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ORIGINAL CONTRIBUTION

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Diet composition and age determine the effects of inulin-type fructans on intestinal calcium absorption in rat

Summary *Background* Although several studies in animals and humans have established that inulin-type fructans (inulin, oligofructose, fructooligosaccharides) enhance intestinal Ca absorption, there are also reports that failed to demonstrate any effects of added fructans on Ca absorption. *Aim of the study* We investigated in a rat model what variables determine inulin actions on mineral absorption with special focus on the basic diet. *Methods* To determine apparent intestinal mineral absorption, whole body retention and mineral accumulation in bone, we performed feeding experiments with a balance technique by which mineral intake, faecal and urinary output are determined. *Results* In a first study we compared the effects of an inulin oligofructose mixture (0 and 10 %, respectively) when added either to a standard diet or a semi-synthetic diet. Rats fed the semi-synthetic diet were younger (adolescent) than rats on standard diet (young adult). We observed that the apparent Ca absorption ratio was significantly increased by inulin and oligofructose only when provided in the semi-synthetic diet and not in the standard diet that intrinsically already contained some fructans from wheat. In our second study with a semi-synthetic diet fed to growing (adolescent) rats, inulin and oligofructose increased not only Ca but also Mg and Zn absorption, whole body mineral retention and femur mineral content. *Conclusion* Inulin-type fructans at dietary levels of 10 % (w/w) do increase mineral absorption, retention and accumulation in bone in the case of Ca, Mg and Zn, but only when the basic diet for the control group contains no intrinsic fructans and when the mineral demand is particularly high as during growth.

Key words apparent absorption – balance – retention – inulin – oligofructose – fructan – femur – calcium – magnesium – zinc – age effects

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Introduction

Inulin-type fructans are linear chains of β 2 → 1 bound fructose monomers found in a variety of plants. The chain length of inulin varies between 2 and 60 or more fructose units, whereas oligofructose has generally shorter chains with 2 to 8 units. Fructooligosaccharides are synthesised from sucrose by adding one or more fructoses resulting in shorter chains than in oligofructose. These fructans are not hydrolysed enzymatically in the small intestine [1] but are fermented by the microflora in the large intestine [2] with a multitude of effects on human and animal health. Fructans, when added to a diet, cause an increase in intestinal mineral absorption as observed in various animal [3–7] and human [8–10] studies. However, there are also reports in which dietary effects of inulin or oligofructose on mineral absorption could not be detected [10–12]. This is in most cases explained by different study designs such as the amount of added inulin-type fructans, age of subjects or animal model and in the type of diet used throughout the experimental period. A careful inspection of the literature on reported studies suggested that the age of the subjects or animals and their different mineral requirements as well as the type of diet could be

EJN 535

the main reasons for the different study outcomes. For example, in human studies investigating the effect of inulin-type fructans on Ca absorption, the amount of inulin-type fructans added to the diets varied between 8 g/d [10] and 40 g/d [8] and the age of subjects ranged from 10 years [10] to 70 years [12]. The duration of the study periods was at minimum 9 days [9] and at maximum 35 days [12] with a highly variable background diet. Three of these human studies failed to demonstrate an effect of added fructans [10–12]. To understand what the variables might be that determine whether dietary fructans are effective in enhancing mineral absorption or not, we conducted a series of experiments with rats in which we compared diets and age of the animals. In the first experiment, young adult rats with 200 g body weight were used and fed a standard diet with naturally occurring fructans or a standard diet enriched with 10 % of an inulin oligofructose mixture (Raftilose Synergy1). In the second experiment adolescent rats with a mean body weight of 100 g received a semi-synthetic diet supplemented with either no or 10 % inulin oligofructose mixture. The rationale for using growing animals was their higher Ca demand for growth that could also boost the effect of fructans on Ca absorption. After obtaining the results that indeed showed a significant increase in apparent Ca absorption ratio by fructans when added to the semi-synthetic diet, we performed a third experiment in which not only the intestinal absorption of Ca but also that of Mg and Zn was measured as well as whole body retention (balance) and accumulation of these minerals in femur.

Materials and methods

Experiment 1

A total of 16 male Sprague Dawley rats (Charles River, Sulzfeld) weighing approx. 200 g were housed in metabolic cages in a temperature- (22 °C) and humidity-controlled (50 %) room. Metabolic cages were metal-free and had mesh bottoms which enabled the collection and separation of faeces and urine by a plastic mesh. The rats received a standard diet (No. 1321, powder, Altromin, Lage) composed of shredded wheat, barley and corn containing 19 % protein, 4 % fat, 50 % carbohydrate, 6 % dietary fibre, 0.9 % Ca, 0.7 % P, 0.2 % Mg and 0.007 % Zn according to the manufacturer and they had free access to deionised water. For the fructan group 10 % Raftilose Synergy1 (Orafti, Tienen), a mixture of inulin and oligofructose, was added. Since fermentation of fructans yields around 20 to 25 % of caloric energy of carbohydrates (mainly in the form of short-chain fatty acids), the control group received 2 % maltodextrine (Orafti, Tienen) for isoenergetic substitution. In the adaptation period of 6 days the Synergy1 content in the diet was gradually elevated and thereafter animals were fed for 28 days with the last 15 days used to collect the 24 h excretion of faeces.

Experiment 2

The 16 male Sprague Dawley rats (Charles River, Sulzfeld) with a mean body weight of around 100 g were housed in metabolic cages as described above. Animals were fed a semi-synthetic diet (No. C 1034 mod., powder, Altromin, Lage) containing 17 % casein, 6 % corn oil, 60 % corn starch, 5 % cellulose, 0.5 % Ca, 0.4 % P, 0.05 % Mg and 0.003 % Zn according to the manufacturer. Raftilose Synergy1 and maltodextrine were added as described above. The adaptation period was 2 days shorter but the experimental period and sample collection were the same as in experiment 1.

Experiment 3

A total of 24 male Sprague Dawley rats (Charles River, Sulzfeld) weighing approx. 100 g were used for this experiment with the same housing and feeding conditions as in experiment 2. The experimental period was shortened to 15 days and 24 h faeces and urine samples were collected for the whole experimental period. On day 16 rats were sacrificed and femoral bones were removed for mineral analysis.

For all experiments, rats were maintained in accordance with the national guidelines for the care and use of laboratory animals.

Analytical procedures

Daily collected faeces and urine samples were pooled (3-day samples) and frozen until analysis. Diet and biological samples (i. e. faeces and total femur) were ashed at 580 °C for 48 h, the ash then dissolved in 6 M HCl (Sigma-Aldrich, Seelze) and filtered through filter paper (No. 1507, Schleicher & Schuell, Dassel). These ashed samples and aliquots of urine were measured after appropriate dilution by flame atomic absorption spectrometry (Model 5100, Perkin-Elmer, Rodgau). For Ca and Mg, samples were diluted with 0.5 % lanthanum ($LaCl_3 \cdot 7H_2O$, Merck, Darmstadt) in 0.6 M HCl. Samples for assaying Zn were diluted only with 0.6 M HCl. Ca was measured at 422.7 nm, Mg at 285.2 nm and Zn at 219.9 nm.

Calculations and statistics

Apparent mineral absorption was calculated as the dietary mineral intake minus faecal mineral excretion. Mineral balance is defined as the dietary mineral intake minus faecal and urinary mineral excretion. Results are expressed as means for the balance period and group ± standard deviation (SD). Differences of means of control and Synergy1 groups were tested for significance using an unpaired student's t test, two-tailed, recognising a P of < 0.05 as significant.

Results

Table 1 summarises the results obtained in experiment 1 and 2 with body weight development, dietary Ca intake and faecal Ca excretion. Feeding of 10 % Synergy1 did not result in any significant changes in the apparent Ca absorption when using the standard diet. For the semi-synthetic diet, however, the apparent Ca absorption ratio significantly increased by 22 % in the Synergy1 group when compared to that of the control group.

The results of the experiment with growing rats including Ca, Mg and Zn balance data are summarised in Table 2. Mineral intakes did not differ between control and Synergy1 groups for each mineral but mineral excretions with faeces were significantly lower in the groups receiving Synergy1. Consequently, apparent absorption of Ca, Mg and Zn increased significantly by feeding the fructans. Urinary mineral excretions also were increased in the case of Ca and Mg but not for Zn when Synergy1 was fed. Finally, absolute mineral balance was significantly increased only for Zn, whereas relative mineral balances showed significant increases for all minerals (Ca, Mg and Zn). In addition to the increased apparent absorption and body retention Ca, Mg and Zn contents in femur were significantly increased when Synergy1 was fed for 15 days with the greatest increase in the case of Zn (Fig. 1).

Discussion

In both, animal [3–7] and human [8–10] studies supplementation of the diet with inulin-type fructans was

Table 1 Apparent Ca absorption in rats fed different diets with or without 10 % Raftilose Synergy1

	Standard diet		Semi-synthetic diet	
	Control (n = 8)	Synergy1 (n = 7)	Control (n = 8)	Synergy1 (n = 7)
Initial body weight, g	191 ± 4	190 ± 5	107 ± 7	105 ± 7
Final body weight, g	284 ± 20	281 ± 25	243 ± 25	233 ± 20
Diet intake, g/d	18.9 ± 1.4	19.0 ± 1.8	13.7 ± 1.3	13.4 ± 1.3
Ca intake, mg/d	179.4 ± 13.3	163.9 ± 15.4	58.5 ± 5.6	53.4 ± 5.3
Faecal Ca excretion, mg/d	136.6 ± 9.8	123.8 ± 12.5*	23.3 ± 4.5	14.3 ± 2.6*
Apparent Ca absorption, mg/d	42.8 ± 4.5	40.1 ± 4.4	35.2 ± 3.8	39.2 ± 3.2
Apparent Ca absorption, %	23.8 ± 1.3	24.4 ± 1.9	60.4 ± 5.5	73.5 ± 3.1*

Mean ± SD; * $P < 0.05$ (t test, two-tailed)

Table 2 Ca, Mg and Zn absorption, balance and content in femur in adolescent rats fed a semi-synthetic diet with or without 10 % Raftilose Synergy1

	Ca		Mg		Zn	
	Control (n = 12)	Synergy1 (n = 12)	Control (n = 12)	Synergy1 (n = 12)	Control (n = 12)	Synergy1 (n = 12)
Initial body weight, g	97 ± 9	101 ± 11	97 ± 9	101 ± 11	97 ± 9	101 ± 11
Final body weight, g	166 ± 16	172 ± 21	166 ± 16	172 ± 21	166 ± 16	172 ± 21
Diet intake, g/d	11.8 ± 1.2	12.2 ± 1.6	11.8 ± 1.2	12.2 ± 1.6	11.8 ± 1.2	12.2 ± 1.6
Mineral intake, mg/d	53.1 ± 5.3	50.1 ± 6.7	7.6 ± 0.8	7.1 ± 0.9	0.512 ± 0.052	0.486 ± 0.065
Faecal mineral excretion, mg/d	15.9 ± 2.7	7.7 ± 2.3*	2.6 ± 0.5	0.9 ± 0.2*	0.356 ± 0.035	0.302 ± 0.043*
Apparent mineral absorption, mg/d	37.1 ± 5.0	42.3 ± 4.9*	5.0 ± 0.5	6.2 ± 0.8*	0.156 ± 0.023	0.184 ± 0.026*
Apparent mineral absorption, %	69.8 ± 5.1	84.9 ± 3.5*	66.1 ± 4.3	86.9 ± 2.6*	30.4 ± 2.7	37.9 ± 2.6*
Urinary mineral excretion, mg/d	0.9 ± 0.3	2.2 ± 0.9*	3.0 ± 0.4	3.8 ± 0.4*	0.005 ± 0.001	0.005 ± 0.002
Mineral balance, mg/d	36.3 ± 4.9	40.1 ± 5.2	2.1 ± 0.3	2.4 ± 0.5	0.151 ± 0.023	0.178 ± 0.026*
Mineral balance, %	68.1 ± 5.0	80.3 ± 2.3*	26.8 ± 2.2	33.1 ± 3.8*	29.4 ± 2.8	36.7 ± 2.3*
Femoral mineral content, mg/g DM	208.7 ± 4.9	213.1 ± 4.3*	4.5 ± 0.2	4.7 ± 0.1*	0.247 ± 0.018	0.269 ± 0.017*

DM dry matter; mean ± SD; * $P < 0.05$ (t test, two-tailed)

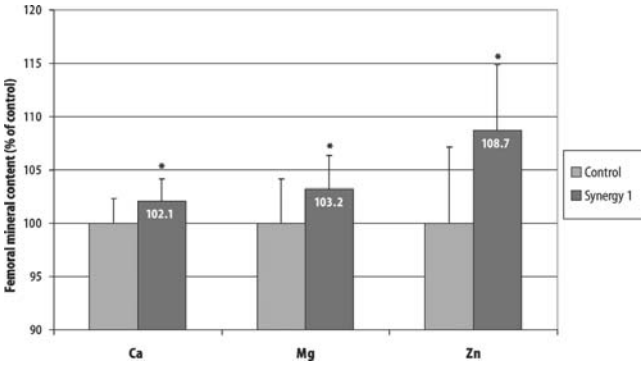

Fig. 1 Bone mineral content (femur) in adolescent rats fed a semi-synthetic diet with or without 10 % Raftilose Synergy1 (Mean ± SD, n = 12, * P < 0.05 (t test, two-tailed))

shown to increase intestinal absorption of Ca and other minerals. Moreover, increased Ca absorption caused by the fructans was found to increase also the Ca content in bone [13] as the major Ca store. We confirm these findings for the minerals Ca, Mg and Zn in adolescent rats fed a semi-synthetic diet supplemented with 10 % Raftilose Synergy1. Despite almost identical mineral intakes along both groups, the apparent absorptions were higher in animals fed Synergy1. The higher absorptions were partly compensated by an elevated urinary excretion of Ca and Mg, but not in case of Zn with a known extremely low urinary excretion contributing to Zn homeostasis. However, the balance ratios for Ca, Mg and Zn were all increased and an increased mineral accumulation in bone (femur) was also observed.

The mechanism for this increase in intestinal mineral absorption induced by fermentable fructans is not clear yet. The fructans are metabolised by the bacteria in the large intestine leading to an increased production of short-chain fatty acids, mainly acetate, propionate and butyrate [14]. Short-chain fatty acid production lowers the luminal pH which increases mineral solubility [15] and which raises the mineral gradient between the luminal and serosal side allowing passive mineral transport to increase. In addition, short-chain fatty acids, especially butyrate, serve as a fuel for mucosal cells and stimulate cell proliferation which in turn could increase the absorptive surface area of the large intestine. A direct coupling of Ca transport with colonic uptake of short-chain fatty acids has also been proposed and experimental data support this coupling theory because the luminal presence of short-chain fatty acids increased the transepithelial Ca transport in rat caecum and colon [16]. In addition, fermentation of fructans could increase intestinal Ca absorption also by effects on gene transcription of proteins involved in mucosal Ca binding and sequestration. Ohta et al. found increased levels of calbindin-D9k in the large intestine of rats receiving fructans in their diet [17].

Based on preliminary findings we addressed the question of whether mineral absorption is dependent on the diet to which the fructans are added and whether the age of the animals is a critical determinant for the fructan effects. We observed that a basic diet containing carbohydrates from wheat failed to induce an effect of added fructans. Analysis of the diet revealed that it contained natural fructans in a concentration of 1.5 % (w/w). This intrinsic content of inulin and oligofructose in the background diet is obviously sufficient to induce effects on mineral absorption and elevate Ca absorption but at the same time prevents any additional effects of extra inulin-type fructans to enhance further Ca uptake. That this low quantity of fructans already affects mineral absorption appears reasonable since it has been shown before that 3 % of inulin-type fructans in the diet can increase significantly apparent Ca absorption in adolescent rats [18]. In contrast, Levrat et al. showed a linear relation (r = 0.99) between the inulin content in diet (0, 5, 10 and 20 %) and the caecal Ca absorption in growing rats [3]. From this, one would expect that increasing the fructan content from 1.5 % to 11.5 % by Raftilose Synergy1 in the diet would further increase Ca absorption, which was not the case in our study (Table 1). The reason might be that Levrat et al. measured specifically caecal Ca absorption, whereas we determined whole intestinal Ca absorption.

A comparison of the animals on standard or semi-synthetic diets (Table 1) reveals a three times higher Ca intake in rats on standard diet. This is due to the higher dietary Ca content and higher food intake and resulted in an apparent Ca absorption of around 40 mg/d (42.8 and 40.1 mg/d). The younger animals on semi-synthetic diet displayed much higher relative absorption rates (60.4 and 73.5 % of intake) but when expressed in mg/d they had nearly equal apparent Ca absorptions (35.2 and 39.2 mg/d) as the older animals fed the standard diet. So, when based on total Ca absorbed per day, fructans added to the semi-synthetic diet increased Ca absorption by 4 mg/d. When compared to the semi-synthetic diet, the standard diet provided obviously an already high Ca availability (Ca absorption was 42.8 mg/d) that may be attributed to its natural fructan content of 1.5 %.

When we determined mineral retention (balance) and mineral contents in femur for the minerals Ca, Mg and Zn in young rats on the semi-synthetic diet, mineral balance ratios were also significantly increased for all elements. Moreover, femur mineral contents (Fig. 1) also increased significantly indicating that not only intestinal absorption but also retention in bone is enhanced by Synergy1. These findings confirm those of Zafar et al. with a higher Ca content in femur in ovariectomized rats fed a diet with 5.5 % inulin and oligofructose [13] and those of Scholz-Ahrens et al. with an increase in bone mineral content (femur) in ovariectomized rats on an oligofructose diet [19]. Kruger et al. reported no diffe-

rences in femur bone mineral content after fructan administration but an increase in that of spine in male rats [20] and Roberfroid et al. demonstrated that whole-body bone mineral content of rats fed inulin was also increased [21].

From the experiments presented here we conclude that inulin-type fructans do increase intestinal mineral absorption and increase whole body retention and accumulation in femur in case of Ca, Mg and Zn. These effects of an addition of inulin or oligofructose may not be seen when the background diet already contains inulin-type fructans – even in quantities as low as 1.5 % – warranting a careful analysis of the diet with respect to its content of highly fermentable carbohydrates when assessing the effects of a dietary intervention with fructans.

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