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ORIGINAL CONTRIBUTION

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Stimulatory effect of inulin on intestinal absorption of calcium and magnesium in rats is modulated by dietary calcium intakes

Short- and long-term balance studies

Summary Previous studies have shown that short-term intake of fermentable oligosaccharides (OS), including inulin, can increase mineral intestinal absorption in humans and animals. While the stimulatory effect of these substances on intestinal magnesium (Mg) absorption is generally high and consistent, their effect on calcium (Ca) seems to depend on experimental conditions, particularly the duration of fermentable OS intake. The aim of this study was to determine how the short- and long-term dietary Ca intake may modulate the effect of inulin on Ca absorption. Sixty male Wistar rats, weighing 275 g, were randomized into two groups to receive or not 10 % of inulin in their diet. Each group was divided into three sub-groups to receive one of the following dietary Ca levels 0.25 %, 0.50 % and 0.75% in their food. The animals were fed fresh food and water *ad libitum* for 40 days. Apparent intestinal absorptions of Ca and Mg were determined at D13 and D36 of the experiment. As expected, inulin feeding increased Ca and Mg absorption in both periods at all dietary Ca levels. However, the effect of inulin on intestinal Ca absorption was dependent on dietary Ca levels and on experiment duration. In the short-term period, the inulin effect was prominent in the groups receiving high or low Ca levels, but in long-term period inulin improved intestinal Ca absorption much more in the group receiving the low Ca level. In addition, efficiency of intestinal absorption of Ca and Mg (%) was negatively affected by Ca intake levels. These results show that the beneficial effect of inulin on intestinal Ca absorption may be more marked in cases where the Ca intake is low or where the organism's Ca requirement is high. Further studies are required to confirm these results in humans.

Key words inulin – intestinal absorption – calcium – magnesium – fermentation – rat

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Abbreviations

Ca	calcium
Mg	Magnesium
OS	oligosaccharides
SCFA	Short-chain fatty acid

Introduction

Non-digestible inulin-type fructans reach the large intestine, where they are fermented by the local microflora. They stimulate the growth of bifidobacteria and lactobacilli in the intestine, which has been suggested to be connected with health-promoting functions (prebiotic property) [1, 2]. Inulin and other fructans are considered as functional food ingredients since they affect physiological and biochemical processes resulting in better health and reduction in the risk of many diseases [3]. Sev-

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eral investigations have demonstrated that rats fed with prebiotic fructans absorbed more Ca and Mg than control rats, despite an increase in total fecal mass [4–6]. Indeed, fermentation products of fructan can influence the intestinal absorption of Ca and Mg in many ways. The short-chain fatty acids (SCFA) are fermentation products that are responsible for lowering the pH of the cecal content, which in turn increases mineral solubility, leading to improved mineral absorption [7]. SCFA can directly influence mineral absorption by forming complexes with the minerals, leading to an increase in their uptake by the intestinal cells [8, 9]. It is thought that the bacterial metabolites (e. g. butyrate) can stimulate the intestinal epithelium and increase its absorptive capacity [10]. These different aspects are closely linked to the nature of the prebiotic carbohydrates and to mineral concentrations [7, 11, 12]. The effects of inulin have been examined in animals and humans and have been shown to be generally high and consistent on intestinal Mg absorption [13], but the effects of inulin on calcium (Ca) absorption seem to be dependant on experimental conditions (inulin dose, Ca content in the diet, experiment duration, animal age and mineral requirements). In this study, we investigated the influence of dietary Ca intakes on the stimulatory effect of inulin on intestinal absorption and retention of Ca and Mg after short- and long-term administration of inulin in rats.

Materials and methods

Animals and diets

Sixty male Wistar rats (from the laboratory animal colony of the National Institute of Agronomic Research, Clermont-Ferrand-Theix, France) were fed a commercial pellet diet (U. A. R, Villemoisson-sur-Orge, France) until body weights reached ~275 g (10 weeks). Six groups of 10 rats each were then formed and fed semi-purified diets containing different levels of Ca (0.25 %, 0.50 % or 0.75 %) with or without inulin. The composition of these six diets is given in Table 1. Normal recommended level of Ca in rat diet is 0.5 %. The Ca was added to the diet in the form of chloride and carbonate (50/50) to avoid major acidifying or alkalinizing diet effect. Tested inulin was purchased from Orafti, Tienen, Belgium (Raftaline®). Chemical analysis of the diets offered to the rats confirmed the Ca content expected in the experimental diets (2510, 4831 and 6942 mg Ca/kg). Chemical analysis showed that the inulin contained approximately 40 mg Ca/kg. Dietary inulin level was 5 % during the first 4 days (D0-D4) and then 10 % until the end of the experiment. The six rat groups were given fresh food and drinks daily, available *ad libitum*. Food and water consumption and body weight were recorded weekly. During the experiment, the rats were housed two per cage (wire-bottomed to limit coprophagy) and maintained in a temperature-controlled room (22 °C) with dark period from 08:00 pm to 08:00 am. For balance studies, food intake was controlled and whole feces and urine were collected for 4 successive days on two occasions during the experiment, on D13–17 and on D36–40, to determine short-term and long-term inulin intake effects, respectively. Total experiment duration was 40 days. All the procedures complied with the Institute's guidelines for the care and use of laboratory animals.

Table 1 Diet composition (g/kg) during the experiment[1]

	Control			Inulin		
	Low Ca	Normal Ca	High Ca	Low Ca	Normal Ca	High Ca
Wheat starch	652.5	650	647.5	552.5	550	547.5
Casein	200	200	200	200	200	200
Corn oil	50	50	50	50	50	50
Cellulose	50	50	50	50	50	50
Mineral mix (AIN 1993)[a]	30	30	30	30	30	30
Vitamin mix (AIN 1993)[b]	10	10	10	10	10	10
dl-Methionine	3	3	3	3	3	3
Choline bi-tartrate	2	2	2	2	2	2
Inulin	0	0	0	100	100	100
Calcium	2.5	5.0	7.5	2.5	5.0	7.5

[a] Mineral mix AIN 1993 ensures the following mineral levels in the diets (mg/kg): Na, 1020; K, 3600; P, 4000; Mg, 500; Zn, 30; Fe, 35; Cu, 6; Mn, 54; Se,0.1; I, 0.2; Cr, 2. Ca was omitted from this mix and added to the different diets in the form of bicarbonate and chloride (50 %50) to achieve the shown final concentrations.

[b] Vitamin mix AIN 1976 ensures the following mineral levels in the diets (mg/kg): thiamine, 6; riboflavine, 6; pyridoxine, 7; nicotinic acid, 30; calcium pantothenate, 16; folic acid, 2; d-biotin: 0.2; and (µg/kg) cyanocobalamine (vitamin B12), 10; vitamin K, 50; and (IU/kg) vitamin A, 4000; vitamin E, 50; vitamin D, 1000.

[1] Rats were fed semi-liquid synthetic experimental diets for 40 days. Each day, diet powder was mixed with distilled water (1 weight/1 weight) and offered fresh to rats

Sampling procedures

The rats were sacrificed just after the dark period (between 08:00 am and 10:00 am) since cecal fermentation is still very active. After anesthesia (40 mg sodium pentobarbital/kg body weight), blood was withdrawn from the abdominal aorta, placed into tubes containing sodium heparin and centrifuged at 1000 g for 10 minutes. Plasma samples were stored at 4 °C for mineral analysis. The cecum, complete with contents, was removed and weighed (total cecal weight). The cecal wall was flushed clean with ice-cold saline, blotted on filter paper and weighed (cecal wall weight). For each rat, duplicate samples of cecal contents were collected into 2 ml microfuge tubes and immediately placed at –20 °C. The pH of cecal content was determined on site using a portative pH-meter Sentron pH-system 1001 (Sentron Europe B. V. Ac Roden, The Netherlands). Supernatants of the digestive contents were obtained by centrifuging one of the two microfuge tubes at 20,000 g for 10 minutes at 4 °C, and then frozen until analysis. One tibia was sampled for Ca and Mg analysis.

Analytical procedures

Ca and Mg concentrations were determined in the plasma and urine after adequate dilution into 0.1 % (w/v) lanthanum chloride. Diet aliquots, fecal materials and tibia were dry-ashed (10 hours at 500 °C), and dissolved with concentrated HNO_3 and H_2O_2, on a heating plate, until complete decoloration. The resulting mineral solutions were set at 10 ml with water and diluted adequately in 0.1 % lanthanum chloride. Mineral concentrations were measured by atomic absorption spectrophotometry (Perkin-Elmer 560, Norwalk, CT, USA) at wavelengths of 422 nm (Ca) and 285 nm (Mg).

Cecal SCFA concentrations, including acetic, propionic and butyric acid, were determined by gas-liquid chromatography on portions of supernatant fractions of cecal contents as previously described [14].

Calculations

Total cecal SCFA content (µmol/cecum) was calculated as the supernatant SCFA concentration (µmol/ml) × cecal water (ml/cecum). Soluble Ca and Mg levels in the cecal contents were determined on the supernatant concentration (µg/ml) and the soluble Ca and Mg contents per cecum was calculated as follows: (µg Ca or Mg/ml) × cecal water (ml).

Net apparent absorptions of Ca and Mg (mg/d) were calculated according to the following equation: mineral absorption = (mineral intake – fecal mineral excretion), whereas efficiency of apparent absorptions of Ca and Mg (%) were calculated according to the following equation: 100 × (mineral absorption/mineral intake).

Net retention of Ca and Mg (mg/d) were determined as follows: mineral retention = mineral intake – (fecal mineral excretion + urinary mineral excretion), whereas efficiency of Ca and Mg retentions (%) were calculated according to the following equation: 100 × (mineral retention/mineral intake).

Data analysis

Values are given as means ± SD and data were tested by 2-way ANOVA using the General Linear Models procedure of the SuperANOVA package (Abacus, Berkeley, CA). Post-hoc comparisons were performed using Fisher's least significant difference procedures. Differences of $p < 0.05$ were considered statistically significant. Simple linear correlation analysis was used to assess the relationship between dietary Ca intake and intestinal Mg absorption. Values of $p < 0.05$ were considered statistically significant.

Results

Food intake and growth rate

Inulin administration decreased food intake rapidly from week 2 until the end of the experiment (from –8.5 % to –13 %). This food intake depression led to a significantly lower growth rate from day 16 to the end of the experiment (Table 2). The lower calorific value of the inulin diets (–5 %) than the control diets may also be responsible for this reduced weight gain in comparison to groups without inulin. In addition, high or low dietary Ca levels were without effect on both food intake and growth rate of rats.

Cecal fermentation parameters

As expected, inulin intake significantly increased cecal content weight and cecal wall weight and significantly decreased cecal content pH (Table 3). However, no significant effect was observed for dietary Ca levels on these variables. In addition, inulin intake significantly increased the overall production of SCFA per cecum, in particular butyrate, whereas low or high dietary Ca intakes were generally linked to non-significant lower cecal SCFA production in comparison to the normal dietary Ca intake.

Table 2 Effect of dietary calcium intakes and inulin administration on food consumption and body weight evolution

Parameters	Ca 0.25%	Ca 0.50%	Ca 0.75%	Ca 0.25% + In	Ca 0.50% + In	Ca 0.75% + In	Inulin effect	Ca effect	Interact
Food consumption, g/d									
D 9	19.1±1.4	19.7±2.2	18.3±2.2	18.1±2.5	18.2±3.0	17.4±3.5	NS	NS	NS
D 16	18.2±2.0	19.2±1.2	18.6±1.3	15.6±1.9	15.8±1.5	14.8±1.4	< 0.0001	NS	NS
D 23	19.0±2.9	19.0±1.8	18.6±1.7	17.1±1.9	17.7±1.9	17.0±2.2	0.0056	NS	NS
D 30	20.5±2.9	19.5±1.5	18.4±1.8	17.2±1.2	16.9±2.5	16.3±2.9	< 0.0001	NS	NS
D 37	18.5±2.6	17.7±1.3	18.2±1.6	15.8±2.6	15.8±1.8	15.5±1.8	< 0.0001	NS	NS
Body weight, g									
D 0	276±5	277±7	277±3	278±4	279±7	280±7	NS	NS	NS
D 9	321±11	327±16	321±9	316±14	316±15	316±15	NS	NS	NS
D 16	343±15	356±15	344±11	329±19	328±19	320±17	< 0.0001	NS	NS
D 21	372±22	383±18	365±12	349±16	345±23	344±20	< 0.0001	NS	NS
D 32	408±28	411±22	392±13	365±30	369±28	371±26	< 0.0001	NS	NS
D 43	437±36	441±22	416±15	388±30	381±28	367±43	< 0.0001	NS	NS

Table 3 Effect of dietary calcium intakes and inulin administration on cecal characteristics and fermentation parameters

Parameters	Ca 0.25%	Ca 0.50%	Ca 0.75%	Ca 0.25% + In	Ca 0.50% + In	Ca 0.75% + In	Inulin effect	Ca effect	Interact
Cecal content weight, g	2.46±0.59	2.57±0.83	2.68±0.48	8.74±2.56	7.45±2.83	7.95±3.44	< 0.0001	NS	NS
Cecal dry materials, %	25.7±2.1	28.0±1.1	28.1±1.1	14.9±1.5	19.9±3.8	22.3±3.9	< 0.0001	< 0.0001	0.0159
Cecal wall weight, g	1.07±0.12	1.08±0.12	1.03±0.12	3.57±1.19	2.91±0.41	3.41±1.09	< 0.0001	NS	NS
Cecal content, pH	7.01±0.18	7.04±0.21	7.17±0.26	5.91±0.37	5.91±0.28	6.09±0.36	< 0.0001	NS	NS
Acetate, mM	114±27	125±37	83±56	89±41a	132±26	73±47	NS	0.0015	NS
Acetate, mol/cecum	0.21±0.08	0.25±0.12	0.17±0.13	0.65±0.48	0.79±0.37	0.45±0.33	< 0.0001	NS	NS
Propionate, mM	28.4±6.3	32.2±12.1	19.4±13.7	21.2±14.9	21.8±7.6	14.4±13.1	0.0201	0.0297	NS
Propionate, µmol/cecum	53.5±22.3	64.3±35.1	41.4±35.2	149.3±149.7	133.6±73.8	87.8±94.1	0.0003	NS	NS
Butyrate, mM	30.2±15.0	29.6±13.4	20.2±13.1	51.3±41.5	54.7±39.0	29.2±36.1	0.0210	NS	NS
Butyrate, µmol/cecum	58.8±43.2	60.3±32.5	42.5±31.1	361±386	380±407	179±243	0.0016	NS	NS
Total SCFA, mM	173±42	187±59	123±77	162±84a	208±43	116±92	NS	0.0042	NS
Total SCFA, µmol/cecum	323±137	372±183	256±188	1161±935	1308±743	715±646	< 0.0001	NS	NS

Total and soluble calcium and magnesium levels in the rat cecum

Total and soluble cecal Ca levels increased proportionally to the dietary Ca intakes, but the soluble fraction of cecal Ca was inversely linked to Ca intake (Table 4). This fraction was 2.5 times higher in the rats receiving the low dietary Ca diet than those receiving the high dietary Ca diet. Inulin greatly increased the soluble fraction of cecal Ca in all dietary Ca intakes. The soluble fraction of cecal Ca was 4 times higher in the groups receiving inulin than those without inulin. In addition, total cecal Mg levels increased proportionally to the dietary Ca intakes but soluble cecal Mg levels were inversely linked to Ca intake. Consequently, the soluble fraction of cecal Mg was 2 times higher in the rats receiving the low dietary Ca diet than those receiving the high dietary Ca diet. In addition, inulin greatly increased the soluble fraction of Mg in the cecum in all dietary Ca intakes. The soluble fraction of cecal Ca was 3 to 4 times higher in the groups receiving inulin than those without inulin.

Intestinal absorption and balance of calcium

In both short- and long-term balance studies, in spite of the large difference in dietary Ca intake, net Ca absorption (mg/d) was statistically similar in the six experimental groups (Table 5). Consequently, the efficiency of Ca absorption (%) was inversely proportional to dietary Ca intakes. As expected, inulin intake significantly increased Ca absorption for all dietary Ca levels in both short- and long-term balance studies. Urinary Ca concentration (mg/l) and its excretion (mg/d) increased under inulin intake and with the increase in dietary Ca levels in both short- and long-term balance studies. Finally, Ca retention efficiency (%) increased with inulin intake and was inversely proportional to dietary Ca intake. However, there was no difference in net Ca retention between the six experimental groups in both short- and long-term balance studies.

Table 4 Effect of dietary calcium intakes and inulin administration on total and soluble cecal concentrations of calcium and magnesium

Parameters	Ca 0.25%	Ca 0.50%	Ca 0.75%	Ca 0.25% + In	Ca 0.50% + In	Ca 0.75% + In	Inulin effect	Ca effect	Interact
Total cecal Ca, mg/g dw	13.9±1.8	26.2±2.3	37.8±4.5	5.33±3.3	17.3±6.7	25.6±7.6	< 0.0001	< 0.0001	NS
Total cecal Ca, mg/cecum	8.66±1.72	18.5±5.5	28.4±5.8	7.1±4.5	29.9±19.9	42.3±16.0	0.0096	< 0.0001	NS
Soluble cecal Ca, mg/mL	0.56±0.15	0.79±0.26	0.76±0.13	0.37±0.08	1.08±0.31	1.42±0.47	0.0123	< 0.0001	0.0028
Soluble cecal Ca, mg/cecum	1.00±0.27	1.59±0.82	1.44±0.30	2.80±1.10	6.19±2.27	8.56±4.36	< 0.0001	0.0002	0.0013
Soluble cecal Ca, %	12.0±3.3	7.94±2.9	5.20±1.11	50.3±23.5	29.9±15.1	19.8±5.1	< 0.0001	< 0.0001	0.0089
Total cecal Mg, mg/g dw	2.48±0.50	2.58±0.33	2.72±0.43	0.94±0.21	1.13±0.21	1.37±0.31	< 0.0001	0.0234	NS
Total cecal Mg, mg/cecum	1.53±0.29	1.81±0.53	2.04±0.43	1.21±0.39	1.88±1.08	2.46±1.29	NS	0.0043	NS
Soluble cecal Mg, mg/L	145±13	128±13	114±6	113±11	125±25	109±11	0.0014	0.0012	0.0064
Soluble cecal Mg, mg/cecum	0.27±0.09	0.25±0.09	0.22±0.04	0.84±0.28	0.73±0.30	0.69±0.33	< 0.0001	NS	NS
Soluble cecal Mg, %	18.2±4.3	13.2±2.5	11.0±2.1	72.3±16.3	46.2±12.6	30.8±10.4	< 0.0001	< 0.0001	< 0.0001

Table 5 Effect of dietary calcium intakes and inulin administration on intestinal absorption and balance of calcium in rats

Parameters	Ca 0.25%	Ca 0.50%	Ca 0.75%	Ca 0.25% + In	Ca 0.50% + In	Ca 0.75% + In	Inulin effect	Ca effect	Interact
Short-term balance study (days 13–17)									
Ca intake, mg/d	59.7±6.6	119.0±7.3	166.0±11.2	50.0±6.3	96.7±9.4	129.7±12.1	< 0.0001	< 0.0001	= 0.0001
Fecal Ca, mg/d	29.2±4.1	79.4±8.0	128.4±17.1	14.1±6.8	53.1±18.8	88.8±11.3	< 0.0001	< 0.0001	0.0115
Ca absorption, mg/d	30.5±7.4	39.6±6.4	37.6±14.6	36.0±6.9	43.6±15.7	41.7±11.7	NS	NS	NS
Ca absorption, %	50.6±8.5	33.3±5.2	22.6±9.2	72.1±12.1	45.6±17.3	31.8±7.8	< 0.0001	< 0.0001	NS
Urinary Ca, mg/L	21.9±7.8	54.5±18.5	68.3±33.1	61.9±28.5	71.8±21.7	99.6±43.4	= 0.0001	< 0.0001	NS
Urinary Ca, mg/d	0.52±0.21	1.43±0.52	1.70±0.84	1.68±0.97	2.35±1.53	2.84±0.85	< 0.0001	0.0010	NS
Ca balance, mg/d	30.0±7.4	38.2±6.4	35.9±14.8	34.3±6.1	41.3±15.2	38.9±12.0	NS	NS	NS
Ca balance, %	49.7±8.5	32.1±5.3	21.6±9.3	68.8±11.3	43.3±17.0	29.6±8.0	< 0.0001	< 0.0001	NS
Long-term balance study (days 36–40)									
Ca intake, mg/d	60.8±8.6	109.7±7.8	162.8±13.9	50.7±8.2	96.4±10.9	136.6±15.9	< 0.0001	< 0.0001	NS
Fecal Ca, mg/d	37.1±6.0	84.1±11.0	132.1±16.2	23.6±5.9	67.2±12.9	104.3±22.4	< 0.0001	< 0.0001	NS
Ca absorption, mg/d	23.7±4.7	25.6±8.0	30.8±15.2	27.1±6.3	29.2±16.5	32.2±15.3	NS	NS	NS
Ca absorption, %	38.9±5.6	23.4±7.5	18.7±8.9	53.4±8.9	29.6±15.9	23.9±12.0	0.0024	< 0.0001	NS
Urinary Ca, mg/L	42.4±28.6	97.6±37.5	101.2±54.5	89.1±26.0	130.1±44.1	201.6±46.4	< 0.0001	< 0.0001	0.0290
Urinary Ca, mg/d	1.27±0.72	2.35±0.56	3.13±0.90	2.15±0.80	3.53±0.90	5.24±1.05	< 0.0001	< 0.0001	NS
Ca balance, mg/d	22.4±4.7	23.2±8.0	27.6±15.3	24.9±5.9	25.7±16.2	27.0±15.7	NS	NS	NS
Ca balance, %	36.8±5.6	21.3±7.5	16.8±9.0	49.2±8.6	25.9±15.7	20.0±12.1	0.0156	< 0.0001	NS

Intestinal absorption and balance of magnesium

In both short- and long-term balance studies, inulin intake significantly increased intestinal Mg absorption (mg/d or %) in comparison to the groups without inulin (Table 6). This increase was greater in the long-term balance study than in the short-term balance study. The same effect was also observed on the urinary Mg concentration and its excretion, which increased significantly under inulin intake in both short- and long-term balance studies. In total, Mg retention efficiency (%) increased significantly under inulin intake whereas net Mg retention (mg/d) increased only non-significantly, in both short- and long-term balance studies.

In both short- and long-term balance studies, intestinal Mg absorption (mg/d or %) was inversely proportional to dietary Ca intakes. The same effect was also observed on the urinary Mg excretion (mg/L or mg/d), which decreased when dietary Ca intake increased in both short- and long-term balance studies. Consequently, net Mg retention (mg/d) and efficiency of Mg retention (%) were not affected by dietary Ca intake in short-term balance study but significantly decreased when dietary Ca intake increased.

Stimulatory effect of inulin according to dietary Ca levels

In the short-term intake of inulin, the highest stimulatory effect of inulin on Ca absorption was observed in the groups receiving the low or the high dietary levels of Ca (+42 %) (Fig. 1). In the long-term intake of inulin, the highest effect of inulin was observed in the group receiving the lowest dietary level of Ca (+37 %). The highest effect of inulin on Mg absorption was observed in the

Table 6 Effect of dietary calcium intakes and inulin administration on intestinal absorption and balance of magnesium in rats

Parameters	Ca 0.25%	Ca 0.50%	Ca 0.75%	Ca 0.25% + In	Ca 0.50% + In	Ca 0.75% + In	Inulin effect	Ca effect	Interact
Short-term balance study (days 13–17)									
Mg intake, mg/d	9.92±1.09	9.95±0.61	9.52±0.64	8.15±1.02	8.06±0.79	7.41±0.69	< 0.0001	NS	NS
Fecal Mg, mg/d	5.03±0.84	5.71±0.50	6.04±0.43	1.67±0.67	2.34±1.10	2.63±0.84	< 0.0001	0.0005	NS
Mg absorption, mg/d	4.90±1.14	4.24±0.43	3.48±0.57	6.48±1.08	5.72±1.07	4.78±0.73	< 0.0001	< 0.0001	NS
Mg absorption, %	49.0±8.4	42.6±3.6	36.4±4.5	79.4±8.3	71.2±12.7	64.8±10.5	< 0.0001	< 0.0001	NS
Urinary Mg, mg/L	90.0±25.0	52.5±24.1	43.2±24.5	103.2±57.3	91.0±47.0	61.1±29.4	0.0182	0.0016	NS
Urinary Mg, mg/d	2.06±0.38	1.31±0.42	0.99±0.30	2.54±1.12	2.44±0.74	1.83±0.49b	< 0.0001	0.0003	NS
Mg balance, mg/d	2.83±0.88	2.92±0.52	2.49±0.42	3.94±0.92	3.28±1.23	2.95±1.11	NS	NS	NS
Mg balance, %	28.3±7.3	29.3±4.7	26.1±3.7	49.2±15.3	41.1±16.0	39.7±14.4	< 0.0001	NS	NS
Long-term balance study (days 36–40)									
Mg intake, mg/d	10.10±1.42	9.17±0.65b	9.34±0.80	8.25±1.34	8.35±0.95	7.97±0.99	< 0.0001	NS	NS
Fecal Mg, mg/d	5.73±0.93	6.03±0.92	6.82±0.82	1.31±0.15	2.94±0.46	3.96±0.75	< 0.0001	< 0.0001	0.0069
Mg absorption, mg/d	4.37±1.09	3.14±0.82	2.52±0.59	6.95±1.31	5.41±0.88	4.01±0.78	< 0.0001	< 0.0001	NS
Mg absorption, %	42.9±7.8	34.2±8.7	27.0±6.0	83.9±2.4	64.6±5.8	50.3±7.7	< 0.0001	< 0.0001	0.0007
Urinary Mg, mg/L	121±47	93±50	56±29	193±81	158±48	139±49	< 0.0001	0.0035	NS
Urinary Mg, mg/d	2.86±0.76	2.10±0.57	1.85±0.95	4.45±1.42	4.21±0.63	3.45±0.65	< 0.0001	0.0028	NS
Mg balance, mg/d	1.51±0.74	1.04±0.87	0.67±0.61	2.50±1.54	1.20±0.47	0.56±0.35	NS	< 0.0001	NS
Mg balance, %	14.5±6.4	11.3±9.6	7.0±6.3	29.8±16.8	14.3±5.2	7.1±4.7	0.0121	< 0.0001	0.0267

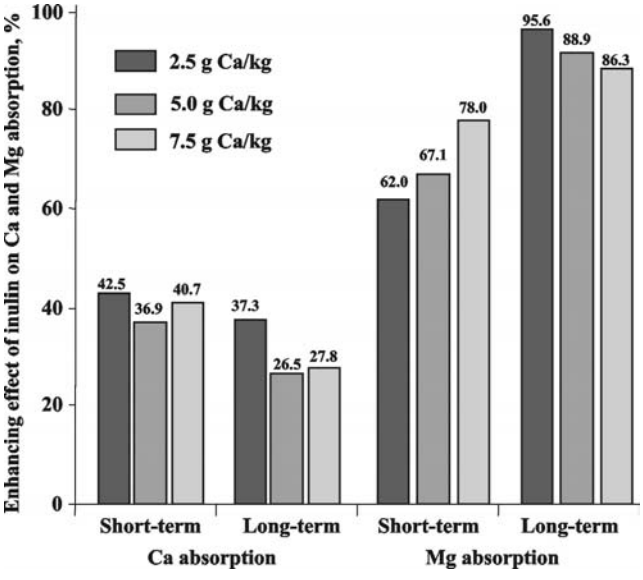

Fig. 1 Influence of dietary calcium levels on the stimulatory effect of inulin on intestinal absorption of calcium and magnesium in rats after short- and long-term intake of inulin. The stimulatory effect of inulin (%) was calculated as follows: 100 · (intestinal absorption with inulin – intestinal absorption without inulin)/intestinal absorption without inulin

group receiving the highest dietary Ca level (+78%) and in the group receiving the lowest dietary Ca level (+96%) for the short-term and the long-term inulin intakes, respectively.

Calcium and magnesium status

Levels of plasma Ca (105 to 108 mg/L), bone Ca (167 to 177 mg/g dry weight) and renal Ca (19.2 to 21.1 mg/g dry weight) did not vary significantly between the six experimental groups (Table 7). In addition, tibia and renal Mg levels remained statistically unchanged in/between the six experimental groups. However, while inulin had no effect on plasma and red blood cell Mg levels, these levels were significantly decreased by increasing dietary Ca levels.

Discussion

Previous studies have repeatedly shown that intake of different inulin-type fructans can increase variably mineral intestinal absorption in humans and animals [4, 5, 12, 15–18]. It is well known that absorption mechanisms of Ca and Mg differ considerably. Indeed, these compounds increase highly and consistently intestinal Mg absorption [13]. However, their effect on Ca absorption seems to depend on experimental conditions, particularly dietary Ca level and duration of fructans intake [18, 19]. In this study, we investigated the importance of dietary Ca levels and the duration of inulin intake on intestinal absorption and balance of Ca and Mg in rats.

Table 7 Effect of dietary calcium intakes and inulin administration on calcium and magnesium status in rats

Parameters	Ca 0.25%	Ca 0.50%	Ca 0.75%	Ca 0.25% + In	Ca 0.50% + In	Ca 0.75% + In	Inulin effect	Ca effect	Interact
Plasma Ca, mg/L	108±6	107±14	108±6	105±9	105±7	108±5	NS	NS	NS
Tibia Ca, mg/g dw	174±14	171±4	177±20	174±7	167±17	175±10	NS	NS	NS
Renal Ca, µg/g dw	19.2±1.4	19.4±2.2	20.5±5.3	20.7±3.3	21.1±3.7	20.0±2.0	NS	NS	NS
Plasma Mg, mg/L	18.3±1.6	17.2±0.6	17.2±0.6	18.8±1.1	17.3±1.0	17.0±1.3	NS	0.0003	NS
Erythrocyte Mg, mg/L	54.6±2.6	55.7±2.3	52.1±3.0	53.7±2.9	54.4±3.3	52.7±2.9	NS	0.0176	NS
Tibia Mg, mg/g dw	3.14±0.23	3.09±0.14	3.16±0.17	3.21±0.14	3.09±0.25	3.22±0.15	NS	NS	NS
Renal Mg, µg/g dw	188±13	186±10	185±15	192±18	190±5	190±11	NS	NS	NS

Impact of experiment duration on the effect of inulin on Ca absorption

Our results showed that short- and long-term intakes of inulin increased the efficiency of intestinal absorption and retention of Ca. The mean increase in Ca absorption in the short-term balance was about 40 % but was only about 30 % in the long-term balance. Thus, the increase in intestinal Ca absorption was lower after the long-term intake than the short-term intake of inulin (–25 %). These results are in agreement with literature data showing that the inulin effect on Ca absorption seems to be optimal in the first two weeks, decreasing then gradually with experiment duration [20, 21]. A possible explanation for this phenomenon is a down-regulation of the active pathway of intestinal Ca absorption after several weeks of feeding inulin as previously reported [20–22].

Impact of dietary Ca levels on the stimulatory effect of inulin on Ca absorption

We hypothesized that inulin intake would increase Ca absorption in the short-term balance whatever the dietary Ca levels, since the adaptive phenomenon has not yet been developed. Our results showed that in the short-term balance, the highest effect of inulin was observed in the group receiving the lowest dietary Ca level (+42.5 %). However, this effect was also higher in the group receiving the high dietary Ca level than in the control group. These results confirmed our hypothesis. We also hypothesized that inulin would increase Ca absorption in long-term balance in the groups receiving low or high dietary Ca levels, because the adaptive phenomenon would not take place in these two groups. In the group receiving the high level of Ca, the high dietary Ca level should depress the active transport of Ca to a point where an additional decrease due to the long-term inulin intake seems improbable. In the group receiving the low level of Ca, the organism had a high Ca requirement, to a point where an increase due to long-term inulin intake is maintained and no adaptive phenomenon may take place under these conditions. In the long-term balance, the highest effect of inulin on Ca absorption was observed in the group receiving the diet with the lowest dietary Ca level (+37 %). In agreement with this are our results showing that the total amount of SCFA per cecum was higher in the group receiving the low Ca diet compared to the group receiving the high Ca diet (1161 versus 715 µmol). Moreover, the soluble fraction of cecal Ca was considerably higher in the group receiving the low Ca diet compared to the groups receiving the control or the high Ca diets (50 %, 30 % and 20 %, respectively). It was also reported that low dietary Ca level accentuated the fall in cecal pH accompanied with higher concentrations of soluble Ca and than its absorption in rats [11]. In another study [23], galacto-OS intake was shown to stimulate Ca absorption when the diet contained 0.5 % Ca but not when the diet contained 0.05 %. Such a result may be due to the very low dietary Ca level tested in their study.

Impact of dietary Ca levels on Ca absorption

In this study, Ca absorption efficiency (%) was highly inversely correlated with the dietary Ca intakes. In the short-term balance, Ca absorption averaged 50 % in the lowest Ca intake, and 22.6 % in the highest intake, and for long-term balance it was 38.9 % and 18.7 % in the lowest and highest Ca intakes, respectively. There are several studies that have investigated this point and are in agreement with our results. Heaney et al. [24] observed that fractional absorption of Ca was highly inversely correlated with the logarithm of Ca load in healthy adult women, and that absorption averaged 64 % at the lowest load (15 mg Ca) and 28.6 % at the highest Ca load (500 mg Ca). Cashman and Flynn [25] reported that fractional absorption of meal Ca decreased with increasing previous dietary Ca intake and with increasing meal Ca content in rats. Miura et al. [26] observed a large reduction in Ca absorption in rats receiving high intake of Ca (1.5 % diet) compared to the control group (26 % versus 10 %). Tryfonidou et al. [27] reported that intestinal Ca absorption in growing dogs was inversely influenced by Ca intake and age but not by growth rate. Ames et al. [28] showed a lower Ca absorption under the high

Ca diet (1180 mg/d) than the low Ca diet (502 mg/d) (36 % *versus* 24 %) in children.

Impact of dietary Ca levels and inulin intake on Mg absorption

Our results clearly showed that short- and long-term balance studies highly and consistently increased intestinal absorption and retention of Mg. The mean increase in Mg absorption in the short-term balance was around 69 %. This increase was about 92 % in the long-term balance, the increase in intestinal Mg absorption was therefore higher after the long-term intake than after the short-term intake of inulin (+33 %). This is in agreement with our previous studies showing a large stimulatory effect of inulin or other oligosaccharides on intestinal Mg absorption, whatever the duration of the experiments and the experimental conditions [12, 13, 29, 30]. This confirms the particular characteristics of Mg homeostasis where intestinal absorption is very weakly-controlled and thus it may not suffer from a negative feedback control as is the case for Ca absorption consequent to long-term inulin intake.

The effect of inulin on Mg absorption was also modulated by dietary Ca levels. In the short-term balance, the highest effect of inulin on Mg absorption was observed in the group receiving the highest dietary Ca level (+78 %). However, in the long-term balance, the highest effect of inulin on Mg absorption was observed in the group receiving the lowest dietary Ca level (+96 %). In agreement with this, we observed that the soluble fraction of cecal Mg was the highest in the group receiving the diet with the lowest level of Ca (data not shown) which may explain the higher effect of inulin on Mg absorption in this group.

In this study, we also clearly showed that intestinal Mg absorption decreased when dietary Ca intake increased. This was true in the presence and absence of inulin, strongly corroborating these results. When Ca intake increased from 0.25 % to 0.75 %, Mg absorption decreased from 49 % to 36 % (–27 %) without inulin and from 79 % to 65 % (–18 %) with inulin, in the short-term balance. In the long-term balance, this became much more clear, with Mg absorption decreasing from 43 % to 27 % (–37 %) without inulin and from 84 % to 43 % (–48 %) with inulin. So, Mg absorption was inversely correlated with Ca intake in both short- and long-term balance studies (Fig. 2). This was confirmed by a significant decrease in urinary Mg excretion and in plasma and red blood cell Mg levels when dietary Ca levels increased (Tables 6 and 7). Some recent studies have investigated the effect of dietary Ca intake on intestinal Mg absorption and homeostasis in animal and man but their results are not consistent. Andon et al. [31] observed that Mg use did not differ in adolescent girls

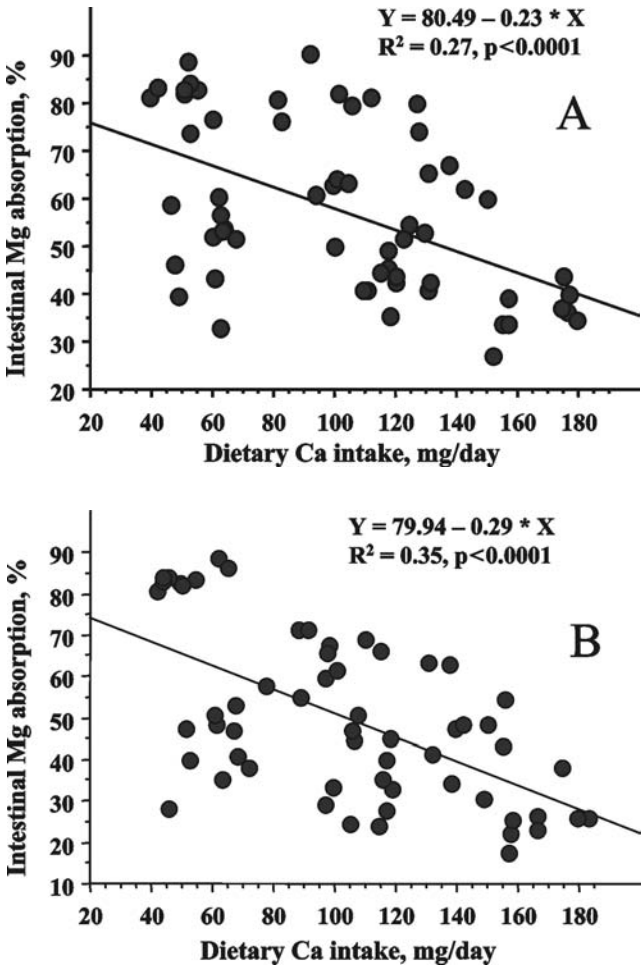

Fig. 2 Negative relationship between dietary Ca intake and intestinal Mg absorption after short- and long-term intake of inulin in rats. **A** Short-term balance corresponding to 13–17 days of inulin intake. **B** Long-term balance corresponding to 36–40 days of inulin intake

when they received low Ca or high Ca (667 *versus* 1667 mg/d) as measured by fecal excretion, absorption and urinary excretion. Yan et al. [32] reported that long-term supplementation with $CaCO_3$ (1 g Ca/d) had no effect on urinary Mg excretion in 60 lactating Gambian women. In a third study, Sojka et al. [33] measured Mg absorption in 5 adolescent girls and found no significant difference between high Ca intakes (1800 mg/d and low 800 mg/d) for percentage Mg absorption or urinary excretion. However, Miura et al. [26] reported that long-term (10 weeks) high intake of Ca (1.5 g/kg diet) reduced Mg utilization in young male rats. Finally, Brink et al. [34] reported that increased intakes of Ca decreased Mg solubility in the ileal lumen and lowered Mg absorption in rats. It is worth noting that in normal human diet the ratio of Ca/Mg is 2 to 3, but it is around 10 in the animal diet. The reported difference in the effect of dietary Ca level on intestinal Mg absorption may be due to species

particularities or simply to the Ca/Mg ratio in human food and in animal diet.

In conclusion, our results confirmed that the effect of inulin on Ca absorption is greater in short-term intake than in long-term intake of inulin, and clearly showed that the effect of inulin is higher when Ca intake is low, in particular under the long-term intake of inulin. These results also confirmed that the large stimulatory effect of inulin on Mg absorption in both short- and long-term inulin intake, and showed that Mg absorption is inversely correlated to dietary Ca levels in rats. Further studies are required to determine the impact of physiological state, in particular animal age, on the effect of inulin on mineral absorption.

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