

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

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The hypothesis was tested that dietary lactose *v.* glucose stimulates Mg absorption in rats because lactose lowers pH of the ileal lumen, which improves Mg solubility which in turn enhances Mg availability for transport across the ileal 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 (100 g/Kg) significantly stimulated apparent absorption of Mg. Apart from Mg absorption, lactulose also significantly enhanced absorption of Ca and phosphate. Lactose *v.* glucose lowered the pH of the ileal 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 Mg, and to a lesser extent also of Ca and phosphate. The smaller fall of ileal pH induced by feeding lactose instead of lactulose may explain why lactose improved Mg absorption only. For all individual rats combined there were negative relationships between ileal pH and apparent absorption of minerals, the relationship being strongest for Mg. Neither lactose nor lactulose was found to raise ileal solubility of minerals, which could relate to the possibility that the time of sampling was not appropriate. It is suggested that lactose-induced stimulation of Mg absorption in rats is caused by a lowering of ileal pH.

Dietary lactose: Lactulose: Magnesium: Rat

Dietary lactose (β -1,4-galactosyl-glucose) *v.* various other carbohydrates influences mineral absorption in rats. Lactose has been consistently shown to improve apparent Mg absorption (Andrieux & Sacquet, 1983; Schaafsma *et al.* 1988; Greger *et al.* 1989; Behling & Greger, 1990; Brink *et al.* 1991), whereas apparent Ca absorption was enhanced in some studies (Fournier *et al.* 1971; Leichter & Tolensky, 1975; Armbrrecht & Wasserman, 1976; Favus & Angeid-Backmann, 1984), but not in others (Greger *et al.* 1987; Sheikh *et al.* 1987; Recker *et al.* 1988; Behling & Greger, 1990). Lactose was found to accelerate the appearance of label in blood when rats were administered lactose and radioactive phosphate together orally (Debiec & Lorenc, 1988). However, in feeding trials no effect of lactose on apparent phosphate absorption was found (Schaafsma *et al.* 1988). The mechanism by which lactose stimulates Mg absorption is not known. Rats become lactase deficient after weaning (De Groot & Hoogendoorn, 1957) and, thus, lactose is poorly digested. This may induce microbial fermentation of lactose in the ileum, as has been shown for arabinose which is also a poorly digestible carbohydrate (Schutte *et al.* 1992).

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Fermentation of lactose may lower the pH in the ileal lumen. Insoluble Ca–Mg–phosphate complexes occur in the ileum (Brink *et al.* 1992), and a lowered pH should prevent the formation of these complexes and, thus, improve solubility of the mineral components (Greenwald *et al.* 1940). Only soluble minerals may cross the ileal epithelium (Bronner, 1987; Hardwick *et al.* 1991), which is the most prominent site in Mg absorption (Hardwick *et al.* 1991). Thus, it could be hypothesized that lactose lowers the pH in the ileal lumen, which enhances ileal solubility of Mg and, thus, stimulates its absorption. However, lactose may not have a major impact on ileal solubility of Ca and phosphate and consequently has no effect on Ca and phosphate absorption either. This hypothesis was tested in *in vitro* experiments and a feeding trial with weanling female rats. To see whether the hypothesis can be generalized, the effects of feeding another poorly digestible carbohydrate, lactulose (β -1,4-galactosyl-fructose; Pomare *et al.* 1985) were studied as well.

MATERIALS AND METHODS

Animals and housing

We used female, outbred Wistar rats (Hsd/Cpb:WU; Harlan, Zeist, The Netherlands), aged about 3 weeks. The experimental protocol was approved by the Animal Experiment Committee of the Department of Laboratory Animal Science, Utrecht State University. On arrival the rats were fed *ad lib.* on a commercial, pelleted diet (RMH-B; Hope Farms, Woerden, The Netherlands) and tap water. They were housed in wire-topped polycarbonate cages (375 × 225 × 150 mm) with a layer of sawdust as bedding. At 4 d after arrival all rats were fed on the purified control diet (Table 1) and demineralized water *ad lib.* After another 8 d (day 0 of the experiment) the rats were divided into three groups of twelve animals each, so that group mean body weights were similar. As from day 0 the rats were housed individually in metabolic cages (31400 mm² × 120 mm). The cages were placed in a room with controlled temperature (20–22°), lighting (light: 06.00–18.00 hours) and relative humidity (50–65%).

Diets

During the experimental period (days 0–21, 0–22 or 0–23) one group of rats remained on the control diet (Table 1). The other groups were fed on diets with 100 g lactose or lactulose/kg. 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 (< 0.01 g/kg) of Mg, Ca and phosphate.

As from day 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° 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 day 17 to day 19 faeces and urine of each rat were collected quantitatively. The cages and tubes for collection of faeces 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 HCl/l and demineralized water.

On day 21, between 08.30 and 11.00 hours, four rats from each dietary group were anaesthetized 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 caecum was removed. The contents in the distal third of the intestine (ileum) were collected in preweighed tubes by gently squeezing the intestine between finger and thumb. The ileal contents were immediately centrifuged (10 min, 10000 g), and the supernatant

Table 1. *Composition of the experimental diets*

	Control*	Lactose	Lactulose
Ingredient (g)			
Glucose†	709.4	609.4	609.4
Lactose‡	—	100.0	—
Lactulose§	—	—	149.9
Constant components	290.6	290.6	290.6
Chemical analysis			
Dry matter (g/kg)	941	941	893
Ca (mmol/kg)	112.5	115.0	107.5
Mg (mmol/kg)	16.4	16.4	16.4
Phosphate (mmol/kg)	125.8	129.0	135.4

* This diet also served as pre-experimental diet.

† Morsweet 01934; Cerestar, Haubourdin, France.

‡ Whey Products, Borculo, The Netherlands.

§ Duphulac; Duphar BV, Amsterdam, The Netherlands; this lactulose preparation contained 333 g water/kg.

|| The constant components consisted of (g): casein 151, maize oil 25, coconut fat 25, cellulose 30, CaCO₃ 12.4, MgCO₃ 1.4, NaH₂PO₄·2H₂O 15.1, KCl 1.0, KHCO₃ 7.7, mineral premix 10, vitamin premix 12. The mineral premix consisted of (mg): FeSO₄·7H₂O 174, MnO₂ 79, ZnSO₄·H₂O 33, NiSO₄·6H₂O 13, NaF 2, KI 0.2, CuSO₄·5H₂O 15.7, Na₂SeO₃·5H₂O 0.3, CrCl₃·6H₂O 1.5, SnCl₂·H₂O 1.9, NH₄VO₃ 0.2, maize 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, cholecalciferol 2, maize meal 9826.15.

fraction and pellet were separated. The weights of pellet and supernatant fraction were determined. The pH of the supernatant fraction was measured directly (Russell combination pH electrode, Type RS-53; Auchtermuchty, Fife, UK). Trichloroacetic acid (TCA) was added to the supernatant fraction to a final concentration of 54 g/l and the TCA-soluble fraction obtained by centrifugation (2 min, 10000 g). Pellet and TCA-soluble fractions were analysed for minerals. Caecum (including its contents), kidney and liver were excised and weighed. On days 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 ileal fluid affects mineral solubility, *in vitro* experiments were carried out. The unique advantage of such experiments is that the influence of pH as the only variable can be ascertained. The molar Ca:phosphate:Mg value 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 (mmol/l) in distilled water either MOPS 50 (pH, 6.4–7.4) or HEPES 50 (pH, 7.6–8.2) and MgCl₂ 3.5, CaCl₂ 20, Na₂HPO₄ 20 and variable amounts of NaCl to maintain the ionic strength at 150 mmol/l. The mixture (0.9 ml) without Na₂HPO₄ was pre-incubated for 10 min at 37° in a shaking water-bath. Then, 0.1 ml Na₂HPO₄ (200 mmol/l) was added and the mixture further incubated for another 15 min. The tubes were then centrifuged (2 min, 10000 g), supernatant fractions collected and the pH measured. After dilution of the supernatant fraction with TCA (final concentration 50 g/l), Mg, Ca and phosphate were analysed.

Chemical analyses

Mg and Ca in samples of ileal contents, faeces, urine, diets and supernatant fractions of *in vitro* incubations were analysed as described elsewhere (Hoek *et al.* 1988; Mars *et al.* 1988). Phosphate was determined in ashed feed samples dissolved in 6 M-HCl with the use of a commercial test combination (Phosphate, MA-KIT 10 ROCHE; Roche Diagnostics,

Table 2. *Effects of dietary lactose and lactulose on growth performance, selected organ weights and excreta production in rats**

(Values are means with their standard errors for twelve rats per dietary group)

Diet ...	Control		Lactose		Lactulose	
	Mean	SE	Mean	SE	Mean	SE
Body wt						
Initial (g)	94	2	94	2	94	2
Final (g)	160 ^b	4	156 ^{ab}	3	148 ^a	2
Feed intake (g/d)	12.6 ^b	0.4	12.1 ^{ab}	0.1	11.4 ^a	0.2
Relative organ wt (g/kg body wt)						
Kidney	3.9	0.1	3.8	0.1	3.6	0.1
Caecum	8.3 ^a	0.5	14.4 ^b	0.9	30.9 ^c	1.6
Liver	42.9	0.8	43.1	0.8	41.2	0.7
Faeces						
Production (g/d)	0.9 ^a	0.1	1.0 ^{ab}	0.1	1.2 ^b	0.1
Dry matter (g/kg)	693 ^c	10	600 ^b	16	534 ^a	11
Urine						
Production (ml/d)	4.1 ^a	0.3	3.8 ^a	0.3	5.8 ^b	0.4

^{a, b, c} Group means with unlike superscript letters were significantly different (Tukey's test; $P < 0.05$).

* For details of diets and procedures, see Table 1 and pp. 748-749.

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 week after dissolution.

Calculations

The distribution of minerals between the solid and liquid phases 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 the liquid phase in the pellet (= total pellet weight minus solid phase) and that of the supernatant fraction. The concentration of minerals in the supernatant fraction 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 fraction by the weight of the liquid phase gave the amount of minerals in the liquid phase. The relative amount of mineral in the liquid phase was computed as a percentage of the total amount in the ileal contents.

Apparent absorption of minerals was calculated as mineral intake minus faecal excretion and expressed as percentage of intake. Retention of minerals was calculated as mineral intake minus faecal 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 on the lactulose diet were significantly lower than those of the control group (Table 2). Relative kidney and liver weights were similar

Table 3. *Effects of dietary lactose and lactulose on the balance of calcium and magnesium in rats**

(Values are means with their standard errors for twelve rats per dietary group)

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

^{a, b, c} Group means with unlike superscript letters were significantly different (Tukey's test; $P < 0.05$).

* For details of diets and procedures, see Table 1 and pp. 748–750.

for all groups. Weight of caecum with contents was significantly elevated in rats fed on lactose or lactulose. Lactulose produced a more than twofold higher caecum weight than did lactose.

Excreta production

Faeces production of rats fed on the lactulose diets was significantly higher than that of rats fed on the control diet containing glucose (Table 2). This was due to the elevated water content of faeces in the rats fed on lactulose. Lactose also produced an increment in the amount of water in faeces but this effect was less pronounced. Group mean urine production was highest in the lactulose group.

Mineral balance

Table 3 shows that intakes of minerals during the balance period (days 17–19) were similar in the dietary groups. Lactose significantly raised apparent Mg absorption, but did not affect that of phosphate. Lactose did not significantly affect urinary mineral excretion. Apparent absorption and retention of Mg and phosphate were significantly higher in the rats fed on lactulose than in those fed on glucose.

Table 4. *Effect of dietary lactose and lactulose on the distribution of calcium and magnesium between the liquid and solid phase in the ileal lumen in rats**

(Values are means with their standard errors for twelve rats per dietary group)

Diet ...	Control		Lactose		Lactulose	
	Mean	SE	Mean	SE	Mean	SE
Liquid phase: wt (g)	0.30 ^a	0.02	0.36 ^{ab}	0.03	0.42 ^b	0.03
pH	7.5 ^b	0.1	7.2 ^{ab}	0.1	7.0 ^a	0.1
Solid phase: wt (g)	0.10	0.01	0.09	0.01	0.10	0.01
Ca						
Amount in liquid phase (μmol)	3.5 ^a	0.3	5.8 ^b	0.6	6.0 ^b	0.8
Amount in solid phase (μmol)	90	12	88	10	62	7
Percentage in liquid phase	4.0 ^a	0.5	6.5 ^{ab}	0.7	9.0 ^b	1.0
Concentration in liquid phase (mmol/l)	12	2	16	1	14	2
Mg						
Amount in liquid phase (μmol)	4.5 ^b	0.3	4.5 ^b	0.3	2.9 ^a	0.3
Amount in solid phase (μmol)	9 ^b	1	8 ^b	1	5 ^a	1
Percentage in liquid phase	35	3	38	3	40	4
Concentration in liquid phase (mmol/l)	16 ^b	1	13 ^b	1	7 ^a	1
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
Percentage 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

^{a, b, c} Group means with unlike superscript letters were significantly different (Tukey's test; $P < 0.05$).

* For details of diets and procedures, see Table 1 and pp. 748–750.

Apparent absorption of Ca was not significantly affected by lactose, whereas Ca absorption was significantly increased in rats fed on lactulose. In the rats fed on the lactulose diet, urinary Ca excretion was significantly elevated.

Minerals in the ileal lumen

Rats fed on lactulose had significantly more liquid phase in their ileal lumen than rats fed on 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.

Ca concentration in the liquid phase did not differ significantly between the dietary groups. However, lactose and lactulose raised the absolute amount of Ca in the liquid phase. The amount of Ca in the solid phase was similar for the three groups. The percentage of total Ca in the liquid phase in the ileal lumen was significantly elevated in the lactulose group and tended to be elevated in the rats fed on lactose when compared with the control group.

Group mean amounts of Mg in the liquid and solid phases in rats fed on the lactulose

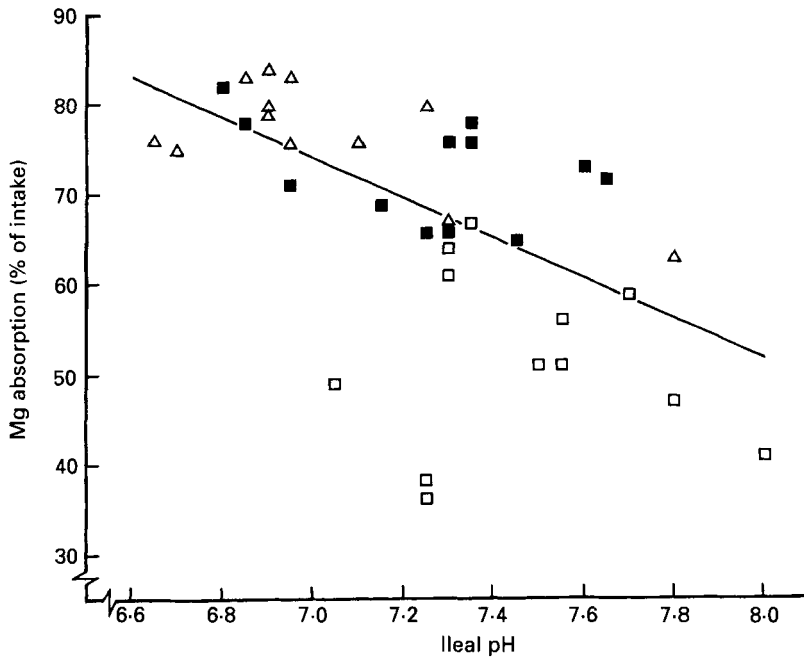


Fig. 1. Relationship between pH in the ileal lumen and percentage apparent magnesium absorption in rats fed on the experimental diets ($y = -22x + 229$, $r = -0.69$, $n = 36$). (□), Control; (■), lactose; (△), lactulose. For details of diets and procedures, see Table 1 and pp. 748–750.

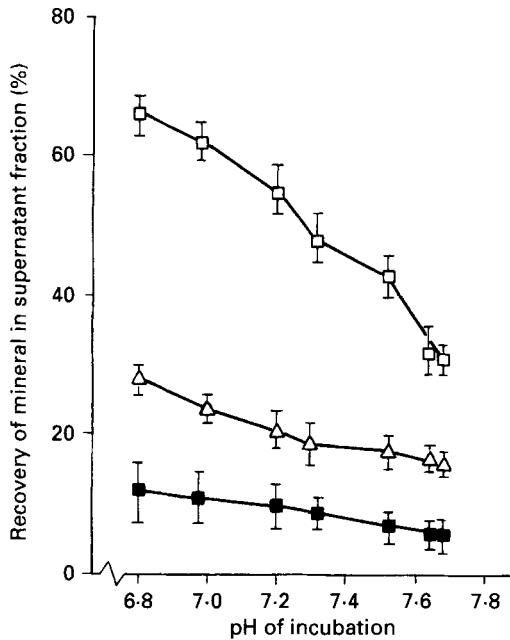


Fig. 2. Magnesium (□), calcium (■) and phosphate (△) in supernatant fractions of *in vitro* incubations of solutions at varying pH. Recovery in the supernatant fraction is expressed as a percentage of the total amount of mineral in the incubation. Values are means and standard deviations represented by vertical bars for six determinations. For details of procedures, see pp. 748–749.

diet were lower than those in rats fed on the other diets. The proportion of soluble Mg 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.

Ileal pH and mineral absorption

There was a negative relationship between the pH in the ileal lumen and percentage apparent Mg absorption (Fig. 1). Similar relationships, although less strong, were calculated for Ca ($r=0.40$, 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 Mg, Ca and phosphate in the supernatant fraction of the incubations were negatively associated (Fig. 2). The increase in solubility with decreasing pH was most pronounced for Mg.

DISCUSSION

As would be expected (Beynen, 1989; Henskens *et al.* 1991), the feeding of either lactose or lactulose produced markedly elevated caecum weights. This substantiates the low digestibility of these carbohydrates (Pomare *et al.* 1985; Andrieux *et al.* 1989) 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 faecal 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.

The present study shows that dietary lactose *v.* glucose significantly enhanced the apparent absorption of Mg, which corroborates other reports (Andrieux & Sacquet, 1983; Schaafsma *et al.* 1988; Greger *et al.* 1989; Behling & Greger 1990; Brink *et al.* 1991). Lactose *v.* glucose caused an increment in group mean Ca absorption, but this effect failed to reach statistical significance. Similar results have been published by others (Fournier *et al.* 1971; Leichter & Tolensky, 1975; Armbrrecht & Wasserman, 1976; Favus & Angeid-Backmann, 1984). Lactose in the diet did not significantly affect apparent phosphate absorption, which agrees with other work (Schaafsma *et al.* 1988). In the present study marked effects of lactulose on mineral absorption emerged. Lactulose *v.* glucose significantly stimulated the apparent absorption of Mg, Ca and phosphate. Another poorly digestible carbohydrate which is structurally related to lactulose, lactitol (β -D-galactopyranosyl-1,4-D-sorbitol), has also been shown to enhance Ca absorption (Ammann *et al.* 1988).

The major objective of the present study was to find out why lactose stimulates Mg absorption. This can be extended by the question why lactulose enhances the absorption of Mg, Ca and phosphate. We had hypothesized that lactose lowers the pH in the ileal lumen, thereby improving the solubility of Mg which in turn elevates the amount of Mg that can cross the ileal epithelium. A similar cascade of events could explain lactulose-induced stimulation of mineral absorption. From the outset a number of limitations of the experimental design used should be discussed. Ileal pH and soluble minerals in ileal digesta were determined at one point of time, whereas apparent mineral absorption was a measure integrated over 3 d. Time-dependent fluctuations in ileal variables could weaken the relationship, if any, between ileal variables and apparent absorption of minerals. Thus, a lack of correlation between ileal pH or ileal soluble Mg and Mg absorption cannot

conclusively disprove our hypothesis. Furthermore, the rats had been fed on an *ad lib.* basis and no attempt was made to standardize the amount and timing of food intake. Thus, any group differences in eating behaviour would hinder the demonstration of a possible relationship between ileal pH or ileal soluble Mg and apparent Mg absorption.

Lactose *v.* 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 Mg. Similar effects, although much less pronounced, were seen for Ca and phosphate. However, dietary lactose did not raise the proportion and concentration of Mg, Ca and phosphate in the liquid phase of ileal digesta. Dietary lactulose significantly lowered the ileal pH from 7.5 to 7.0. In keeping with the effects seen *in vitro*, dietary lactulose *v.* glucose caused a significant increment in the proportion of Ca in the ileal liquid phase but it did not affect Ca concentration. Lactulose also raised group mean proportions of soluble Mg and phosphate. Due to the large within-group variation these effects did not reach statistical significance. Thus, there is no solid evidence that lactulose raised ileal solubility of Mg, Ca and phosphate.

For all individual rats combined, negative relationships between pH in the ileal lumen and apparent absorption of Mg, Ca and P could be demonstrated. As would be expected on the basis of *in vitro* observations (Fig. 2), the relationship was strongest for Mg (Fig. 1). The observed negative relationship between ileal pH and apparent Mg absorption is consistent with the first part of our hypothesis. However, the second part of our hypothesis, that is the existence of a positive relationship between ileal soluble Mg and Mg absorption, is not supported by the present study. As mentioned previously it is possible that the experimental design used prevented the demonstration of a relationship between ileal soluble Mg and apparent Mg absorption.

The concentration of minerals in the liquid phase of the ileal contents may determine mineral absorption rather than the proportions of minerals in the liquid phase. Within this concept, lactulose should elevate the concentration of minerals in the liquid phase. However, as mentioned previously, this was not seen. As to Mg, the concentration in the liquid phase was even significantly lowered after feeding lactulose. This may relate to differences in timing of food intake or intestinal transit time between rats fed on either glucose or lactulose. This is supported by the observation that the intestine of rats fed on the lactulose diet tended to contain less minerals. If non-absorbable markers for the solid and liquid phases of digesta had been added to the diets it would have been possible to express ileal minerals relative to the markers. This would have facilitated interpretation of the results. Unfortunately we did not use non-absorbable markers.

In the present study we focussed on the ileum as predominant site in Mg absorption. Thus, any diet effects on Mg solubility in digesta from other sites of the gastrointestinal tract remain unknown. Mg (Hardwick *et al.* 1991), Ca (Petith & Schedl, 1976; Beynen *et al.* 1990) and phosphate (Beynen *et al.* 1990) can be absorbed in the caecum and/or colon. Given the marked influence of lactose and lactulose on caecum weight it is likely that these disaccharides alter the milieu of the caecum and/or colon. Whether this influences overall mineral absorption is not known.

In conclusion, under the experimental conditions used the stimulatory effect of lactose on Mg absorption was not associated with increased solubility of Mg in the ileal lumen. However, the observed negative relationship between pH in the ileal lumen and intestinal Mg absorption indicates that the decrease in ileal pH after lactose feeding is responsible for the improved Mg absorption.

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