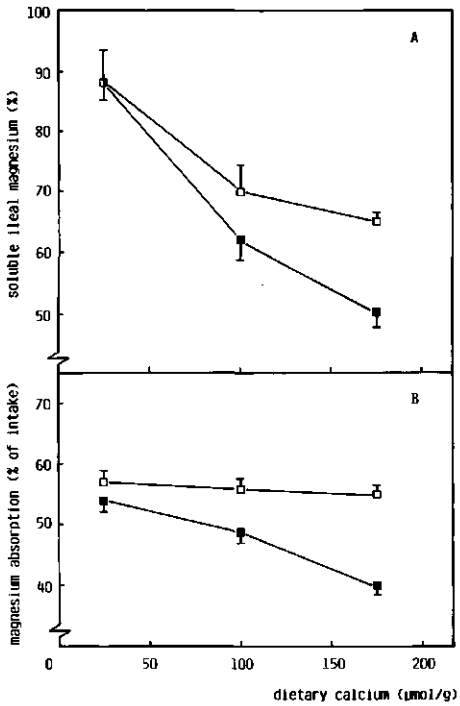

Figure 3. *In vivo* effects of calcium concentration and phosphate:magnesium ratio in the diet on soluble magnesium in the ileal lumen (panel A) and on apparent magnesium absorption in rats (panel B). Results are expressed as means ± SEM (n=8). The dietary magnesium concentration was kept constant at 16.4 µmol/g. Molar phosphate:magnesium ratios were 3.5 (□) and 6.3 (■). Analysis of variance: panel A, calcium effect, $P < 0.001$; phosphate effect, $P < 0.01$; interaction (calcium x phosphate), not significant; panel B, calcium effect, $P < 0.001$; phosphate effect, $P < 0.001$; interaction (calcium x phosphate), $P < 0.05$.

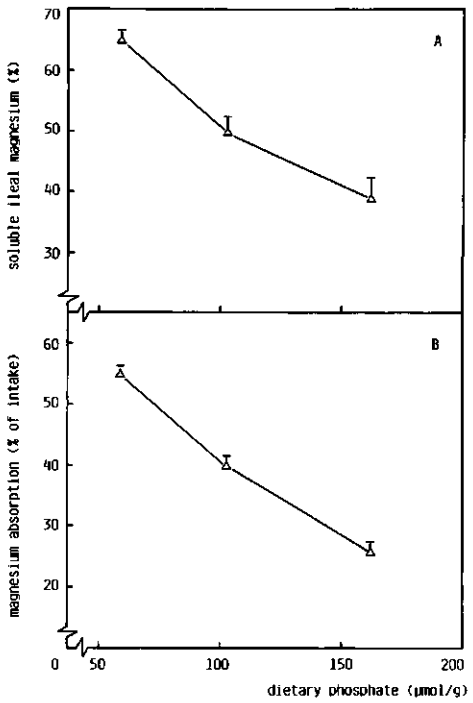

Figure 4. *In vivo* effects of dietary phosphate concentration, at constant calcium (175 µmol/g) and magnesium (16.4 µmol/g) concentrations, on soluble magnesium in the ileal lumen (panel A) and on apparent magnesium absorption in rats (panel B). Results are expressed as means ± SEM (n=8). Within each panel, group means were significantly different ($P < 0.05$; Tukey's test).

percentage of intake (Figures 3 and 4). Increasing dietary calcium concentrations did not affect apparent magnesium absorption at the low phosphate:magnesium ratio of 3.5 (Figure 3B). At the phosphate:magnesium ratio of 6.3, increasing the dietary calcium concentration from 100 to 175 µmol/g significantly ($P < 0.05$) decreased apparent magnesium absorption. This effect of calcium on magnesium absorption was associated with decreased urinary magnesium excretion (Table 2). Magnesium concentration in plasma was not significantly affected by dietary calcium concentration (Table 2).

Dietary phosphate and solubility of magnesium in the ileal lumen. Increasing the amount of dietary phosphate at the highest dietary calcium concentration (175 µmol/g) significantly decreased the ileal solubility of magnesium (Figure 4A).

Table 2. *Effect of dietary calcium and phosphate on magnesium metabolism*[1].

	Dietary treatment[2]						
	LCaLP	MCaLP	HCaLP	LCaMP	MCaMP	HCaMP	HCaHP
Mg intake (μmol/d)	280±13	272±14	288±15	273±9	285±12	296±14	283±19
Urinary Mg (μmol/d)	123±8[c]	144±25[c]	128±8[c]	119±5[c]	120±5[c]	90±3[b]	62±2[a]
Plasma Mg (mmol/L)	0.99±0.04	0.99±0.07	0.91±0.03	0.95±0.04	0.93±0.04	1.00±0.07	0.82±0.03
Ileal Mg (μmol/g)[3]	21±1	21±1	20±1	22±1	21±1	20±1	20±1
Ileal pH	7.0±0.1	7.1±0.2	7.0±0.3	6.9±0.1	7.0±0.3	7.1±0.2	7.1±0.2

[1] Values are means ± SEM for eight rats per dietary group. Means within a row not sharing the same superscript are significantly different ($P < 0.05$, Tukey's test).
[2] See Table 1.
[3] μmol magnesium per g wet total ileal contents.

Dietary phosphate and magnesium balance. Increasing the phosphate concentration in the diet at the highest dietary calcium concentration (175 μmol/g) significantly decreased magnesium absorption (Figure 4B). The decreased magnesium absorption was associated with decreased urinary magnesium excretion (Table 2). Magnesium concentration in plasma was not significantly influenced by dietary phosphate concentration.

Solubility of magnesium in the ileal lumen and magnesium absorption. The relationship for group means of magnesium concentrations in liquid phase of ileal contents and apparent magnesium absorption is illustrated in Figure 5. Whereas a positive, pseudo-linear relationship was observed for soluble ileal magnesium concentrations between 10 and 18 mmol/L, it should be noted that absorption became saturated at higher soluble magnesium concentrations.

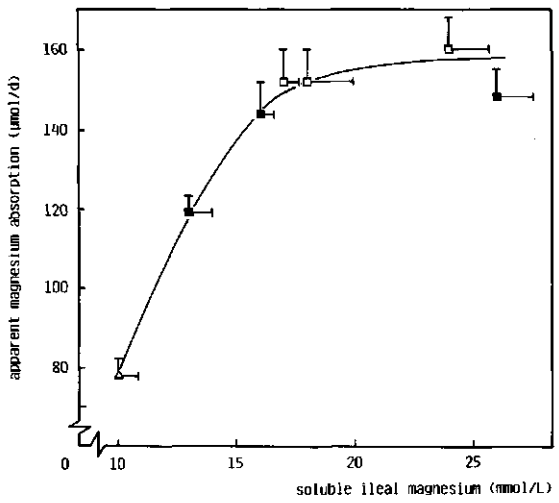

Figure 5. Relationship between soluble magnesium in the ileal lumen and intestinal magnesium absorption in rats (means ± SEM, n=8), at a constant dietary magnesium concentration (16.4 μmol/g). Symbols: □, dietary phosphate = 58 μmol/g and dietary calcium = 25, 100 or 175 μmol/g; ■, dietary phosphate = 103 μmol/g and dietary calcium = 25, 100 or 175 μmol/g; ▲, dietary phosphate = 161 μmol/g and dietary calcium is 175 μmol/g.

Discussion

The present study shows that the inhibiting effect of dietary calcium on magnesium absorption depended on the dietary concentration of phosphate. Likewise, the effect of dietary phosphate on magnesium absorption was determined by the dietary concentration of calcium. Separate inhibitory effects of calcium (1-5) and phosphate (20-24) on apparent magnesium absorption have been reported before, but adequate explanations for these effects have not been given (11).

The observed relationship between soluble magnesium in intestinal lumen and apparent magnesium absorption (Figure 5) suggests that magnesium solubility is an important determinant of magnesium absorption. In this light, the *in vitro* and *in vivo* experiments might explain why there is an interaction of dietary calcium and phosphate concentrations with regard to the effects of these minerals on magnesium absorption. In *in vitro* incubations, increasing the calcium concentration reduced magnesium solubility. This effect did not occur in absence of phosphate but became greater with increasing amounts of phosphate (Figure 1). Phosphate-induced lowering of magnesium solubility was seen only if sufficient calcium was present in the incubation (Figure 2). This indicates that precipitation of a calcium-magnesium-phosphate complex determines magnesium solubility. Variation of dietary calcium and phosphate concentrations had similar effects on soluble magnesium in the intestinal lumen of rats as did variation of calcium and phosphate concentrations on magnesium solubility under *in vitro* conditions. This suggests that formation of a calcium-magnesium-phosphate complex in the intestine determines magnesium solubility which in turn determines the amount of magnesium absorbed. The efficiency of magnesium absorption and the concentration of soluble magnesium in the ileal lumen differed between the dietary groups, but the total amount of magnesium in the ileum was almost identical for all seven dietary groups. This suggests that at the time and site of sampling the amount of magnesium absorbed was very small compared to that present in the intestinal lumen.

The reported lack of effect of dietary calcium on magnesium absorption in humans (6-10) is probably caused by both the relatively low calcium concentration and low phosphate:magnesium ratio in human diets. This low calcium concentration in human diets, as compared with rat diets, might result in limited supersaturation of the human intestinal lumen, and consequently lead to sparse formation of insoluble calcium-magnesium-phosphate complexes. Based on the observation that formation of insoluble calcium-magnesium-phosphate complexes is critically dependent on the concentration of calcium as well as on the phosphorus:magnesium ratio (Figure 1), it is anticipated that only small amounts of these complexes will be formed in the human intestine. At the low dietary phosphate:magnesium ratio of 3.5, which occurs in human diets (10), increasing dietary calcium concentration from 100 to 175 μmol/g had a negligible effect on magnesium solubility in the intestinal lumen (Figure 3A) and did not affect magnesium absorption at all (Figure 3B). At the dietary phosphate:magnesium ratio of 6, which generally occurs in rat diets (15), it is likely that abundant amounts of calcium-magnesium-phosphate complexes can be formed in the intestine

after increasing dietary calcium concentrations from 100 to 175 μmol/g. As a result, the amount of soluble magnesium in the intestinal lumen and apparent intestinal magnesium absorption should be decreased, as was indeed observed (Figure 3).

As to the impact of the present results on magnesium absorption from cow's milk versus human milk we can only speculate. The molar phosphorus:magnesium ratios in cow's milk and human milk are 6 and 3, respectively (25). The calcium concentration in cow's milk is almost fourfold higher than in human milk, the concentrations being 31 and 8 mmol/L (25). According to the results of this study, magnesium absorption would be significantly lower in infants fed cow's milk as sole source of food than in infants fed human milk. In practice, however, cow's milk as such is not used; special formulas based on cow's milk may rather be used. The composition of the whole formula will determine magnesium absorption.

It is generally assumed that magnesium is absorbed by two mechanisms: a non-saturable, linear transport and a saturable, carrier-mediated process involving facilitated diffusion (11, 26). *In vitro* studies (13, 14) indicated that there is a non-saturable, paracellular component of magnesium absorption, implying that absorption of magnesium is linearly dependent on its luminal concentration. However, the observed relationship between the concentration of soluble magnesium in ileal lumen and apparent absorption of magnesium (Figure 5) suggests that magnesium absorption *in vivo* is determined by a saturable, carrier-mediated process. There may be a direct competition between magnesium and calcium for a common carrier system in the ileum (13, 27, 28) but this could not be demonstrated for the duodenum (29) or colon (30). However, in a recent study (31) competition between magnesium and calcium for ileal uptake could not be confirmed. It has been suggested that competition for carrier mediated transport is responsible for the reduction of magnesium absorption by increased calcium intakes in rats (26, 32). This mechanism does not explain the lack of effect of dietary calcium on magnesium absorption in humans. Our observation that increased intakes of calcium did not reduce magnesium absorption at a relatively low dietary phosphate concentration (Figure 3B) suggests that there is no common mechanism for transport of magnesium and calcium across the intestinal mucosa. Our results indicate that the inhibitory effect of dietary calcium on magnesium absorption depends on the phosphate:magnesium ratio of the diet which determines whether or not an insoluble calcium-magnesium-phosphate complex is formed in the intestine.

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References

1. Schaafsma, G., Duursma, S.A., Visser, W.J. & Dekker, P.R. (1985) The influence of dietary calcium on kidney calcification and renal function in rats fed high-phosphate diets. *Bone* **6**: 155-163.

2. Greger, J.L., Krzykowski, C.E., Khazen, R.R. & Krashoc, C.L. (1987) Mineral utilization by rats fed various commercially available calcium supplements or milk. *J. Nutr.* **117**: 717-724.
3. Behling, A.R., Kaup, S.M. & Greger, J.L. (1990) Changes in intestinal function of rats initiated with DMH and fed varying levels of butterfat, calcium and magnesium. *Nutr. Cancer* **13**: 189-199.
4. Kaup, S.M., Behling, A.R., Choquette, L. & Greger, J.L. (1990) Calcium and magnesium utilization in rats: Effect of dietary butterfat and calcium and of age. *J. Nutr.* **120**: 266-273.
5. Hoek, A.C., Lemmens, A.G., Mullink, J.W.M.A. & Beynen, A.C. (1988) Influence of dietary calcium:phosphorus ratio on mineral excretion and nephrocalcinosis in female rats. *J. Nutr.* **118**: 1210-1216.
6. Lewis, N.M., Marcus, M.S.K., Behling, A.R. & Greger, J.L. (1989) Calcium supplements and milk: effects on acid-base balance and on retention of calcium, magnesium and phosphorus. *Am. J. Clin. Nutr.* **49**: 527-533.
7. Storey, M.L., Greger, J.L., Kiratli, B.J. & Smith, E.L. (1988) Urinary calcium and magnesium excretion by women in response to short-term calcium supplementation. *Nutr. Res.* **8**: 617-624.
8. Spencer, H., Schwartz, R., Norris, C. & Osis, D. (1985) Magnesium-28 studies and magnesium balances in man. *J. Am. Coll. Nutr.* **4**: 316.
9. Spencer. H. (1979) Calcium and magnesium interaction man. *Clin. Chem.* **25**: 1043.
10. Van der Meer, R., Welberg, J.W.M, Kuipers, F., Kleibeuker, J.H, Mulder, N.H., Termont, D.S.M.L., Vonk, R.J., de Vries, H.T. & de Vries, E.G.E. (1990) Effects of supplemental dietary calcium on the intestinal association of calcium, phosphate, and bile acids. *Gastroenterology* **99**: 1653-1659.
11. Hardwick, L.L., Jones, M.R., Brautbar, N. & Lee, D.B.N. (1991) Magnesium absorption: mechanisms and the influence of vitamin D, calcium and phosphate. *J. Nutr.* **121**: 13-23.
12. Boskey, A.L. & Posner, A.S. (1974) Magnesium stabilization of amorphous calcium phosphate. *Mat. Res. Bull.* **9**: 907-916.
13. Care, A.D. & van 't Klooster, A.Th. (1965) In vivo transport of magnesium and other cations across he wall of the gastro-intestinal tract of the sheep. *J. Physiol.* **177**: 174-191.
14. Ross, D.B. (1962) In vitro studies on the transport of magnesium across the intestinal wall of the rat. *J. Physiol.* **160**: 417-428.
15. American Institute of Nutrition (1977) Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. *J. Nutr.* **117**: 1340-1348.
16. Brink, E.J., Dekker, P.R., van Beresteijn, E.C.H. & Beynen A.C. (1991) Inhibitory effect of dietary soybean protein versus casein on magnesium absorption in rats. *J. Nutr.* **121**: 1374-1381.
17. Brink, E.J., Dekker, P.R., van Beresteijn E.C.H. & Beynen, A.C. Bioavailability of magnesium and calcium from cow's milk and soybean beverage in rats. *Br. J. Nutr.*: in press.
18. Fiske, C.H. & Subbarow, Y. (1925) The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**: 375-400.
19. Tukey, J.W. (1953) The problem of multiple comparisons. Princeton, N.J.: Princeton University.
20. Mars, Y.W.H.M., Lemmens, A.G. & Beynen, A.C. (1988) Dietary phosphorus and nephrocalcinosis in female rats. *Nutr. Rep. Int.* **38**: 249-258.
21. Forbes, R.M. (1963) Mineral utilization in the rat. I. Effects of varying dietary ratios of calcium, magnesium and phosphorus. *J. Nutr.* **80**: 321-326.
22. Toothill, T. (1963) The effect of certain dietary factors on the apparent absorption of magnesium by the rat. *Br. J. Nutr.* **17**: 121-143.
23. O'Dell, B.L. & Morris, E.R. (1963) Relationship of excess calcium and phosphorus to magnesium requirement and toxicity in guinea pigs. *J. Nutr.* **81**: 175-181.
24. Bunce, G.E., Chiemchaisri, Y. & Phillips, P.H. (1962) The mineral requirement of the dog. IV. Effect of certain dietary and physiologic factors upon magnesium deficiency syndrome. *J. Nutr.* **76**: 23-29.
25. Greer, F.R., Calcium, phosphorus and magnesium: How much is too much for infant formulas. *J. Nutr.* **119**: 1846-1851.
26. Ebel, H. & Gunther, T. (1980) Magnesium metabolism: a review. *J. Clin. Chem. Clin. Biochem.* **18**: 257-270.
27. Alcock, N. & Macintyre, I. (1962) Inter-relation of calcium and magnesium absorption. *Clin. Sci.* **22**: 185-193.
28. Behar, J. (1975) Effect of calcium on magnesium absorption. *Am. J. Physiol.* **229**: 1590-1595.
29. O'Donell, J.M. & Smith, M.H. (1973) Uptake of calcium and magnesium by rat duodenal mucosa analyzed by means of competing metals. *J. Physiol.* **229**: 733-749.

30. Karbach, U. (1989) Cellular-mediated and diffusive magnesium transport across the colon descendens of the rat. *Gastroenterology* **96**: 1282-1289.
31. Karbach, U. & Rummel, W. (1990) Cellular and paracellular magnesium transport across the terminal ileum of the rat and its interaction with the calcium transport. *Gastroenterology* **98**: 985-992.
32. Alcock, N. & Macintyre, I. (1960) Interrelation of calcium and magnesium absorption. *Biochem. J.* **76**: 19-20.

Chapter 7

Inhibitory effect of soybean protein versus casein on apparent absorption of magnesium in rats is due to raised fecal excretion of endogenous magnesium

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Abstract. Apparent magnesium absorption, that is magnesium intake minus fecal excretion, is depressed in rats fed diets containing soybean protein instead of casein or fed diets enriched with sodium phytate. However, it cannot be excluded that changes in apparent absorption are caused by changes in fecal excretion of endogenous magnesium. In this study, we measured true magnesium absorption with the use of oral and intraperitoneal administration of tracer doses of ^{28}Mg. Fecal excretion of endogenous magnesium was calculated from magnesium intake and true absorption. True magnesium absorption was not affected by either substitution of soybean protein for casein or by the addition of sodium phytate to a diet containing casein. Endogenous magnesium excretion in feces was significantly increased by soybean protein and sodium phytate. Thus, the observed impairment of apparent magnesium absorption in rats fed soybean protein or sodium phytate is due to enhanced fecal excretion of endogenous magnesium. With other dietary treatments, enhanced fecal excretion of endogenous magnesium was not associated with a discrepancy between apparent and true magnesium absorption. Dietary lactose versus dextrose and supplemental phosphate both stimulated fecal excretion of endogenous magnesium, but lactose raised both true and apparent magnesium absorption and phosphate depressed both true and apparent magnesium absorption.

Introduction

Soybean protein in comparison with casein in the diet lowers apparent magnesium absorption in rats (1). This effect is most likely caused by phytate (1), which is present in significant amounts in soybean protein (2). Apparent absorption of magnesium is calculated as intake minus fecal excretion and thus includes fecal excretion of endogenous magnesium. It has been shown using various animal species (3-6) including man (7-9), that a significant portion of magnesium in feces is of endogenous origin. The amount of endogenous magnesium can be affected by diet composition. Guenther and Sell (10) showed in chickens that apparent magnesium absorption was consistently lower than true absorption, and that the difference, which is caused by fecal output of endogenous magnesium, differed between diets containing different foodstuffs.

In this study, we re-examined the influence of dietary soybean protein versus casein and sodium phytate supplementation of the diet on apparent magnesium absorption in rats, and compared it with that on true magnesium absorption. The latter was determined with the use of the radiotracer ^{28}Mg which was administered orally or intraperitoneally. From the two retention curves of the administered isotope, true magnesium absorption was calculated with the method of Heth and Hoekstra (11). Fecal excretion of endogenous magnesium was calculated from the difference between true and apparent absorption. For comparison, we also determined the effects of dietary lactose versus dextrose and supplemental phosphate. Replacement of dextrose in the diet by lactose stimulates apparent magnesium absorption (1, 12-15), while addition of phosphate to the diet inhibits it (16-19).

Materials and Methods

The experimental protocol was approved by the animal welfare officer of the Agricultural University, Wageningen, The Netherlands.

Animals and housing. Outbred, male Wistar rats (Hsd/Cpb:WU, Harlan, Zeist, The Netherlands) aged 10 wk, and with mean body weight of about 300 g were used. The rats were housed individually in metabolic cages in a room with controlled temperature (20-22 °C), relative humidity (50-60 %) and light cycle (light, 06.00-18.00 h). They were randomly divided into 5 dietary groups of 8 animals each and were fed different experimental diets for 30 d.

Experimental diets. The composition of the diets is shown in Table 1. The control diet contained casein (acid casein, DMV, Veghel, The Netherlands) as protein source and the composition was essentially according to that of the AIN-76 diet (20). To formulate the soybean-protein diet, casein in the control diet was replaced by soybean protein (soy isolate, Purina protein, Hofhuis Pentacon, Bunschoten, The Netherlands). Sodium phytate was added to the control diet to a concentration of 4 mmol/kg to simulate the amount in the diet containing soybean protein which was 3.78 mmol/kg (Table 1). Lactose was added to the control diet to a concentration of 100 g/kg at the expense of dextrose. The analyzed concentration of lactose in the lactose diet was 283 mmol/kg. Phosphate was added to the control diet to a final analyzed concentration of 190 mmol/kg diet in the form of sodium, calcium and potassium dihydrogen. The phosphate concentration of the control diet was 105 mmol/kg diet. All diets were balanced for magnesium, calcium, sodium and potassium. Phosphate concentration was also equal for all diets, except for the high-phosphate diet.

The diets were stored in powdered form at 4 °C until feeding. Diets and demineralized water were freely available. Feed consumption was recorded daily and body weights were measured weekly.

Experimental design. A period of 13 d was used to accustom the animals to their diets. ^{28}Mg administrations were performed on d 14 and d 21. On d 14, after overnight fasting, four animals of each dietary group received $^{28}MgCl_2$ (Interfaculty Reactor Institute, University of Technology, Delft, The Netherlands) with an extrinsically labeled meal. The remaining four animals of each group were injected with the radiotracer intraperitoneally. To equalize handling and treatment of each rat, the rats receiving the radiotracer orally were injected intraperitoneally with distilled water and the rats that were injected with ^{28}Mg were given a meal without the radiotracer. On d 21, the route of administration of radiotracer for each animal was alternated. On the days of radiotracer administration, treatment order of the rats was randomized.

The radioactive meals were prepared by adding 100 μl of 97.6 mmol/L $^{28}MgCl_2$ (1.3 GBq/mol) in distilled water to 3 g of experimental diet. The added solution was dried,

Table 1. *Composition of the experimental diets.*

	Control	Soybean protein	Phytate	Lactose	High phosphate
			g/kg		
Ingredient					
Casein	180	-	180	180	180
Soybean protein	-	200	-	-	-
Dextrose	404.51	389.30	405.12	305.73	397.53
Lactose	-	-	-	100	-
Sodium phytate	-	-	4.2	-	-
$MgCl_2.6H_2O$	3.95	3.27	3.95	3.95	3.95
$Na_2HPO_4.2H_2O$	6.30	-	-	5.88	-
$NaH_2PO_4.H_2O$	-	4.89	-	-	10.98
Na_2CO_3	0.53	-	1.84	0.78	-
$CaCO_3$	2.25	1.30	2.12	1.69	-
CaH_2PO_4	-	-	-	-	3.03
KH_2PO_4	-	-	1.50	-	4.01
K_2CO_3	1.96	0.74	0.77	1.47	-
Constant components[1]	400.5	400.5	400.5	400.5	400.5
Total	1000	1000	1000	1000	1000
			mmol/kg		
Chemical analysis					
Nitrogen	1838	1955	1843	1868	1846
Lactose	0	0	0	283	0
Phytate	0.00	3.78	4.01	0.00	0.00
Magnesium	18.1	17.7	17.3	18.1	18.1
Calcium	141.8	142.5	142.3	141.0	140.5
Phosphorus	104.5	108.1	103.5	107.7	189.7
Sodium	106.5	105.2	102.2	106.1	104.0
Potassium	104.9	104.4	103.8	105.6	106.9

[1] The constant components consisted of (g/kg diet): DL-methionine, 3; wheat starch, 150; cellulose, 50; palm oil, 150; mineral premix, 35; vitamin premix, 10; retinyl acetate, 0.25 (4000 IU); cholecalciferol, 0.25 (1000 IU); choline chloride, 2. The mineral premix consisted of the following (g): $CaCO_3$, 11.94; KH_2PO_4, 3.54; KCl, 4.90; $FeSO_4.7H_2O$, 0.18; $MnSO_4.H_2O$, 0.24; $ZnSO_4.7H_2O$, 0.48; $CuSO_4.5H_2O$, 0.11; KIO_3, 0.0004; $Na_2SeO_3.5H_2O$, 0.0004; $CrK(SO_4)_2.12H_2O$, 0.02; dextrose, 13.60. The vitamin premix consisted of the following (mg): thiamin, 6; riboflavin, 6; pyridoxin, 7; nicotinamide, 30; DL-calcium pantothenate, 16; folic acid, 2; D-biotin, 0.2; cyanocobalamine, 0.01; DL-α-tocopherylacetate, 100; menadione, 0.05; dextrose, 9833.

and then mixed with the diet. For intraperitoneal administration, the 100 μl of radiotracer solution was injected. The oral or intraperitoneal administration of $MgCl_2$ instead of $^{28}MgCl_2$

was carried out in a comparable manner. The meals with or without radiotracer were presented to the rats after a 16-h fast. The meals were consumed within 15 min. Subsequently, the intraperitoneal injection was given. Radioactivity in individual rats was counted in a specially designed whole-animal gamma scintillation detector (21) within 4.5 h after administration of ^{28}Mg. Thereafter, all rats received their normal diets. For another 4 d, the animals were counted at regular intervals. All animals were also measured on d 20, one d before the second administration of the radiotracer; whole-body activity was found not to differ from background measurements. The efficiency of the whole-body counter for detection of ^{28}Mg was 65%, and its stability was monitored by counting a ^{65}Zn source.

From d 14 to d 17 and d 21 to d 24 of the experiment, feces and urine of each animal were collected separately and quantitatively.

Analyses. Diet samples and feces were freeze-dried, homogenized and wet ashed with nitric acid (14 mol/L) plus perchloric acid (12 mol/L). After appropriate dilution, magnesium was analyzed in ashed samples by atomic absorption spectrophotometry (AAS)(Perkin Elmer 1100, Perkin Elmer & Co GmbH, Uberlingen, Germany). Diet samples were also analyzed for calcium by AAS, for sodium and potassium by atomic emission spectrophotometry (Perkin Elmer 1100) and for phosphate by the Fiske-Subbarow method (22). Nitrogen was determined by the macro-Kjeldahl method (23) and lactose by high performance liquid chromatography (24). The phytate content of the diets was analyzed as described by Slump et al. (25). Urinary creatinine concentrations were determined with the use of a commercial test combination (Creatinine, MA-KIT 10 ROCHE, Roche Diagnostics, Basel, Switzerland) and a COBAS-BIO autoanalyzer (Hoffmann - La Roche BV, Mijdrecht, The Netherlands).

Calculations. True magnesium absorption was calculated according to Heth & Hoekstra (11). Counting measurements were corrected for background and radioisotope decay, and then expressed as percentage of administered dose. Plots of the logarithm of percentage radioactivity retention after intraperitoneal and oral ^{28}Mg administration versus time were constructed. The zero-time intercepts were determined by extrapolating the linear parts of the curves. Percentage true absorption was calculated by dividing the intercept of the retention curve for oral ^{28}Mg by that of the retention curve for intraperitoneal ^{28}Mg and multiplying by 100. This calculation was executed for each animal. Absolute true magnesium absorption was calculated by multiplying magnesium intake and percentage true magnesium absorption.

Apparent intestinal absorption of magnesium was calculated as magnesium intake minus fecal magnesium excretion, and was expressed as such and as percentage of intake.

Fecal excretion of endogenous magnesium (in μmol/d) was calculated as absolute true absorption minus absolute apparent absorption of magnesium.

Statistics. Within dietary groups, the results of radiotracer administration on d 14 versus d 21, including the slopes of the retention curves for the same administration route, were compared using Student's t test with the level of significance preset at $P < 0.05$. There were

no significant differences between the radiotracer administration intervals, and thus the data were pooled. The same held for the results of the two balance periods. Dietary treatments were evaluated using Bonferroni's test for multiple comparisons (26) with *a priori* defined contrasts. The level of statistical significance adjusted for multiple comparisons was preset at $P < 0.05$. The following contrasts were tested: soybean-protein diet versus control diet; control diet with added sodium phytate versus control diet; control diet with added lactose versus control diet; control diet with added phosphate versus control diet; control diet with added phytate versus soybean-protein diet. Only those contrasts that reached statistical significance are indicated in Table 2.

Results

Body weight gain and feed intake. Body weight gain and feed intake did not differ significantly between the dietary groups (results not shown). Final body weight for pooled rats was on average 351 ± 3 g and feed consumption was 19.7 ± 0.2 g/d (means ± SEM, n = 40). Magnesium intake did not differ significantly between the experimental groups (Table 2).

Retention curves for ^{28}Mg. For each dietary treatment, the semi-logarithmic retention curves for oral and intraperitoneal ^{28}Mg were found to be linear between 35 and 90 h post-administration (Figure 1). The slopes of the linear portions of the two retention curves were not significantly different within each dietary treatment.

Magnesium absorption. True magnesium absorption was not affected by soybean protein versus casein, whereas rats fed the soybean-protein diet had a significantly lower efficiency

Table 2. *Influence of the differential experimental diets on apparent and true absorption of magnesium*[1].

	Control	Soybean protein	Phytate	Lactose	High phosphate
Magnesium intake (μmol/d)	391±13	366±13	373±10	371±8	376±15
Apparent absorption (μmol/d)	207±11	161 ± 10^{A}	170 ± 11^{B}	248 ± 8^{C}	154 ± 14^{D}
Apparent absorption (%)	52±2	46 ± 2^{A}	46 ± 2^{B}	68 ± 1^{C}	40 ± 2^{D}
True absorption (%)	54±3	55±5	53±3	77 ± 4^{C}	48 ± 0.4^{D}
Endogenous Mg in feces (μmol/d)	8±4	33 ± 7^{A}	25 ± 4^{B}	31 ± 10^{C}	28 ± 8^{D}
Urinary excretion (μmol Mg/μmol creatinine)	1.03±0.05	0.66 ± 0.08^{A}	0.90±0.09	1.64 ± 0.10^{C}	0.83 ± 0.03^{D}

[1] Values are means ± SEM for eight rats per dietary group. Superscripts refer to significantly different contrasts (Bonferroni's test, $P < 0.05$): A = soybean protein versus control; B = sodium phytate versus control; C = lactose versus control; D = phosphate versus control.

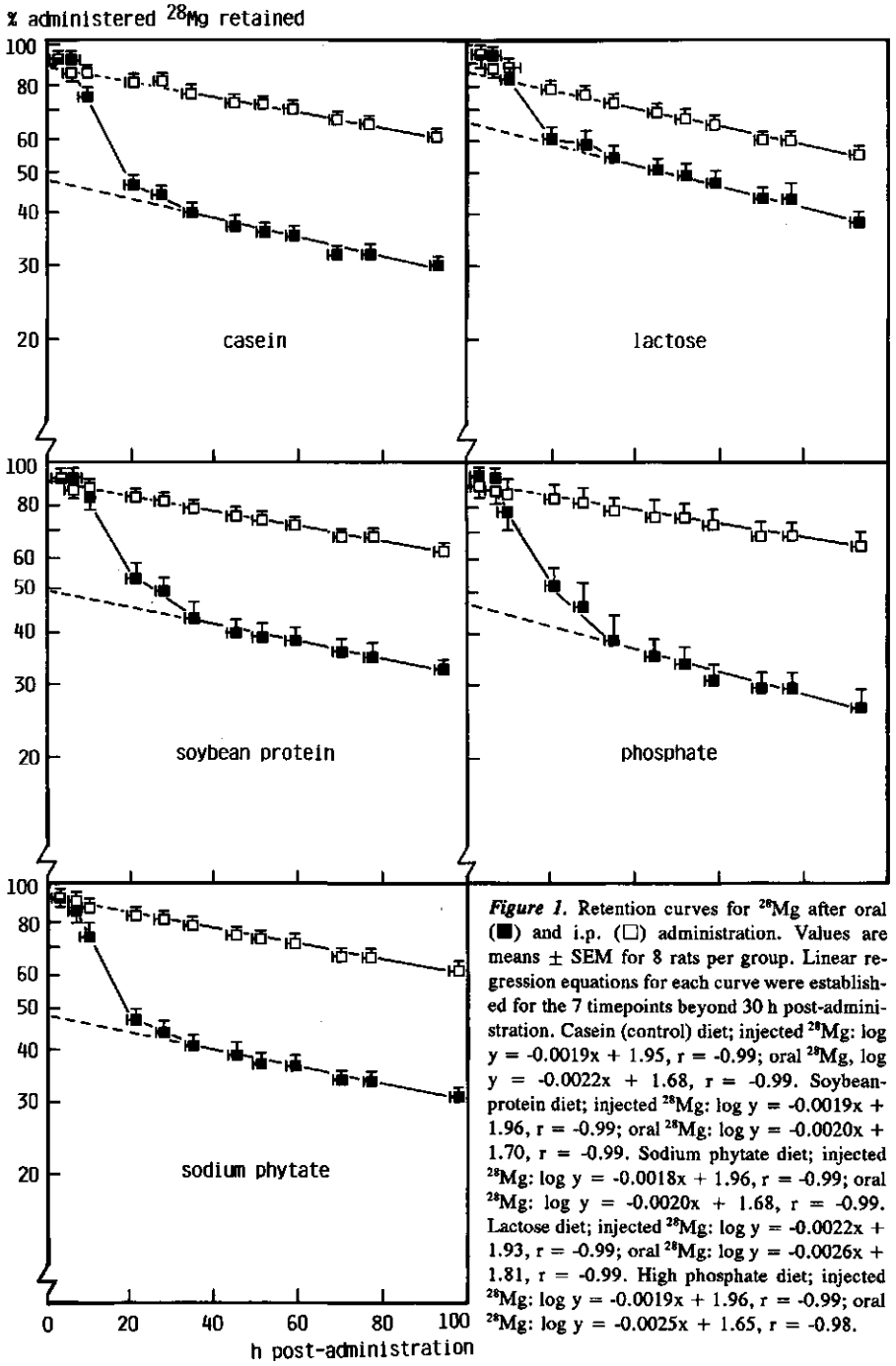

Figure 1. Retention curves for ^{28}Mg after oral (■) and i.p. (□) administration. Values are means ± SEM for 8 rats per group. Linear regression equations for each curve were established for the 7 timepoints beyond 30 h post-administration. Casein (control) diet; injected ^{28}Mg: log y = -0.0019x + 1.95, r = -0.99; oral ^{28}Mg, log y = -0.0022x + 1.68, r = -0.99. Soybean-protein diet; injected ^{28}Mg: log y = -0.0019x + 1.96, r = -0.99; oral ^{28}Mg: log y = -0.0020x + 1.70, r = -0.99. Sodium phytate diet; injected ^{28}Mg: log y = -0.0018x + 1.96, r = -0.99; oral ^{28}Mg: log y = -0.0020x + 1.68, r = -0.99. Lactose diet; injected ^{28}Mg: log y = -0.0022x + 1.93, r = -0.99; oral ^{28}Mg: log y = -0.0026x + 1.81, r = -0.99. High phosphate diet; injected ^{28}Mg: log y = -0.0019x + 1.96, r = -0.99; oral ^{28}Mg: log y = -0.0025x + 1.65, r = -0.98.

of apparent magnesium absorption and depressed urinary excretion of magnesium (Table 2). Addition of sodium phytate to the control diet did not affect true magnesium absorption, but significantly lowered apparent magnesium absorption. Sodium phytate had no significant impact on urinary magnesium excretion. Lactose, when compared with dextrose, raised both true and apparent magnesium absorption, and stimulated magnesium excretion in urine. Supplemental phosphate lowered true and apparent magnesium absorption, as well as urinary magnesium excretion (Table 2).

Excretion of endogenous magnesium. In rats fed the experimental diets, fecal excretion of endogenous magnesium was significantly higher than in rats fed the control diet (Table 2).

Discussion

Three assumptions underly the use of ^{28}Mg to determine the intestinal absorption of magnesium. The first is that ^{28}Mg given either orally or intraperitoneally behaves similarly once it has entered the circulation. While such an effect has been demonstrated for zinc (11) no literature data are available to validate or repudiate this assumption for magnesium. However, for each dietary treatment the linear portion of the two semi-logarithmic retention curves had similar slopes (Figure 1). This indicates that ^{28}Mg once inside the body is handled independent of the route of administration. Secondly, it was assumed that the radiotracer given orally reflects the behavior of nonradioactive magnesium in food. Schwartz *et al.* (27, 28) have demonstrated that the absorptive efficiency from an oral test dose of ^{28}Mg is similar to that of ^{26}Mg intrinsically incorporated into vegetables. Likewise, Liu *et al.* (29) did not show any difference in true magnesium absorption after feeding intrinsically or extrinsically labeled milk products. Thus, our method of oral administration of ^{28}Mg may be considered valid to determine magnesium absorption. Thirdly, it was assumed that ^{28}Mg rapidly equilibrates with nonradioactive magnesium in the body. Orally and intravenously administered ^{28}Mg rapidly disappears from the circulation (8, 9, 30) and initially accumulates in soft tissues (31, 32). Rogers and Mahan (33) reported that most of the ^{28}Mg administered intravenously to rats equilibrated among the tissues and plasma within 25 h. Thus, the intercept determined by extrapolation of the retention curves between 35 and 90 h post-administration (Figure 1) most likely holds for equilibrium.

The rapid initial loss of total body activity after oral administration of ^{28}Mg is caused by passage of the radiotracer through the intestine and its excretion in feces. Compared with the control diet, neither the soybean-protein diet nor the sodium-phytate diet affected the initial loss of label (Figure 1). This indicates, as was indeed found after calculation, that true magnesium absorption was not altered by these dietary treatments. In keeping with earlier work (1), the supplementation of the control diet with sodium phytate, to a concentration identical to that in the diet containing soybean protein, significantly lowered apparent absorption of magnesium to a level seen after feeding soybean protein (Table 2). Thus, phytate in soybean protein appears responsible for the reduction in apparent magnesium

absorption as induced by soybean protein when compared with casein. A significant rise in fecal excretion of endogenous magnesium was observed in rats fed either soybean protein or sodium phytate (Table 2). It would seem that the inhibitory influence of soybean protein and sodium phytate on apparent magnesium absorption does not represent the inhibition of intestinal magnesium absorption, but rather stimulation of fecal excretion of endogenous magnesium. The extra loss of endogenous magnesium is compensated for by a depressed urinary excretion of magnesium (Table 2).

In rats fed the lactose diet, the early loss of total body radioactivity after oral administration of ^{28}Mg was reduced. Indeed, calculated true magnesium absorption was significantly raised by lactose. Both fecal excretion of endogenous magnesium and apparent magnesium absorption were significantly elevated by lactose. Lactose has been frequently reported to raise apparent absorption of magnesium in rats (1, 12-14), but until now, data on true magnesium absorption were not available. The lactose-induced stimulation of magnesium absorption is reflected by enhanced urinary excretion of magnesium. Evidently, the extra amount of magnesium absorbed was larger than that of endogenous magnesium excreted in feces.

Increasing intakes of phosphate have been reported to depress apparent magnesium absorption in rats (16-19), but the influence on true absorption was unknown. Phosphate loading significantly reduced true magnesium absorption and stimulated fecal output of endogenous magnesium. The diminished absorption of dietary magnesium and the enhanced loss of endogenous magnesium in the feces was compensated for by a fall of magnesium output in urine.

We conclude that the earlier observed reduction of apparent magnesium absorption and lowered urinary magnesium excretion in rats fed diets containing soybean protein and/or sodium phytate (1) is not associated with impairment of true magnesium absorption. This may be caused by enhanced loss of endogenous magnesium in feces as induced by dietary soybean protein and phytate. However, enhanced fecal excretion of endogenous magnesium is not invariably related with depressed apparent magnesium absorption and unaltered true absorption. Fecal excretion of endogenous magnesium was raised in rats fed either the lactose or high-phosphate diet, whereas true magnesium absorption was stimulated and depressed, respectively. It is not known why all four dietary treatments produced a rise of fecal excretion of endogenous magnesium. It might be caused by increased turnover of epithelial cells, but the basis of this effect may just as well differ between treatments.

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References

1. Brink, E.J., Dekker, P.R., van Beresteijn, E.C.H. and Beynen, A.C. (1991) Inhibitory effect of dietary soybean protein vs casein on magnesium absorption in rats. *J. Nutr.* **121**: 1374-1381.
2. Reddy, N.R., Sathe, S.K. & Salunkhe, P.K. (1982) Phytates in legumes and cereals. *Adv. Food Res.* **28**: 1-92.
3. Guenther, W. & Sell, J.L. (1973) Magnesium absorption and secretion along the gastrointestinal tract of the chicken. *J. Nutr.* **103**: 875-881.
4. Smith, R.H. (1959) Absorption of magnesium in the large intestine of the calf. *Nature* **184**: 821-822.
5. Aikawa, J.K. (1959) Gastrointestinal absorption of ^{28}Mg in rabbits. *Proc. Soc. Exp. Biol. Med.* **100**: 293-295.
6. Chutkow, J. (1964) Sites of magnesium absorption and excretion in the intestinal tract of the rat. *J. Lab. Clin. Med.* **63**: 71-79.
7. Schuette, S.A., Ziegler, E.E., Nelson, S.E. & Janghorbani, M. (1990) Feasibility of using the stable isotope ^{25}Mg to study Mg metabolism in infants. *Pediatr. Res.* **27**: 36-40.
8. Silver, L., Robertson, J.S. & Dahl, L.K. (1960) Magnesium turnover in the human studied with ^{28}Mg. *J. Clin. Inv.* **39**: 420-425.
9. Avioli, L.V. & Berman, M. (1966) Mg^{28} kinetics in man. *J. Appl. Physiol.* **21**: 1688-1694.
10. Guenther, W. & Sell, J.L. (1974) A method for determining "true" availability of magnesium from foodstuffs using chickens. *J. Nutr.* **104**: 1446-1457.
11. Heth, D.A. & Hoekstra, W.G. (1965) Zinc-65 absorption and turnover in rats. I. A procedure to determine zinc-65 absorption and the antagonistic effect of calcium in a practical diet. *J. Nutr.* **85**: 367-374.
12. Andrieux, C. & Sacquet, E. (1983) Effect of microflora and lactose on the absorption of calcium, phosphorus and magnesium in the hindgut of the rat. *Repr. Nutr. Dev.* **23**: 258-271.
13. Fournier, P., Dupuis, Y. & Fournier, A. (1971) Effect of lactose on the absorption of alkaline earth metals and intestinal lactase activity. *Isr. J. Med. Sci.* **7**: 389-391.
14. Greger, J.L., Gutkowski, C.M. & Khazen, R.R. (1989) Interactions of lactose with calcium, magnesium and zinc in rats. *J. Nutr.* **119**: 1691-1697.
15. Brink, E.J., Dekker, P.R., van Beresteijn, E.C.H. & Beynen, A.C. Bioavailability of magnesium and calcium from cow's milk and soybean beverage in rats. *Br. J. Nutr.*: in press.
16. Mars, Y.W.H.M., Lemmens, A.G. & Beynen, A.C. (1988) Dietary phosphorus and nephrocalcinosis in female rats. *Nutr. Rep. Int.* **38**: 249-258.
17. Toothill, T. (1963) The effect of certain dietary factors on the apparent absorption of magnesium by the rat. *Br. J. Nutr.* **17**: 121-143.
18. Brink, E.J., Beynen, A.C., Dekker, P.R., van Beresteijn, E.C.H. & van der Meer, R. Interaction of calcium and phosphate decreases ileal magnesium solubility and apparent magnesium absorption in rats. *J. Nutr.*: in press.
19. Hardwick, L.L., Jones, M.R., Brautbar, N. & Lee, D.B.N. (1991) Magnesium absorption: Mechanisms and the influence of vitamin D, calcium and phosphate. *J. Nutr.* **121**: 13-23.
20. American Institute of Nutrition (1977) Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. *J. Nutr.* **117**: 1340-1348.
21. Barneveld, A.A. & van den Hamer, C.J.A. (1984) Intestinal passage of simultaneously administered 64-Cu and 65-Zn and the effect of feeding in mouse and rat. *Nutr. Rep. Int.* **29**: 173-182.
22. Fiske, C.H. & Subbarow, Y. (1925) The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**: 375-400.
23. International Dairy Federation (1962) Determination of total nitrogen content of milk by the Kjeldahl method. FIL-IDF 20.
24. Brons, C. & Olieman, C. (1983) Study of high performance liquid chromatographic separation of reducing sugars applied to the determination of lactose in milk. *J. Chrom.* **259**: 79-86.
25. Slump, P., Bos, K.D. & Wolters, M.G.E. (1987) Improved determination of phytate by HPLC. In: Euro Food Chem. IV. Rapid analysis in food processing and food control, Vol. 1. Loen, Norway: Norwegian Food Research Institute, pp. 151-155.
26. Fleiss, J.L. (1986) The design and analysis of clinical experiments. New York: John Wiley and Sons, pp. 103-107.
27. Schwartz, R., Spencer, H. & Welsh, J. (1984) Magnesium absorption in human subjects from leafy vegetables, intrinsically labeled with the stable isotope ^{26}Mg. *Am. J. Clin. Nutr.* **39**: 571-576.

28. Schwartz, R., Grunes, D., Wentworth, R. & Wien, E. (1980) Magnesium absorption from leafy vegetables, intrinsically labeled with the stable isotope ^{26}Mg. *J. Nutr.* **110**: 1365-1371.
29. Liu, Y., Neal, P., Ernst, J., Weaver, C., Rickard, K., Smith, D.L. & Lemons, J. (1989) Absorption of calcium and magnesium from fortified human milk by very low birth weight infants. *Pediatr. Res.* **25**: 496-502.
30. Aikawa, J.K., Gordon, G.S. & Rhoades, E.L. (1960) Magnesium metabolism in human beings: studies with Mg^{28}. *J. Appl. Physiol.* **15**: 503-507.
31. Brandt, J.L., Glaser, W. & Jones, A. (1958) Soft tissue distribution and plasma disappearance of intravenously administered isotopic magnesium with observations on uptake in bone. *Metabolism* **7**: 355-363.
32. Field, A.C. & Smith, B.S.W. (1964) Effect of magnesium deficiency on the uptake of ^{28}Mg by the tissues in mature rats. *Br. J. Nutr.* **18**: 103-112.
33. Rogers, T.A. & Mahan, P.E. (1959) Exchange of radioactive magnesium in the rat. *Proc. Soc. Exp. Biol. Med.* **100**: 235-239.

Chapter 8

Lactose intake does not affect the apparent absorption of magnesium and calcium in healthy adults

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Abstract. The effect of lactose on the absorption of magnesium and calcium was studied in a double-blind, cross-over study, covering three periods of one wk. Twenty-four healthy, lactose-tolerant, adult volunteers maintained their habitual diets with the exception that all lactose-containing dairy products in the diet were replaced by 600 g/d of three specially prepared dairy products. These products were based on either lactose-enriched cow's milk or lactose-enriched, lactase-treated cow's milk with or without added magnesium and were given in turn during one wk. Lactose intake was increased by 127 mmol/d (46 g/d) while taking the lactose-enriched products. While taking the magnesium-enriched products, magnesium intake was increased by 2.8 mmol/d (69 mg/d), which was equivalent to 17% of the habitual magnesium intake. Apart from the lactose and magnesium intake, nutrient intake was comparable during the three dietary periods. Urinary excretions of magnesium and calcium were used as indicators for their absorption. Magnesium supplementation significantly increased urinary magnesium excretion by 0.97 mmol/d (equivalent to an increase of 18%, $P < 0.001$), indicating that urinary magnesium excretion is a valid indicator for intestinal magnesium absorption. Hydrolysis of lactose did not affect urinary excretion of magnesium and calcium, which implies that lactose intake does not affect the absorption of magnesium and calcium in healthy adults.

Introduction

Marginal intakes and impaired intestinal absorption of magnesium and calcium may contribute to the pathogenesis of osteoporosis, coronary heart disease and cancer (1, 2). Thus it is of interest to identify nutrients that either reduce or improve absorption of these minerals. The milk sugar, lactose, enhances intestinal absorption of magnesium in rats (3-9). Such an effect also occurs in infants (10-12) but it is not known whether lactose influences magnesium absorption in adults. The effect of lactose on calcium absorption in rats and lactose-tolerant humans is controversial with stimulation (4, 7, 8, 13-21) and no effect (22-25) being reported.

The lack of conclusive information about the effect of lactose on the absorption of magnesium and calcium in lactose-tolerant, adult humans prompted us to perform the present study. We determined urinary excretion of magnesium and calcium in adults given in a cross-over design lactose-enriched cow's milk and cow's milk with hydrolyzed lactose either without or with added magnesium. The latter treatment served as a positive control for the detection of an increased urinary excretion of magnesium. Under steady state conditions and at normal intakes of magnesium and calcium, urinary excretion of these minerals is a valid indicator for their absorption (26, 27).

Subjects and methods

Subjects. Twenty-four healthy subjects (14 men and 10 women), all employees of The Netherlands Institute for Dairy Research, participated in this study. They were 21 - 43 y of

age, 54 - 91 kg in body weight, did not take any medication and were apparently lactose-tolerant. All participants gave their informed consent, and the study protocol was approved by the Medical Ethical Committee of the Wageningen Agricultural University. All participants finished the study.

Experimental protocol. The subjects were asked to maintain their usual lifestyle habits for the duration of the study. They were instructed to comply with their habitual diet with the exception that all lactose-containing dairy products were replaced by specially prepared (see below) chocolate milk (400 g/d) and vanilla custard (200 g/d). These products were based on either lactose-enriched cow's milk or lactose-enriched, lactase-treated cow's milk with or without added magnesium. The chocolate milk was consumed with breakfast (200 g/d) and lunch (200 g/d) and the vanilla custard with dinner (200 g/d). The calculated daily lactose intake from the lactase-treated products was 8.6 (products without added magnesium) or 9.2 mmol/d (products with added magnesium) and 135.6 mmol/d from the lactose-enriched products. The calculated extra intake of magnesium from the magnesium-enriched products was 3.1 mmol/d.

To eliminate any bias due to subjects' and investigators' attitudes and to control for time trends and carry-over effects, a double-blind cross-over design was used in which each treatment followed each other the same number of times. The subjects were divided into six groups of four individuals each. Each group underwent one of the six treatment orders. The groups were stratified for body weight, age and male-to-female ratio. Each dietary treatment was given for one wk and the entire study was conducted over a period of three wk. During each one-wk period, the subjects recorded their actual intake of nutrients on three arbitrary d (two working-d and one weekend-d) in specially designed diaries. Two samples of 24-h urine were collected: on d 5 and d 7 of each wk. Body weight was measured once a wk.

Experimental products. The experimental dairy products were manufactured by the Technology Department of The Netherlands Institute for Dairy Research. Sterilized, semi-skimmed cow's milk was supplemented with 116 mmol/L lactose (Pharmatose, DMV, Veghel, The Netherlands). Milk with hydrolyzed lactose was prepared by adding 400 mg (2000 NLU) lactase (Maxilact LX 5000, Gist-Brocades, Delft, The Netherlands) to one L of this product. After incubation for 24 h at 10 °C, about 92% of the lactose was found to be hydrolyzed. Part of the lactase-treated milk was supplemented with magnesium carbonate (Fluka, Buchs, Switzerland). In order to ensure a double-blind study design, chocolate milk and vanilla custard were prepared from the lactose-enriched milk, the lactose-enriched, lactase-treated milk and the lactose-enriched, lactase-treated, magnesium-enriched milk using standard recipies. The analyzed lactose and mineral concentrations in the experimental dairy products are given in Table 1.

Analyses. Nutrient intakes were calculated from the computerized Dutch food composition table with adjustments for the experimental dairy products. The experimental products and

Table 1. *Analyzed concentrations of nitrogen, fat, lactose and minerals in the experimental dairy products per 100 g product.*

	Chocolate milk			Vanilla custard		
	Control[1]	Lactose-enriched	Mg-enriched[2]	Control[1]	Lactose-enriched	Mg-enriched[2]
Nitrogen (g)	0.55	0.55	0.54	0.50	0.51	0.50
Fat (g)	1.7	1.7	1.6	1.5	1.4	1.5
Lactose (mmol)	1.4	22.9	1.5	1.5	22.0	1.6
Calcium (mmol)	2.63	2.65	2.61	2.50	2.52	2.53
Magnesium (mmol)	0.72	0.72	1.20	0.40	0.40	0.97
Phosphorus (mmol)	2.96	2.97	2.95	2.54	2.55	2.56
Water (g)	84	84	84	80	79	80

[1] Control = lactose-enriched, lactase-treated.
[2] Mg-enriched = lactose-enriched, lactase-treated and supplemented with magnesium.

urine samples were analyzed for magnesium and calcium by atomic absorption spectrophotometry (Perkin Elmer 1100, Bodenseewerk Perkin Elmer, Uberlingen, Germany) and for phosphorus by the Fiske Subbarow method (28). Lactose in the experimental dairy products was analyzed by high performance liquid chromatography (29) and nitrogen by the macro-Kjeldahl method (30). Urine was analyzed for sodium by atomic emission spectrophotometry (Perkin Elmer 1100). Urine samples were also analyzed for creatinine and urea by a colorimetric method (31, 32) with the Ektachem 700 XR (Kodak, Rochester, USA). The urinary excretion of minerals was expressed relative to that of creatinine.

Statistics. Changes in body weight, dietary and urinary variables were evaluated for statistically significant differences between males and females and between time intervals by analysis of variance; no such differences ($P > 0.99$) were found. There were no significant differences in urinary mineral excretion between d 5 and d 7 of urine collection ($P > 0.67$, Student's paired t test) within dietary periods. The effects of lactose and magnesium were then evaluated for pooled subjects and time intervals with the use of Student's paired t-test. In case of urinary variables, average values of d 5 and d 7 of each dietary period were used. The level of significance was preset at $P < 0.05$.

Results

Body weight. Body weight was not influenced by dietary treatments and remained stable throughout the experiment. Initial and final mean body weight was 71.8 $\pm$ 1.9 kg and 71.7 $\pm$ 1.9 kg (mean $\pm$ SEM, n = 24), respectively.

Table 2. *Nutrient intake of subjects when taking the lactose-enriched, lactase-treated products (control treatment), the lactose-enriched, or the lactose-enriched, lactase-treated, magnesium-enriched products*[1].

	Dietary treatment		
	Control[2]	Lactose-enriched	Magnesium-enriched[3]
Energy (MJ/d)	11.9±0.4	11.5±0.5	11.5±0.5
Protein (energy %)	13.9±0.4	14.0±0.4	14.2±0.3
Fat (energy %)	34.1±0.9	32.9±0.9	32.6±1.3
Carbohydrates (energy %)	49.7±0.9	51.3±1.2	50.4±1.4
Lactose (mmol/d)	8.6±0	135.6±0*	9.2±0
Ethanol (energy %)	2.3±0.6	2.7±0.8	2.8±0.8
Fiber (g/d)	35.8±1.6	36.0±2.0	35.2±2.2
Magnesium (mmol/d)	16.8±0.7	17.5±0.8	19.7±0.6*
Calcium (mmol/d)	37.0±1.6	36.4±1.7	36.8±1.9
Phosphate (mmol/d)	62.7±2.3	62.7±2.5	62.6±2.5
Potassium (mmol/d)	114±3	117±4	112±3
Sodium (mmol/d)	167±10	155±8	174±12

[1] Values are expressed as means ± SEM (n = 24).
[2] Control = lactose-enriched, lactase-treated.
[3] Mg-enriched = lactose-enriched, lactase-treated and supplemented with magnesium.
* Significant difference ($P < 0.001$, one-sided one-sample *t* test).

Nutrient intake (Table 2). Table 2 shows that the actual lactose intake was increased by 127 mmol/d after consumption of the lactose-enriched products. The actual magnesium intake was significantly increased by 2.8 mmol/d during the period that the lactose-enriched, lactase-treated, magnesium-enriched products were consumed. This was associated with a negligible increase in lactose intake when compared with the lactose-enriched, lactase-treated period (control treatment). Otherwise, there were no significant differences in nutrient intake between the dietary periods.

Urinary parameters (Table 3). Urinary excretions of creatinine, urea, phosphate and sodium were not significantly affected by increased lactose or increased magnesium intake. Likewise, extra lactose did not significantly influence the urinary excretion of magnesium and calcium. However, urinary magnesium excretion was significantly increased when the lactose-enriched, lactase-treated, magnesium-enriched products were consumed.

Table 3. *Urinary excretion of subjects when taking the lactose-enriched, lactase-treated products (control treatment), the lactose-enriched, or the lactose-enriched, lactase-treated, magnesium-enriched products*[1]

	Dietary treatment		
	Control[2]	Lactose-enriched	Magnesium-enriched[3]
Volume (L/d)	1.39±0.09	1.40±0.11	1.41±0.08
Creatinine (mmol/d)	14.1±0.5	14.0±0.5	13.8±0.6
Urea (mmol/d)	402±14	400±16	403±18
Magnesium/creatinine (mmol/mmol)	0.38±0.02	0.37±0.01	0.46±0.02*
Calcium/creatinine (mmol/mmol)	0.32±0.03	0.31±0.03	0.32±0.02
Phosphate/creatinine (mmol/mmol)	2.20±0.07	2.22±0.08	2.19±0.08
Sodium/creatinine (mmol/mmol)	11.2±0.5	11.1±0.7	11.8±0.9

[1] Values are expressed as means ± SEM (n = 24).
[2] Control = lactose-enriched, lactase-treated.
[3] Mg-enriched = lactose-enriched, lactase-treated and supplemented with magnesium.
* Significant difference ($P < 0.001$, one-sided one-sample t test).

Discussion

On the assumption that subjects are in steady state, differences in urinary magnesium excretion should reflect magnesium absorption. Indeed, a positive relationship between absorption and urinary excretion of magnesium has been demonstrated (26). As would be anticipated, the moderately increased magnesium intake while consuming the lactose-enriched, lactase-treated, magnesium-enriched products caused an increase in urinary magnesium excretion (Table 3). This indicates that our study design can be considered valid, which strengthens our observation that lactose consumption did not affect urinary magnesium excretion (Table 3). The increase in magnesium intake by 2.8 mmol/d (Table 2) produced an increase in urinary magnesium excretion by 0.97 mmol/d (cf. Table 3), indicating that magnesium absorption from the magnesium-enriched experimental dairy products was 35 %. In our study, an increase in urinary magnesium excretion of only 0.3 mmol/d would have reached statistical significance at the $P < 0.05$ level. In other words, we would have been able to detect a lactose-induced increase in percentage apparent magnesium absorption from the complete diet by as low as 1.8 %.

Our study with humans is in contradiction with studies using rats and showing that lactose increases intestinal absorption of magnesium (3-9). Rats become lactase-deficient after weaning (33), and thus are not capable to hydrolyze lactose in the intestine. Possibly, microbial fermentation of lactose in the intestine lowers luminal pH. This would result in

increased solubility of intestinal magnesium (34), leading to enhanced magnesium absorption (35). Our subjects were lactose-tolerant and thus such a mechanism for lactose to stimulate magnesium absorption could not occur.

The lack of effect of lactose on magnesium absorption in our study is at variance with studies in infants (10-12). Kobayashi *et al.* (12) reported that lactase-treated lactose increased magnesium absorption compared with intact lactose. This is an unexpected finding. However, magnesium intake was not reported and differences in magnesium intake between experimental groups cannot be excluded. Wirth *et al.* (11) reported that lactose increases magnesium absorption, but lactose ingestion was associated with an increased magnesium intake. As shown in our study and in others (36, 37), a higher magnesium intake by itself results in increased absolute magnesium absorption so that an independent effect of lactose cannot be determined.

There is controversy about the influence of lactose on calcium absorption in humans (17-21, 24, 25). In our lactose-tolerant subjects, intact lactose versus hydrolyzed lactose did not affect calcium absorption as assessed by urinary calcium excretion, whereas an increase of urinary calcium excretion of 0.5 mmol/d would have reached statistical significance at the $P < 0.05$ level. Thus, in our study a lactose-induced increase in percentage apparent calcium absorption from the complete diet by as low as 1.3 % would have been detected. Some investigators (18-20) showed that lactose increases calcium absorption in lactose-tolerant subjects whereas it causes a decrease in lactose-intolerant subjects. Other investigators (12, 22) demonstrated that hydrolyzed lactose produces higher rates of calcium absorption than intact lactose. These results indicate that hydrolysis of lactose in the intestine is a prerequisite for increased calcium absorption. In keeping with this, Birlouez-Aragon (38) showed that after milk consumption lactose-intolerant subjects absorb less calcium than lactose-tolerant subjects. Furthermore, lactase-treated milk compared with normal milk enhanced calcium absorption in lactose-intolerant subjects, but had no effect in lactose-tolerant subjects (38). The present study supports the latter observation. In lactose-tolerant young adults with a high calcium intake, intact lactose had no specific effect on calcium absorption when compared with its galactose and glucose components.

Apparent magnesium and calcium absorption from the whole diet was on average 30 and 12%. Reported percentages of magnesium absorption range from 30 to 40 % (39, 40) and those of calcium from 20 to 30 % (39) at common intakes of these minerals (10-20 mmol/d for magnesium and 20-25 mmol/d for calcium). The relatively low calcium absorption observed in our experiment might be explained by the high calcium intake of 37 mmol/d. Heaney *et al.* (41) showed that an increase in calcium intake markedly decreased the absorption efficiency for calcium. Calcium intake was equal for each dietary period (Table 2) and thus the low calcium absorption may not have affected the treatment comparisons.

From our results, we conclude that intact lactose versus hydrolyzed lactose does not affect the apparent absorption of magnesium and calcium in healthy, lactose-tolerant adults.

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References

1. Shills, M.E. (1988) Magnesium in health and disease. *Ann. Rev. Nutr.* **8**: 429-460.
2. Berner, L.A., McBean, L.D. & Lofgren, P.A. (1990) Calcium and chronic disease prevention: challenges to the food industry. *Food Technol.* **44**: 50-70.
3. Brink, E.J., Dekker, P.R., van Beresteijn, E.C.H. & Beynen, A.C. (1991) Inhibitory effect of dietary soybean protein vs casein on magnesium absorption in rats. *J. Nutr.* **121**: 1374-1381.
4. Andrieux, C. & Sacquet, E. (1983) Effect of microflora and lactose on the absorption of calcium, phosphorus and magnesium in the hindgut of the rat. *Repr. Nutr. Dev.* **23**: 258-271.
5. Behling, A.R. & Greger, J.L. (1990) Importance of lactose in yogurt for mineral utilization. *J. Agr. Food Chem.* **38**: 200-204.
6. Fournier, P., Dupuis, Y. & Fournier, Y. (1971) Effect of lactose on the absorption of alkaline earth metals and intestinal lactase activity. *Isr. J. Med. Sci.* **7**: 103-107.
7. Greger, J.L., Gutkowski, C.M. & Khazen, R.R. (1989) Interactions of lactose with calcium, magnesium and zinc in rats. *J. Nutr.* **119**: 1691-1697.
8. Schaafsma, G., Dekker, P.R. & de Waard, H. (1988) Nutritional aspects of yogurt. 2. Bioavailability of essential minerals and trace elements. *Neth. Milk Dairy J.* **42**: 135-146.
9. Brink, E.J., Dekker, P.R., van Beresteijn, E.C.H. & Beynen, A.C. Bioavailability of magnesium and calcium from cow's milk and soy beverage in rats. *Br. J. Nutr.*: in press.
10. Ziegler, E.E. & Fomon, S.J. (1983) Lactose enhances mineral absorption in infancy. *J. Pediatr. Gastr. Nutr.* **2**: 288-294.
11. Wirth, Jr. F.H., Numerof, B., Pleban, P. & Neylan, M.J. (1990) Effect of lactose on mineral absorption in preterm infants. *J. Pediatr.* **117**: 283-287.
12. Kobayashi, A., Kawai, S., Ohbe, Y. & Nagashima, Y. (1975) Effects of dietary lactose and a lactase preparation on the intestinal absorption of calcium and magnesium in normal infants. *Am. J. Clin. Nutr.* **28**: 681-683.
13. Fournier, P. (1954) L'effet protecteur du lactose vis-a-vis du squelette de la ratte allaitante. *C. R. Acad. Sci.* **238**: 509-511.
14. Pansu, D., Chapuy, M.C., Milani, M. & Bellaton, C. (1975) Transepithelial calcium transport enhanced by xylose and glucose in the rat jejunal ligated loop. In: Mielsen, P., Hjoring-Hansen, E. Symp. Calc. Tissue Proc. XIth Europ., Fald's Copenhagen pp. 45-51.
15. Armbrecht, H. & Wasserman, R. (1979) Enhancement of Ca^{2+} uptake by lactose in the rat small intestine. *J. Nutr.* **106**: 1265-1271.
16. Vaughan, O. & Filer, J. (1960) The enhancing action of certain carbohydrates on the intestinal absorption of calcium in rats. *J. Nutr.* **71**: 10-14.
17. Fournier, P. & Dupuis, Y. (1960) Pouvoir antirachitique de composes divers dits de structure lactose, glucosamine, L-xylose, mannitol. *C. R. Acad. Sci.* **250**: 3050-3052.
18. Pansu, D. & Chapuy, M. (1970) Calcium absorption enhancement by lactose and xylose. *Calcif. Tiss. Res.* **4**: 155-156.
19. Cochet, B., Jung, A., Griessen, M., Bartholdi, P., Schaller, P. & Donath, A. (1983) Effects of lactose on intestinal calcium absorption in normal and lactase-deficient subjects. *Gastroenterology* **84**: 935-940.
20. Condon, J., Nassim, J.R., Hilbe, A., Millard, F.J.H. & Stainhope, E.M. (1970) Calcium and phosphorus metabolism in relation to lactose tolerance. *Lancet* **16**: 1027-1029.
21. Kocian, J., Skala, I. & Bakos, K. (1973) Calcium absorption from milk and lactose-free milk in healthy subjects and patients with lactose intolerance. *Digestion* **9**: 317-324.
22. Debongnie, J.C., Newcomer, A.D., McGill, D.B. & Phillips, S.F. (1979) Absorption of nutrients in lactase deficiency. *J. Dig. Dis. Sci.* **24**: 225-231.
23. Greger, J.L., Krzykowski, C.E., Khazen, R. & Krashoc, C. (1987) Mineral utilization by rats fed commercially available calcium supplements or milk. *J. Nutr.* **117**: 717-724.

24. Recker, R.R., Bammi, A., Barger-lux, J. & Heaney R. (1988) Calcium absorbability from milk products, an imitation milk and calcium carbonate. *Am. J. Clin. Nutr.* **47**: 93-95.
25. Sheikh, M.S., Santa Ana, C.A., Nicar, M.J., Schiller, L.R. & Fordtran, J.S. (1987) Gastrointestinal absorption of calcium salts. *N. Engl. J. Med.* **317**: 532-536.
26. Morris, E.R., Ellis, R., Steele, P. & Moser, P.B. (1988) Mineral balance of adult men consuming whole or dephytinized bran. *Nutr. Res.* **8**: 445-458.
27. Spencer, H., Kramer, L. & Osis, D. (1984) Effect of calcium on phosphorus metabolism in man. *Am. J. Clin. Nutr.* **40**: 219-225.
28. Fiske, C.H. & Subbarow, Y. (1925) The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**: 375-400.
29. Brons, C. & Olieman, C. (1983) Study of high performance liquid chromatographic separation of reducing sugars applied to the determination of lactose in milk. *J. Chromatogr.* **259**: 79-86.
30. International Dairy Federation (1986) Determination of nitrogen content (Kjeldahl method) and calculation of crude protein content. International IDF standard no 20A. International Dairy Federation. Brussel. FIL-IDF 20.
31. Goren, M.P., Osborne, S. & Wright, R.K. (1986) A peroxidase coupled kinetic enzymatic procedure evaluated for measuring serum and urinary creatinine. *Clin. Chem.* **32**: 548-551.
32. Spayd, R.W., Bruschi, B., Burdick, B.A., Dappen, G.M., Fikenberry, J.N., Esders, T.W., Figueras, J. *et al* (1978) Multilayer film elements for clinical chemistry. *Clin. Chem.* **24**: 1343-1344.
33. De Groot, A.P. & Hoogendoorn, P. (1957) The detrimental effect of lactose.II. Quantitative lactase determinations in various mammals. *Neth. Milk Dairy J.* **11**: 290-303.
34. Shiga, A., Sasaki, T. & Horii, N. (1987) Correlations among pH and Mg, Ca, P, Na, K, Cl^- and HCO_3^- contents of digesta in the gastrointestinal tract. *Jpn. J. Vet. Sci.* **49**: 973-979.
35. Hardwick, L.L., Jones, M.R., Brautbar, N. & Lee, D.B.N. (1991) Magnesium absorption: mechanisms and the influence of vitamin D, calcium and phosphate. *J. Nutr.* **121**: 13-23.
36. Hodgkinson, A. & Heaton, F.W. (1965) The effect of food ingestion on the urinary excretion of calcium and magnesium. *Clin. Chim. Acta* **11**: 354-362.
37. Schwartz, R., Walker, G., Linz, M.D. & MacKellar, I. (1973) Metabolic responses of adolescent boys to two levels of dietary magnesium and protein. I. Magnesium and nitrogen retention. *Am. J. Clin. Nutr.* **26**: 510-518.
38. Birlouez-Aragon, I. (1988) Effect of lactose on calcium absorption during duodenal milk perfusion. *Repr. Nutr. Develop.* **28**: 1465-1472.
39. Nordin, B.E.C. (1976) Calcium, phosphate and magnesium metabolism. Churchill Livingstone, New York.
40. Shills, M.E. (1984) Magnesium. In: Nutrition Reviews. Present knowledge in nutrition. Washington DC: Nutrition Foundation.
41. Heaney, R.P., Weaver, C.M. & Fitzsimmons, M.L. (1990) Influence of calcium load on absorption fraction. *J. Bone Min. Res.* **11**: 1135-1138.

Chapter 9

General discussion

General discussion

There is an increasing use of soybean products as substitute for animal protein in human nutrition (1), whereas the impact of this dietary change on magnesium absorption is not clear. Earlier studies in rats indicate that feeding of soybean protein instead of casein does not affect magnesium concentrations in serum, femur, kidney and liver (2, 3), which could imply that magnesium absorption is not affected by the type of dietary protein. However, Chapter 3 demonstrates that soybean protein versus casein decreased apparent magnesium absorption and that this effect is caused by the phytate component of soybean protein; the addition of sodium phytate to a casein diet, to a concentration identical to that in the soybean protein diet, reduced apparent magnesium absorption to the same extent as did the diet containing soybean protein. Therefore, it is suggested that soybean protein lowers apparent absorption of magnesium through its phytate component. At neutral pH, as it occurs in the intestine, phytate can form an insoluble calcium-magnesium-phytate complex (4) or a protein-magnesium-phytate complex (5). Formation of such complexes might decrease intestinal magnesium solubility. Because magnesium absorption is directly correlated with the concentration of soluble magnesium in the intestine, at least at lower concentrations (Chapter 6), the formation of insoluble magnesium complexes could decrease magnesium absorption (6). Indeed, Shinoda and Yoshida (7) showed that dietary phytate lowered magnesium solubility in the gut. However, in view of our results it is unlikely that the observed soybean protein-induced decrease in apparent magnesium absorption is caused by the formation of an insoluble complex in the intestine. In Chapter 7, it is shown that true magnesium absorption in rats was not altered by soybean protein versus casein. Likewise, the addition of sodium phytate to the diet did not affect true magnesium absorption. On the other hand, a significant rise in fecal excretion of endogenous magnesium was observed in rats fed either soybean protein or sodium phytate. It seems that the inhibitory influence of soybean protein and sodium phytate on apparent magnesium absorption reflects stimulation of fecal excretion of endogenous magnesium. However, this was compensated for by a reduction in urinary excretion of magnesium.

Lactose has frequently been reported to increase apparent magnesium absorption in rats (Chapter 2). The studies described in this thesis are confirmatory (Chapters 3, 5 and 7). Chapter 7 shows that true magnesium absorption was significantly raised by lactose. Although fecal excretion of endogenous magnesium was enhanced by lactose, apparent magnesium absorption was also significantly higher in rats fed lactose versus dextrose. The lactose-induced stimulation of magnesium absorption is reflected by enhanced urinary magnesium excretion. Evidently, the extra amount of magnesium absorbed was higher than that of endogenous magnesium excreted in feces. The lactose-induced increase of magnesium absorption in rats may be related to the lactase-deficiency of these animals. Rats become lactase-deficient after weaning (8) and thus are not capable to hydrolyze lactose in the intestine. Possibly microbial fermentation of lactose in the intestine lowers luminal pH. This could increase solubility of magnesium in the intestine, leading to enhanced magnesium

absorption. Chapter 5 documents that feeding of lactose to rats decreased intestinal pH from 7.5 to 7.2. Such a decrease would clearly increase magnesium solubility *in vitro* (Chapter 5). *In vivo*, an increase in magnesium solubility in the intestinal lumen could not be demonstrated. This might be due to differences in transit time between rats fed either lactose or glucose. However, the lactose-induced decrease in intestinal pH was associated with an increase in apparent magnesium absorption from 52 % to 70 %, suggesting that intestinal pH is an important determinant in magnesium absorption.

Based on above reasoning, it could be hypothesized that lactose has no effect on magnesium absorption in lactose-tolerant humans. Data from literature cannot prove or disprove this hypothesis. Experiments with lactose-tolerant infants have indicated that lactose stimulates apparent magnesium absorption (9-11). However, in one study magnesium intake was not reported and thus differences in magnesium intake cannot be excluded (11), while in another study lactose ingestion was associated with increased magnesium intake (10). As shown in Chapter 8, a higher magnesium intake by itself results in increased absolute magnesium absorption so that in the study of Wirth *et al.* (10) an independent effect of lactose could not be determined. In Chapter 8 evidence is presented that in lactose-tolerant humans with constant magnesium intake lactose may not affect apparent magnesium absorption.

From the effects of lactose present in cow's milk products versus that of the phytate component of soybean products, it can be predicted that substitution of soybean beverage for cow's milk lowers apparent magnesium absorption in rats. However, it is possible that lactose and phytate do not exhibit the anticipated effects when present in the matrix of the intact products. In Chapter 4 it is shown that these compounds as present in cow's milk or soybean beverage still exert their influence on magnesium absorption in rats. Soybean beverage versus cow's milk reduced apparent magnesium absorption. As to the impact of substitution of soybean beverage for cow's milk on apparent magnesium absorption in humans only speculation is opportune. The lactose component of milk does probably not affect apparent magnesium absorption (Chapter 8). Literature data (Chapter 2) indicate that pure sodium phytate as well as naturally occurring phytate decrease apparent magnesium absorption in humans.

The relationship between the intestinal absorption of magnesium and calcium has been subject of considerable controversy. Studies in rats have demonstrated that apparent absorption of magnesium is depressed by high calcium intake (Chapter 2). This has often been explained by competition between magnesium and calcium for a common carrier system in the ileum (12-14). However, in a recent study in rats (15), competition between magnesium and calcium for ileal uptake could not be confirmed. The observation described in Chapter 6 that increased intake of calcium does not reduce magnesium absorption at a relatively low dietary phosphate concentration, suggests that there is no common mechanism for transport of magnesium and calcium across the intestinal mucosa. Results from human studies are contradictory (Chapter 2). Some studies show a decreased apparent magnesium absorption at high calcium intakes, but most studies with healthy adult subjects indicate that

high calcium intake does not affect intestinal magnesium absorption. An explanation for the controversy between rat and most human experiments is offered in Chapter 6. The inhibitory effect of dietary calcium on apparent magnesium absorption appears to depend on the phosphate:magnesium ratio of the diet which determines whether or not an insoluble magnesium-calcium-phosphate complex is formed in the intestine. The relatively low phosphate:magnesium ratio and low calcium concentration in human diets, as compared with rat diets might result in less formation of insoluble complexes in the human intestine after ingestion of extra calcium. This would explain why addition of calcium to human diets does not affect apparent magnesium absorption, whereas addition of calcium to rat diets inhibits it. This explanation is confirmed by the study of Giles *et al.* (16). In infants consuming diets with a high phosphate:magnesium ratio similar to that of rat diets, an increase in calcium intake decreased apparent magnesium absorption.

References

1. Erdman J.W. & Fordyce E.J. (1989) Soy products and the human diet. *Am. J. Clin. Nutr.* **49**: 725-737.
2. Lo, G.S., Steinke, F.H. & Hopkins, D.T. (1980) Effect of isolated soybean protein on magnesium bioavailability. *J. Nutr.* **110**: 829-836.
3. Forbes, R.M., Weingarter, K.E., Parker, H.M., Bell, R.R. & Erdman, J.W. (1979) Bioavailability to rats of zinc, magnesium and calcium in casein-, egg- and soy protein-containing diets. *J. Nutr.* **109**: 1652-1660.
4. Cheryan, M., Anderson, F.W. & Grynspan, F. (1983) Magnesium-phytate complexes: Effect of pH and molar ratio on solubility characteristics. *Cereal Chem.* **69**: 235-237.
5. Champagne, E.T. (1988) Effect of pH on mineral-phytate, protein-mineral-phytate, and mineral-fiber interactions. Possible consequences of atrophic gastritis on mineral bioavailability from high-fiber foods. *J. Am. Coll. Nutr.* **7**: 499-508.
6. Hardwick, L.L., Jones, M.R., Brautbar, N. & Lee, D.B.N. (1991) Magnesium absorption: Mechanisms and the influence of vitamin D, calcium and phosphate. *J. Nutr.* **121**: 13-23.
7. Shinoda, S. & Yoshida, T. (1989) Influence of sodium phytate and gut microflora on the solubility of Ca, Mg and Zn in the rat digestive tract. *Nutr. Rep. Int.* **40**: 909-922.
8. De Groot, A.P. & Hoogendoorn, P. (1957) The detrimental effect of lactose. II. Quantitative lactase determinations in various mammals. *Neth. Milk Dairy J.* **11**: 290-303.
9. Ziegler, E.E. & Fomon, S.J. (1983) Lactose enhances mineral absorption in infancy. *J. Pediatr. Gastroenterology Nutr.* **2**: 288-294.
10. Wirth, F.H., Numerof, B., Pleban, P. & Neylan, M.J. (1990) Effect of lactose on mineral absorption in preterm infants. *J. Pediatr.* **117**: 283-287.
11. Kobayashi, A., Kawai, S., Ohbe, Y. & Nagashima, Y. (1975) Effects of dietary lactose and a lactase preparation on the intestinal absorption of calcium and magnesium in normal infants. *Am. J. Clin. Nutr.* **28**: 681-683.
12. Alcock, N. & MacIntyre, I. (1962) Inter-relation of calcium and magnesium absorption. *Clin. Sci.* **22**: 185-193.
13. Behar, J. (1975) Effect of calcium on magnesium absorption. *Am. J. Physiol.* **229**: 1590-1595.
14. Care, D.A. & van 't Klooster, A.Th. (1965) *In vivo* transport of magnesium and other cations across the wall of the gastrointestinal tract of the sheep. *J. Physiol.* **177**: 174-191.
15. Karbach, U. & Rummel, W. (1990) Cellular and paracellular magnesium transport across the terminal ileum of the rat and its interaction with the calcium transport. *Gastroenterology* **98**: 985-992.
16. Giles, M.M., Laing, I.A., Elton, R.A., Robins, J.B., Sanderson, M. & Hume, R. (1990) Magnesium metabolism in preterm infants: Effect of calcium, magnesium, and phosphorus, and of postnatal gestational age. *J. Pediatr.* **117**: 147-154.

Summary

The influence of various nutrients present in dairy products and soybean-based products on absorption of magnesium has been investigated. The studies demonstrate that soybean protein versus casein lowers apparent magnesium absorption in rats through its phytate component. However, true magnesium absorption was neither affected by soybean protein in the diet nor by supplemental phytate. The inhibitory influence of soybean protein and phytate on apparent magnesium absorption was found to be caused by stimulation of fecal excretion of endogenous magnesium. The loss of magnesium was compensated for by depressed urinary magnesium excretion so that whole-body magnesium retention was left unchanged. Lactose in the diet raised both apparent and true magnesium absorption in rats. Phytate and lactose, as intrinsic component of milk and soybean beverage still exerted their influence on apparent magnesium absorption in rats. The stimulatory effect of lactose is most likely due to the lactase-deficiency of these animals. Microbial fermentation of lactose in the intestine may have caused the observed decrease in intestinal pH. This might result in increased magnesium solubility in the ileal lumen, which in turn stimulates magnesium absorption. The concentration of soluble magnesium in the intestinal lumen, at least at low concentrations, is the major determinant of the amount of magnesium absorbed. In lactose-tolerant humans possessing lactase in the intestine, lactose did not affect magnesium absorption. Increasing intakes of calcium may reduce apparent magnesium absorption, depending on the phosphate:magnesium ratio in the diet. High ratios favor the formation of an insoluble magnesium-calcium-phosphate complex in the intestine after consumption of extra calcium, so that magnesium absorption is impaired. This mechanism speaks against a common mechanism for transport of magnesium and calcium across the intestinal mucosa.

Samenvatting

In dit proefschrift is de invloed van verschillende nutrienten, die voorkomen in melk- en sojaprodukten, op de absorptie van magnesium bestudeerd. De experimenten laten zien dat sojaeiwit in vergelijking met caseine de schijnbare magnesiumabsorptie bij ratten verlaagt door de aanwezigheid van fytaat in dit eiwit. Noch sojaeiwit in de voeding, noch suppletie van fytaat hadden invloed op de 'werkelijke' absorptie van magnesium. Er werd aangetoond dat de vermindering van de schijnbare magnesiumabsorptie door sojaeiwit en fytaat werd veroorzaakt door een verhoogde uitscheiding van endogeen magnesium in de faeces. Dit verlies van magnesium werd gecompenseerd door een verlaging van de magnesium uitscheiding in de urine, waardoor de magnesium retentie in het lichaam niet werd beinvloed. Lactose in de voeding verhoogde zowel de schijnbare als de 'werkelijke' absorptie van magnesium in ratten. Fytaat en lactose, indien aanwezig in de complete produkten sojadrank en melk, behielden hun effect op de schijnbare magnesiumabsorptie in ratten. Er zijn aanwijzingen dat het stimulerende effect van lactose wordt veroorzaakt door het feit dat de rat lactase-deficient is. De waargenomen pH daling in de darm wordt mogelijk veroorzaakt door microbiële fermentatie van lactose. Deze pH daling zou kunnen resulteren in een verhoogde oplosbaarheid van magnesium in het darmlumen waardoor de magnesiumabsorptie wordt gestimuleerd. De concentratie oplosbaar magnesium is, in ieder geval bij lage concentraties, de belangrijkste determinant voor de hoeveelheid magnesium die wordt opgenomen. Bij lactose-tolerante volwassenen, die in bezit zijn van het enzym lactase in de darm, had lactose geen effect op de schijnbare magnesiumabsorptie. Een verhoging van de calciuminneming kan, afhankelijk van de fosfaat:magnesium verhouding in de voeding, de schijnbare absorptie van magnesium verminderen. Hoge fosfaat:magnesium verhoudingen bevorderen de vorming van een onoplosbaar calcium-magnesium-fosfaat complex in de darm na consumptie van extra calcium, waardoor de absorptie van magnesium wordt verlaagd. In dit onderzoek zijn geen aanwijzingen gevonden voor een gemeenschappelijk transport-mechanisme van calcium en magnesium over de darmmucosa.

Curriculum vitae

Lisette Brink werd geboren op 4 augustus 1962 te Heusden. In 1980 behaalde zij het diploma gymnasium-ß aan het Mgr. Zwijsen College te Veghel. Aansluitend begon zij de studie Humane Voeding aan de toenmalige Landbouwhogeschool te Wageningen en behaalde daar in september 1984 het kandidaatsexamen. Tijdens de doctoraalfase werkte zij gedurende acht maanden op het laboratorium voor Pathologie van het Rijksinstituut voor Volksgezondheid en Milieuhygiëne (RIVM) te Bilthoven. In januari 1987 slaagde zij voor het ingenieurs-examen met als hoofdvak Toxicologie en als bijvakken Voedingsleer en Dierfysiologie. Op 15 mei 1987 begon zij als wetenschappelijk medewerkster bij de afdeling Voedingsfysiologie van het Nederlands Instituut voor Zuivelonderzoek (NIZO) te Ede met het onderzoek waarvan de resultaten in dit proefschrift staan beschreven. In 1988 behaalde zij het Certificaat Onderzoeker ex artikel 9 van de Wet op de Dierproeven. Sinds 15 november 1991 is zij werkzaam als verteringsfysiologe bij het Unilever Research Laboratorium te Vlaardingen.