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ORIGINAL CONTRIBUTION

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Effects of inulin-type fructans of different chain length and type of branching on intestinal absorption and balance of calcium and magnesium in rats

Summary *Background* Inulin-type fructans or chains with mainly β[2–1] linked fructose molecules escape the ingestion procedure in the small intestine and are fermented by the microflora, and are known to increase colonic absorption of minerals in animals. The fermentation rate in the large bowel into short-chain fatty acids depends on the molecular mass and the structure of these food ingredients. It is thought that this colonic fermentation is the basis for the reported increase in mineral absorption. *Aim of the study* The purpose of the present study was twofold: a) to compare different types of fructans that differ in the sugar chain length and in chain branching; b) to determine the potential synergistic effect of a combination of inulin-type fructans with different chain lengths. *Methods* For this purpose, 50 adult male Wistar rats weighing 170 g each were used in this study. The rats were distributed into 5 groups and fed for 28 days a fiber-free basal purified diet or diet containing 10 % oligofructose (OF) (DP_{av} 4), or 10 % HP-inulin (DP_{av} 25), a blend of 50 % OF and 50 % HP-inulin, or a branched-chain inulin. *Results* During the first period, the rats went into a gradual adaptation, during which the rats received 2.5 % for 1 week and then 5 % for 1 week of the tested products. During the last 4 days of the experiment, feces and urine were monitored for mineral balance study. The animals were then sacrificed and blood, cecum and tibia were sampled for mineral status assessment. Our results showed that the ingestion of all the tested fructans led to a considerable cecal fermentation. All tested compounds increased the intestinal absorption and balance of Mg significantly. Interestingly, in the present experimental set-up, all tested compounds increased the intestinal absorption and balance of Ca numerically, but only the blend OF + HP-inulin increased apparent intestinal absorption and balance of Ca significantly. *Conclusions* The different types of fructans studied in the present experiment seem to have similar activity on mineral absorption. However, the combination of OF and HP-inulin showed synergistic effects on intestinal Ca absorption and balance in rats. Further studies with other combinations of fructans need to be done to extend these findings.

Key words oligofructose – inulin – short-chain fatty acids – calcium and magnesium – intestinal absorption – rat

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Abbreviations

BC-inulin, branched-chain inulin
DP, degree of polymerization or number of fructose molecules bound together in a fructan chain
DP_{av}, average DP
HP-inulin, high-performance inulin, which is a long-chain fraction of chicory inulin (DP 10–65, DP_{av} 25)
OF, oligofructose, which is a short-chain fraction of chicory inulin (DP 2–8, DP_{av} 4)
SCFA, short-chain fatty acids
Synergy1 is a 1/1 mixture of OF and HP-inulin

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Introduction

Inulin-type fructans or β[2–1] fructans are non-digestible in the upper intestinal tract, and reach the large intestine, where they are fermented selectively (prebiotic property) by the local microflora. They stimulate the growth of bifidobacteria and lactobacilli in the intestine, which has been proposed to be connected with health-promoting functions [1]. Many other health-beneficial effects for fermentable fructans have been reported, concerning diabetes, lipid metabolism and cancer prevention [2, 3]. Several investigations have demonstrated that rats fed the prebiotic fructans absorbed more Ca and Mg than control rats, despite an increase in total fecal mass [4, 5]. Indeed, fermentation 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 its turn increases mineral solubility, leading to improve mineral absorption. The SCFA can influence mineral absorption directly by forming complexes with the minerals, leading to an increase in their absorption. It is thought that the bacterial metabolites (e. g., butyrate) can stimulate the intestinal epithelium and increase its absorptive capacity. These different aspects are closely linked to the nature of the prebiotic carbohydrates and to mineral concentrations [4–6]. The enhancing effect of inulin on Ca and Mg absorption has been investigated several times in animals [4, 7] and in man [8–10]. The effects of OF have been examined in animals and man, and shown to enhance effects on Ca and/or Mg absorption [11–13]. Only a few studies have tried to compare different fractions of inulin-type fructans or their combination on SCFA production and mineral absorption [14–16].

The aim of the present study was to compare the effects of different types of fructans and the potential synergistic effect of a blend of two fructans on fermentation parameters, and on the apparent intestinal absorption of Ca and Mg and on their status in rats.

Materials and methods

Experimental products

The experimental products, OF, high-performance inulin (HP-inulin), Synergy1 and branched-chain inulin (BC-inulin), were supplied by ORAFTI, Tienen, Belgium. Native inulin is obtained industrially from chicory roots by extraction with water, followed by refining and spray-drying. It is a polydisperse mixture of linear molecules, all with the same basic chemical structure, which is symbolized as G-F_n (G, glycosyl moiety; F, fructosyl moiety; n, number of fructose units linked together through β(2–1) bonds). The DP varies between 3 and 65, and DP_{av} is 10. OF is obtained by partial enzymatic hydrolysis of native inulin. It is a mixture of short-chain GF_n molecules. Its DP ranges between 2 and 8 with a DP_{av} of 4. HP-inulin is obtained by physically removing the lower-DP fraction from native inulin. The DP of HP-inulin ranges from 10 to 65, with a DP_{av} of 25. Synergy1 is a 1/1 mixture of OF and HP-inulin. The products can be quantitatively analyzed by gas chromatography: glucose and fructose are quantified before and after complete enzymatic hydrolysis, which allows the inulin content to be calculated. As shown in Fig. 1, the distribution of the various DPs is best analyzed by means of high performance anion exchange chromatography (HPAEC-PAD) [17]. It should be noted that DP = 10 is an important physicochemically barrier. Inulin chains with DP < 11 have high solubility in water (up to 85 %) and are very rapidly fermented. They were shown to be very 'bifidogenic'. Inulin chains with DP > 10 are hardly soluble in water (up to 5 %) and are in vitro 5 times more slowly fermented than OF by fecal slurry. A BC-inulin was included in this study. The branching occurs as a β(6–1) fructosyl bond at every third fructose unit. The DP_{av} of the BC-inulin is 15. Highly branched inulin is readily accessible for bacterial exo-inulinases or even invertase activity. *In vitro*, BC-inulin is fermented as rapidly as OF, while HP-inulin is fermented the slowest.

Animals and diets

Fifty male Wistar rats (derived from the colony of laboratory animals of the National Institute of Agronomic Research, Clermont-Ferrand/Theix, France) were fed a commercial pellet diet (U. A. R., Villemoisson s/Orge, France) until body weights reached ~170 g. Groups of 10 rats were then formed and fed for 28 days a basal (fructan-free) purified diet, or diets containing OF, or HP-inulin, or a blend of OF + HP-inulin (Synergy1) or BC-inulin. Diet compositions are given in detail in Table 1. The first week, the animals received these products at 2.5 % (w/w) in their diets. The second week, the animals received these products at 5 % (w/w) in their diets. During the third and the fourth weeks, the animals received these products at 10 % (w/w) in their diets. The levels of Ca and Mg in the diet were about 5400 and 520 mg/kg, respectively. The rats were provided with fresh food and distilled water daily; these were available *ad libitum*. During the period of adaptation (the first 21 days), the rats were housed two per cage (wire-bottomed to limit coprophagy) and maintained in a temperature-controlled room (22 °C) with the dark period from 08:00 pm to 08:00 am. Following the adaptation phase, rats were housed individually for an additional 7 days (the experimental phase) in metabolic cages fitted with urine/feces separators to collect feces and urine. Food consumption and body weight were recorded once a week during the

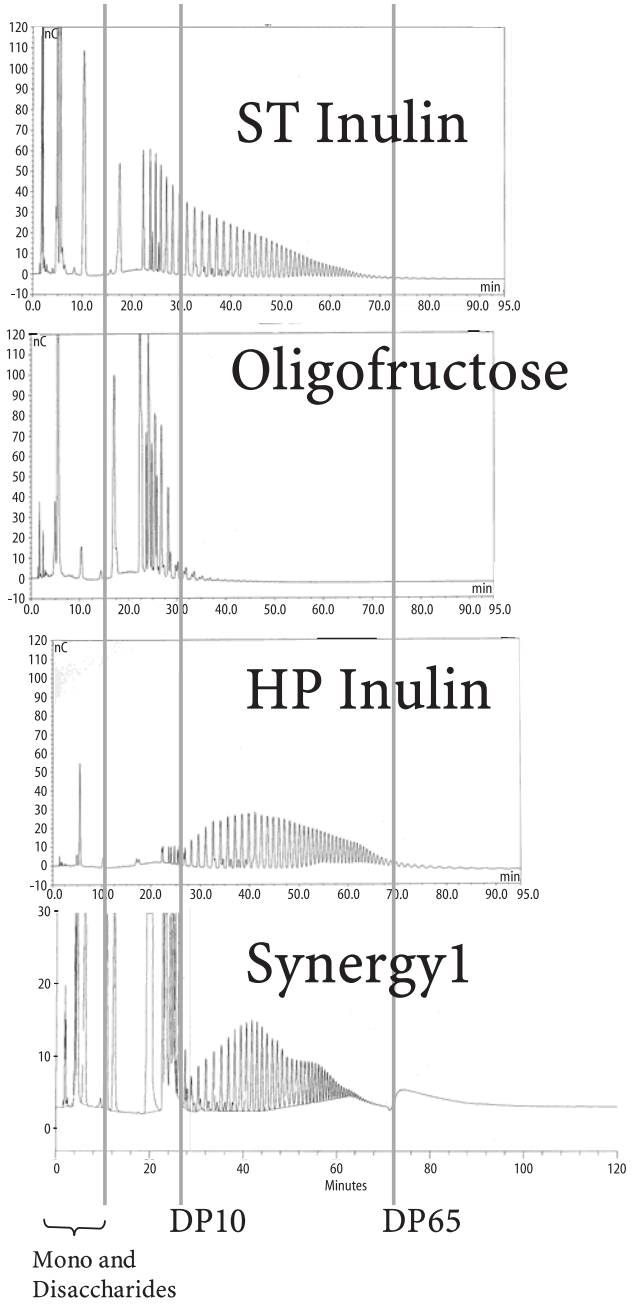

Fig. 1 High performance anion exchange chromatograms with PAD detector of the different chicory inulin fractions tested (see reference 17). The technique is not quantative, but shows accurately the distribution of various chain lengths. The mono- and disaccharides are fructose, glucose and sucrose. ST-inulin is the inulin as extracted from the chicory root. Degree of polymerization (DP) varies from 3 to 65; average DP (DPav) is 10. Oligofructose is partially hydrolyzed ST-inulin (DP between 2 and 8, DPav = 4). HP-inulin is ST-inulin devoid of its oligofructose fraction (DP between 10 and 65; DPav = 25). Synergy1 is a 1/1 mixture of Oligofructose and HP-inulin

adaptation phase. Food consumption was then monitored daily during the 4-day balance period. Urine (acidified by HCl, final 5 mmol/l) and feces were collected during the last 4 days of the experimental phase for determination of mineral balance. The animals were maintained and handled according to the recommendations of the Institutional Ethic Committee of the University of Clermont-Ferrand.

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

	Week 1	Week 2	Weeks 3 and 4
Casein	200	200	200
Wheat starch	**625**	**600**	**550**
Corn oil	50	50	50
Cellulose	50	50	50
Mineral mix (AIN 1976)	35	35	35
Vitamin mix (AIN 1976)	10	10	10
dl-Methionine	3	3	3
Choline bi-tartrate	2	2	2
Tested Fructans	**25**	**50**	**100**

Mineral mix AIN 1976 ensures the following mineral levels in the diets (mg/kg): Na, 1020; K, 3600; Ca, 5200; P, 4000; Mg, 500; Zn, 30; Fe, 35; Cu, 6; Mn, 54; Se, 0.1; I, 0.2; Cr, 2.
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

Sampling procedures

The rats were sacrificed just after the dark period (between 08:00 am and 10:00 am) because cecal fermentation was still very active. After anesthesia (40 mg/kg of sodium pentobarbital), blood was withdrawn from the abdominal aorta and placed into microfuge tubes containing sodium heparin and centrifuged at 10,000 *g* for 2 minutes. Plasma samples were stored at 4 °C for mineral analysis. One tibia was sampled. The cecum, complete with contents, was removed and weighed (total cecal weight). Duplicate samples were collected into 2 ml microfuge tubes that were placed immediately at –20 °C. The cecal walls were flushed clean with ice-cold saline, blotted on filter paper and weighed (cecal wall weight). 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.

Analytical procedures

SCFA were measured by gas-liquid chromatography on aliquots of supernatants of cecal contents as described [18]. Ca and Mg were determined on the plasma, cecal supernatants (soluble) and urine after adequate dilution into 0.1 % (w/v) lanthanum chloride. Untreated cecal samples (total), fecal materials and tibia were first dry-ashed (10 hours at 500 °C), and then dissolved with HNO_3 and H_2O_2, on a heating plate, until complete de-

coloration. The resulting solutions of minerals were made to 10 ml with water and diluted adequately in 0.1 % lanthanum chloride. Mineral concentrations were then measured by atomic absorption spectrophotometry (Perkin-Elmer 560, Norwalk, CT, USA) at wavelengths of 422 nm (Ca) and 285 nm (Mg).

Calculation and data analysis

The SCFA content of the cecum was calculated as the supernatant concentration (µmol/ml) × cecal water (ml). Soluble cecal mineral content was calculated as supernatant concentration (µmol/ml) × cecal water (ml). Total cecal mineral content was calculated as cecal concentration (µmol/ml) × cecal content (g). Cecal water was determined as cecal content x fractional cecal humidity.

Values are given as the mean ± SD and, where appropriate, the significance of differences between mean values was determined by ANOVA and multiple-range comparisons by Fisher's least-significant-difference procedures. Statistics have been done on the five experimental groups to compare the inulin-type fructan groups to the control group, and done on the four inulin-type fructan groups to compare them together. ANOVA assumes that the data are sampled from populations that follow a Gaussian distribution. This assumption was tested using the method of Kolmogorov & Smirnov. ANOVA assumes that the data are sampled from populations with identical SDs. This assumption was tested using the Bartlett-test. Values of $p < 0.05$ were considered significant.

Results

Growth rate, food intake and cecal fermentation parameters

The growth rate of the groups consuming the fructans was similar to that of the control animals during the 3 first weeks of the protocol. At the 28th day, the body weight of the animals of the BC-inulin group was significantly higher ($p < 0.05$) than that of the animals of the HP-inulin group (321 ± 27 g and 285 ± 25 g, respectively). Although the HP-inulin diet was consumed the least, we did not note a significant difference concerning food consumption among the five experimental groups until the end of the experiment (18.4 to 19.4 g/day).

The weight of the cecal wall (100 %) and the weight of the cecal contents (350 %) were significantly higher and the pH of the cecal content (–20 %) was lower in the four fructan groups than in the control rats (Cecal content: 1.53 ± 0.31 g; Cecal wall: 0.82 ± 0.20 g; Cecal pH: 7.12 ± 0.15 for control group). However, no significant difference among the groups consuming the prebiotics was observed for these variables.

The results showed that both acetate and butyrate levels in the cecum were similar in the 5 groups (42 to 55 mmol/L and 6 to 11 mmol/L, respectively), whereas cecal propionate level was higher in the fructan groups than in the control group (20 versus 35–40 mmol/L). Given the higher cecal mass in the fructan groups than in the control, the various inulin-type fructans were responsible for a significant increase in the overall production of SCFA (acetate, propionate and butyrate) in the cecum ($p < 0.05$) compared to the control rats (Fig. 2). It was also noted that the consumption of the OF was responsible for the greatest production of butyrate, whereas HP-inulin produced less ($p < 0.05$).

Total and soluble calcium and magnesium concentration in the cecum

The total Ca pool was 128 % to 158 % higher in rats consuming the fructans than in rats fed the control diet (Fig. 3). The soluble Ca pool was largely increased by dietary fructans (20–30 times). This augmentation was most pronounced (30 times) in the presence of Synergy1. However, there was no significant difference among the different tested fructans. In rats fed the control diet, only 3.5 % of cecal Ca was soluble, whereas 23–44 % of the cecal Ca was in a soluble form in rats fed the fructans. The total Mg pool was significantly larger in rats fed the inulin fractions (27 % to 53 %) than in those fed the control diet (Fig. 3). The cecal soluble Mg pool was 10 times higher than in the control group. In

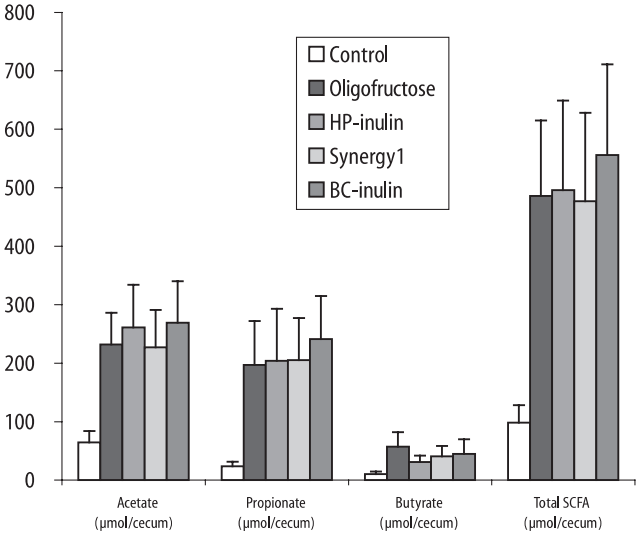

Fig. 2 Effects of different inulin-type fructans on cecal fermentation variables. Values are mean ± SD, n = 10. Control means differ significantly from all fructan group means. Amongst the fructan groups, OF mean differs significantly from HP-inulin mean

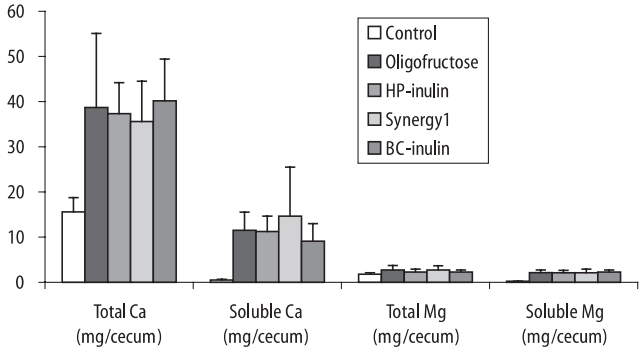

Fig. 3 Effects of different inulin-type fructans on total and soluble calcium and magnesium levels in the rat cecum. Values are mean ± SD, n = 10. Control means differ significantly from all fructan group means. Among the fructan groups, there are no significant differences

rats fed the control diet, only 13.1 % of cecal Mg was soluble, whereas 75 to 95 % of the cecal Mg was in a soluble form in rats fed the fructans.

Intestinal absorption and balance of calcium and magnesium

The chemical analysis of the diets offered to the rats confirms the content of Ca expected in the experimental diets (5278–5488 mg/kg). We analyzed the fructans, which contained between 40 and 100 mg Ca/kg. The OF was completely deprived of Ca. The amount of Ca ingested during the 4 days of the balance study varied from 400 mg (HP-inulin group) to 425 mg (OF group), without reaching significance. The Ca fecal content was approximately 40 mg/g in the control group and about 30 mg/g in the fructan groups. Given the significant increase in the weight of feces in the fructan groups, the total amount of Ca excreted in the feces was similar in the five experimental groups and varied from 43 to 52 mg/day.

As for the amount of Ca absorbed, it varied from 47.5 (control group) to 60 mg/day (Synergy1 group). Only the rats consuming Synergy1 absorbed significantly more Ca than the control rats (Table 2). We observed that the relative absorption of Ca (%) in the rats consuming Synergy1 was statistically higher ($p < 0.05$) than that observed in the control rats. The concentration of urinary Ca varied between 61 and 125 mg/l without significant differences among the five experimental groups. The amount of Ca excreted in the urine varied between 1.1 and 1.8 mg/day, and remained statistically similar among the different experimental groups (Table 2). Lastly, Ca retention in the Synergy1 group was significantly higher than that in the control group.

The chemical analysis of the diets offered to the rats confirmed the content of Mg expected in the experimental diets (507–539 mg/kg). The contribution of Mg from the fructans was low, and did not exceed 5 mg/kg diet for the Synergy1 group, which was the product richest in Mg. The quantity of Mg ingested during the 4 day balance study varied from 10 mg (control group) to 11 mg (OF group). The fecal Mg content was approximately 3.6 mg/g in the control group and only about 1.7 mg/g in the experimental groups. In spite of the significant increase in the weight of feces in the experimental groups, the total amount of Mg excreted in the feces remained significantly lower in those four fructan groups than in that of the control rats. It was noted that

Table 2 Effects of different fructo-oligosaccharides on intestinal absorption and retention of calcium and magnesium in rats

	Control	Oligofructose	HP-inulin	Synergy1	BC-inulin
Calcium					
Intake, mg/day	100 ± 9a	107 ± 4a**	99 ± 5a*	104 ± 5a*,**	104 ± 7a*,**
Fecal level, mg/g	38.4 ± 2.0a	31.1 ± 2.2b**	29.7 ± 2.0bc*,**	28.3 ± 2.7c*	31.6 ± 2.0b**
Fecal excretion, mg/day	52.2 ± 7.5a	50.5 ± 7.3a*	45.5 ± 6.8a*	43.5 ± 7.5a*	50.0 ± 7.0a*
Absorption, mg/day	48.0 ± 6.5b	56.0 ± 6.3ab*	53.3 ± 5.2ab*	60.3 ± 8.5a*	54.0 ± 7.0ab*
Absorption, %	47.9 ± 5.5b	52.7 ± 6.0ab*	54.1 ± 5.6ab*	58.1 ± 7.4a*	51.9 ± 6.0ab*
Urinary level, mg/l	61.0 ± 38.0a	87.5 ± 32.5a*	126 ± 114a*	69.0 ± 32.0a*	92.3 ± 59.1a*
Urinary excretion, mg/day	1.12 ± 0.57a	1.84 ± 0.79a*	1.77 ± 0.54a*	1.76 ± 0.82a*	1.76 ± 0.81a*
Balance, mg/day	46.8 ± 6.3b	54.2 ± 6.5ab*	51.5 ± 5.2ab*	58.5 ± 8.2a*	52.2 ± 7.0ab*
Magnesium					
Intake, mg/day	9.50 ± 0.85a	10.48 ± 0.40b**	9.53 ± 0.52a*	10.20 ± 0.47ab**	9.83 ± 0.62ab*,**
Fecal level, mg/g	3.57 ± 0.20a	1.86 ± 0.23b**	1.46 ± 0.38c*	1.53 ± 0.37c*	1.89 ± 0.18b**
Fecal excretion, mg/day	4.85 ± 0.62a	3.00 ± 0.50bc*	2.25 ± 0.75c*	2.38 ± 0.70c*	3.00 ± 0.47b*
Absorption, mg/day	4.62 ± 0.65c	7.45 ± 0.53ab*,**	7.25 ± 0.65ab*,**	7.83 ± 0.73a**	6.83 ± 0.50b*
Absorption, %	48.8 ± 5.3c	71.3 ± 4.5ab*,**	76.4 ± 7.3a**	76.7 ± 6.7a**	69.6 ± 3.8b*
Urinary level, mg/l	70.5 ± 20.6a	92.1 ± 25.9a*	161 ± 147a*	93.8 ± 32.0a*	130 ± 73a*
Urinary excretion, mg/day	1.29 ± 0.21c	1.87 ± 0.41b*	2.24 ± 0.67ab*	2.29 ± 0.27ab*	2.40 ± 0.35a*
Balance, mg/day	3.32 ± 0.55c	5.57 ± 0.80a**	5.02 ± 0.77ab*,**	5.55 ± 0.72a**	4.42 ± 0.35b*

Values are mean ± SD, n = 10.
Means sharing the same letter do not differ significantly.
Means of inulin-type fructan groups sharing the same number of asterisks (*) do not differ significantly

the total amount of Mg excreted in the feces of the HP-inulin and Synergy1 groups was significantly lower than that excreted in the feces of the BC-inulin group (Table 2).

The amount of Mg absorbed varied from 5 (control group) to 8.1 mg/day (Synergy1 group). All the rats consuming the fructans absorbed significantly more Mg than the control rats did, and the rats consuming Synergy1 absorbed significantly more Mg than the rats consuming BC-inulin (Table 2). The relative absorption of the Mg (%) varied between 49 (control rats) and 77 (HP-inulin and Synergy1 groups). This absorption was significantly higher in the rats consuming the experimental fructans compared to the control rats. Moreover, this percentage of Mg absorption was significantly higher in the rats consuming the HP-inulin or the Synergy1 than in the rats consuming BC-inulin (Table 2).

The urinary Mg level varied between 71 and 161 mg/l with a tendency to increase in the groups receiving the fructan diets without observing significant difference among these fructan groups. On the other hand, the amount of Mg excreted in the urine was significantly higher in the rats consuming the fructan-containing diets (1.9–2.4 mg/day) compared to the control rats (1.29 mg/day). Lastly, the retention of Mg in the rats receiving the prebiotic diets was higher than that in the control rats. Moreover, this retention was significantly higher in the rats consuming OF, HP-inulin or Synergy1 than in the rats consuming BC-inulin (Table 2).

Status indices of calcium and magnesium

Plasma (95.1 to 97.6 mg/L) and bone Ca (230 to 234 mg/g dry weight) levels did not vary significantly among the five experimental groups. On the other hand, the significant increase in the absorption and the retention of Mg in the rats consuming fructans resulted only in a non-significant increase in plasma Mg level (17.4 to 18.7 mg/L) compared to the control rats (17.0 mg/L), which was highest in the rats consuming BC-inulin (+10 %). However, the bone Mg level remained statistically similar among the five groups in this study (4.44 to 4.65 mg/g dry weight).

Discussion

Although the indigestibility and the fermentability of many inulin-type fructans have been well examined, there are still very few investigations comparing their effects separately or combined on mineral absorption [13, 16, 19]. In this study, we investigated the effects of β[2–1] fructans of different chain length and chain branching type, and a combination on intestinal absorption and balance of Ca and Mg in rats. Our results showed that the ingestion of all tested fructans led to a high production of SCFA in the cecum (5 times), and resulted in an increase of 4 to 5 times the cecal contents and of twice the weight of the cecal wall, as well as in a considerable decrease of the pH of the cecal contents compared to the control group. This was expected and confirmed the good fermentability of these various compounds. The important results of this work concerned the effect of these prebiotic carbohydrates on mineral absorption and retention. The ingestion of all these products increased Mg absorption and retention in rats. However, only the ingestion of Synergy1 led to a significant increase in Ca absorption (26 %) and retention (25 %) in rats. The other fructans trended to increase Ca absorption between 11 and 17 % without reaching significance ($p < 0.1$). Because the urinary Ca excretion was quite similar in the five groups, the Ca retention presented the same profile as the Ca absorption and only the ingestion of Synergy1 led to a significant increase in Ca retention in rats ($p < 0.05$). The BC-inulin fructan was not better at promoting mineral absorption than the other two fructans, although its structure should facilitate the access of hydrolytic enzymes in the rat large intestine. Indeed, fractional intestinal Mg absorption was significantly lower with BC-inulin than with the HP-inulin or Synergy 1.

The mechanisms and sites of intestinal Ca absorption are different from those of intestinal Mg absorption [20]. This may explain the different impact that fructans can have on the whole and on the segmented intestinal absorption of these two major minerals. Intestinal Ca absorption involves two distinguishable mechanisms. The main absorptive pathway of Ca is a saturable transcellular process, regulated by vitamin D *via* Ca-binding protein [21]. This is a highly regulated process, occurring mainly in the duodenum. The second process is a non-saturable, para-cellular transport that occurs throughout the length of the intestine [22]. The mechanisms involved in intestinal Mg absorption are a saturable process (facilitated diffusion or active absorption) and a passive diffusion. Thus, intestinal Mg absorption by passive diffusion from the distal part of the intestine is very important. The intestinal Mg absorption is controlled very weakly, if at all [23]. Thus, a possible explanation of the weak effect of fructans on intestinal Ca absorption in the present study is a down-regulation of the active pathway of the small intestinal Ca absorption after several weeks of feeding fructan. Therefore, a potential positive effect of inulin-type fructans on colonic Ca absorption might be masked when the overall intestinal Ca absorption is considered. This adaptation could be mediated through changes in the amounts of intestinal calcium-binding protein (CaBP) in response to a decreased serum concentration of 1,25-dihydroxy vitamin D, the main regulator of intestinal Ca absorption [21]. There are some experimental data that

support this assumption of adaptation. Chonan et al. [24] have noted that galacto-oligosaccharide supplementation increases intestinal Ca absorption in ovariectomized rats initially (9 days), but the effect had disappeared at 28 days. Ohta et al. [19] measured Ca and Mg absorption in rats at 10 and 24 days of feeding fructo-oligosaccharides (FOS). Their results indicated clearly that the effect of FOS on Ca absorption was lower at day 24 than at day 10, whereas the effect on Mg absorption was similar at both periods. Other investigators have reported a reduction in the Calbindin D9K level in the small intestine in rats after feeding FOS or lactose, supporting the occurrence of such an adaptive phenomenon [25, 26]. In human studies, OF, inulin and Synergy1 were shown to increase intestinal Ca absorption in adolescents when the supplementation lasted 9 days [10, 11], 21 days [13] or 28 days [8]. In one study with healthy young adult volunteers, no effect was observed with inulin or with OF [10]. More recently, two studies have investigated several weeks ingestion of a FOS in healthy men [27] or a short-chain FOS in post-menopausal women [28], without observing any positive effect of these FOS on intestinal absorption of Ca.

The results of this study showed that among the fructans tested, only the group of animals receiving the Synergy1 presented higher intestinal Ca absorption compared to the control group. Given that the urinary excretion of Ca did not differ among the five experimental groups, Ca retention was significantly higher only with Synergy1 (25 %) compared to the control group. The first speculation we can make to explain this better effect of Synergy1 on Ca absorption is that the actual activity of this product may be less important than that of the other products and that the feed-back phenomenon was less effective in the Synergy1 group than in the other fructans tested. This hypothesis is not supported, however, by the other results of our study. The production of SCFA in the group consuming Synergy1 was not lower than in the other groups. Moreover, the highest value of fractional intestinal Mg absorption was observed in the rats receiving Synergy1. It is thus highly possible that Synergy1 has higher activity than OF or HP-inulin when administered singly. The results obtained recently with rats in our laboratory [16] and in human volunteers [13] are in line with the results of the present study. In our previous study [16], we compared the effect of standard chicory inulin and resistant starch, and their combination, on Ca and Mg absorption in rats. We observed that the blend of these two fermentable carbohydrates had synergistic effects on intestinal absorption of Ca and on plasma levels of Mg in rats. In a recent human study, where volunteers were administered saturation dose of Ca (1500 mg Ca/day), Griffin et al. [13] reported that 3 weeks feeding of Synergy1 but not OF alone, increased the intestinal absorption of Ca significantly in adolescents. It is possible that OF, a short chain sugar, serves as a starter for selective bifidogenic fermentation in the proximal colon, whereas the long sugar chain (HP-inulin), which is fermented more slowly, can maintain the metabolic activity of the improved flora in more distal parts of the colon (J. Van Loo, personal communication). The presence of short and long sugar chains should thus allow maintenance of high fermentation activity throughout the large intestine, which can increase the beneficial effects on mineral absorption.

In conclusion, the four fructans studied were equally effective on the parameters of fermentation as on the intestinal absorption and retention of Mg. However, the overall intestinal absorption of Ca was improved significantly only with Synergy1, and little improved with the other fructans. This may result from a feed-back phenomenon concerning the absorption of Ca in the upper parts of the intestine. Taking into account the whole of the results, we observed that Synergy1 presented the best efficacy on mineral absorption among the fructans studied. Other studies are still needed to determine the potential synergistic effects of other types of prebiotic fructan combinations on mineral absorption.

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