Inulin and Oligofructose and Mineral Metabolism: The Evidence from Animal Trials[1–4]

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Abstract

Nondigestible oligosaccharides have been shown to increase the absorption of several minerals (calcium, magnesium, in some cases phosphorus) and trace elements (mainly copper, iron, zinc). Inulin-type fructans including oligofructose and fructooligosaccharides derived from sucrose by enzymatic transfructosylation are the best investigated food ingredients in this respect. The stimulation of absorption was more pronounced when the demand for calcium was high, i.e., in animals in the rapid growing stage and in animals with impaired calcium absorption because of either ovariectomy or gastrectomy. Even a small stimulation of calcium absorption increased the mineral accumulation in the skeleton because of its persisting effect over months. Inulin-type fructans stimulated mineral absorption and bone mineral accretion when combined with probiotic lactobacilli and in the presence of antibiotics. Direct comparison of different inulin-type fructans revealed a more pronounced effect by inulin or a mixture of long-chain inulin and oligofructose than by oligofructose alone. Mechanisms on how inulin-type fructans mediate this effect include acidification of the intestinal lumen by short-chain fatty acids increasing solubility of minerals in the gut, enlargement of the absorption surface, increased expression of calcium-binding proteins mainly in the large intestine, modulated expression of bone-relevant cytokines, suppression of bone resorption, increased bioavailability of phytoestrogens, and, via stimulation of beneficial commensal microorganisms, increase of calcium uptake by enterocytes. Under certain conditions, inulin-type fructans may improve mineral absorption by their impact on the amelioration of gut health including stabilization of the intestinal flora and reduction of inflammation. The abundance of reports indicate that inulin-type fructans are promising substances that could help to improve the supply with available calcium in human nutrition and by this contribute to bone health. J. Nutr. 137: 2513S–2523S, 2007.

Introduction

The prevention of diseases of the musculoskeletal system, which increase with age, is a challenge for aging societies (1). In Germany, annual costs for diseases of the musculoskeletal system were €24.5 billion in 2004, with most of the total costs per inhabitant in subjects >65 y old (2). Age-related osteoporosis affects ~28 million Americans (3). In the United States the direct cost from musculoskeletal conditions for the health services was 1.2%, and the indirect costs for the loss of productivity and wages mounted up to another 1.3% of the gross national product (4,5). The direct costs for osteoporotic hip fractures alone reached $18 billion in the United States in 2002 (6).

Prevention is the most effective means to conserve cost and to prevent individual suffering, as can be seen from lower total life span morbidity in subjects with healthier lifestyles (7). Providing adequate nutrients with high bioavailability is 1 option that can contribute to a preventive public health concept. This may also include functional ingredients in an expanding market of functional foods that may contribute to reduction of risk for certain diseases.

Inulin-type fructans are the most investigated prebiotics with respect to functionality (see this Supplement). Among other physiological properties, their effect on mineral metabolism and bone health has been investigated using different approaches,

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[4] In these proceedings, the term inulin-type fructan shall be used as a generic term to cover all β–(2←1) linear fructans. In any other circumstances that justify the identification of the oligomers vs. the polymers, the terms oligofructose and/or inulin or eventually long-chain/or high-molecular-weight inulin will be used, respectively. Even though the oligomers obtained by partial hydrolysis of inulin or by enzymatic synthesis have a slightly different DP_{av} (4 and 3.6, respectively), the term oligofructose shall be used to identify both. Synergy will be used to identify the 30/70 mixture (wt:wt) of oligofructose and inulin HP otherwise named oligofructose-enriched inulin.

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including in vitro and cell-culture studies, experiments in animal models, and ultimately in human clinical trials. Human studies have been performed mainly in the short term. Several target groups have been included, and different substances were tested, such as inulin, oligofructose, or a mixture of short- and long-chain products (Synergy). Meanwhile, a long-term study with an intervention >1 y was performed with repeated calcium balances to study the persistence of the effect in girls (8). In addition, bone mineral density (BMD),[5] which is an accepted surrogate marker for the risk of bone fracture, was determined as a primary outcome. Results of human studies, however, are the topic of separate articles of this issue of the Journal (see Abrams et al. and Coxam, this volume).

Although they are only indicative for effects in humans, studies in animal models may provide valuable information that is difficult to obtain in human studies, partly for practical and ethical reasons. This is especially true for long-term studies with strictly controlled dietary and environmental factors, but it is true for mechanistic studies as well. The present article is mainly limited to recent reports and to animal experiments that investigated the effects of inulin-type fructans on the metabolism of minerals that are important for bone health with special emphasis on calcium and phosphorus. In addition, the experimental background that favors a significant effect on mineral balance, bone mineral content (BMC), or bone structure, and thus contributes to explain the underlying mechanism, is discussed.

Effect of inulin-type fructans on trace elements (copper, iron, manganese, and zinc)

In 5-wk-old growing rats that were pair-fed an experimental semipurified diet over a period of 3 wk that contained 6 g calcium/kg diet and 3.5 g phosphorus/kg diet, no significant effect of 10, 30, or 50 g oligofructose/kg diet on the absorption and retention of iron, manganese, and zinc was observed, but copper absorption and balance were significantly reduced (9); these results are unexpected and in contrast to reports by others (**Table 1**). Vanhoof and DeSchrijver (10) used 60 g/kg inulin and found no effect on the absorption and retention of iron and zinc in rats with nonpurified diets. The cecal pH was not affected either. In pigs on the same diet, zinc absorption was significantly higher, and zinc retention tended to be higher compared with control animals. In contrast to the report by Wolf et al. (9), there was a positive effect of 75 g/kg of oligofructose but not of inulin at the recommended intake of calcium and phosphorus on iron absorption, as shown by recovery from anemia in gastrectomy-induced iron-deficient rats (11).

When rats were fed 100 g inulin/kg diet as part of a semipurified diet, the absorption of iron and copper was higher compared with the control group (12). Raschka and Daniel (13) confirmed the stimulating effect of inulin-type fructans (100 g/kg semipurified diet of Synergy) on the absorption and balance of iron and zinc for growing rats, which was also observed earlier (14) when 100 g oligofructose/kg diet was given. For copper, absorption was only a trend for a stimulating effect.

Recently, Coudray et al. (15) reported that 75 g/kg inulin in a semipurified diet stimulated the absorption of copper and zinc in rats at different ages, as assessed by stable-isotope technique. Despite this, the level of zinc in plasma, liver, and bone and the content of copper in liver and bone were not significantly affected. The lack of effect on the concentration of these trace elements in organs may be attributable to the relative shortness of the intervention, which lasted for only 30 d.

In summary, there is some evidence of a stimulatory effect of inulin-type fructans on the absorption of the trace elements iron, copper, and zinc. The magnitude of a stimulating effect on trace element absorption is affected by the type of fructan and its dose. Moreover, its content in the background diet is important: As part of nonpurified diets based on grain and soy, fructans yielded small or no effects. This may be attributable to the lack of an additional effect on luminal pH, thus indicating a different fermentation pattern, as reported by Vanhoof and DeSchrijver (10). In addition, the body store of a trace element seems to be important in affecting its absorption capacity or ratio. Differences in these factors among studies as well as limited sample size may explain the different outcomes that have been reported.

Effect of inulin-type fructans on minerals

Magnesium. When 5-wk-old growing rats were fed an experimental diet that contained 6 g calcium/kg diet, the magnesium absorption as a percentage of intake was higher in animals on the diets containing inulin-type fructans, but the retention was not affected (9). Vanhoof and DeSchrijver (10) used 60 g/kg inulin as part of a nonpurified diet and found no effect on the absorption of magnesium in growing rats.

Takahara et al. (16) reported a 27% increase of magnesium absorption and a 20–40% increase of magnesium incorporation into the femur in growing rats after 2 wk on a semipurified diet with 50 g/kg oligofructose and a calcium content of 5 g/kg. Lobo et al. (17) reported that 50 g oligofructose/kg as part of a semipurified diet in the presence of 7.5 g calcium/kg diet increased the absorption of magnesium in healthy growing rats. The content of magnesium in the femur or tibia was not affected after 3 wk of intervention.

Using stable isotopes, Coudray et al. (18) reported higher magnesium absorption and retention of similar magnitude in rats of different ages (2, 5, 10, and 20 mo) that received 75 g/kg inulin with their semipurified diet. When the metabolic balance was computed, magnesium absorption but not retention (mg/d) was significantly higher in rats on a diet that contained 75 g/kg inulin with their semipurified diet in the presence of varying contents of calcium (19). Younes et al. (20) observed a stimulation of magnesium absorption in adult rats by inulin, resistant starch, and inulin plus resistant starch compared with a control diet with wheat starch and 7.5 g/kg calcium, without difference between the substances.

In 8-wk-old intact and ovariectomized mice, 5 g/kg oligofructose in a semipurified diet containing 5 g/kg calcium and 3 g/kg phosphorus only slightly increased the magnesium content in the femur compared with the control diet (21). The stimulatory effect of oligofructose on magnesium absorption was also observed in dogs (22). In hens the absorption was not measured, but the incorporation of magnesium into bone was not improved in animals that were fed 10 g/kg diet oligofructose, although that of calcium and phosphorus was (23).

In summary, in animal trials, using mainly semipurified diets, a significant stimulation of magnesium absorption by inulin-type fructans was demonstrated, an observation that was made in humans as well (24). The effect of inulin-type fructans on magnesium retention or its incorporation into bone was less consistent, and it has to be clarified at which experimental conditions the effect becomes significant. It might be that animals in a physiological state with a higher demand will benefit more from inulin-type fructans (25) than animals that have been exposed to a normal or high magnesium supply.

[5] Abbreviations used: BMC, bone mineral content; BMD, bone mineral density; DEXA, dual-energy x-ray absorptiometry; NDO, nondigestible oligosaccharides.

TABLE 1 Effect of inulin-type fructans on trace element absorption in animal models[1]

	Effect	Animals	Age (*wk*) or BW [*g*] at start	*n*	Duration, *wk*	Element, *mg/kg*	Ca, *g/kg*	Dietary ITF, *g/kg*	Exp. diet	Type of ITF	Literature cited
Copper											
Retention, *%*	(↑)	Male rats	[100]	10	3.5	10	7	10	sp	OF	14
Balance, *μg/d*	↓	Male rats	5	10	3	6	6	10, 30, 50	sp	OF	9
Retention, *% and nmol*	↑	Male rats	2–20 mo	10	4	7	5	75	sp	IN	15
Iron											
Retention, *%*	↑	Male rats	[100]	10	3.5	70	7	10	sp	OF	14
Balance, *μg/d*	ns	Male rats	5	10	3	47	6	10, 30, 50	sp	OF	9
Absorption, *%*	ns	Male rats	3	8	5	169	8	60	np	IN	10
Status, *Hb, Ht*	↑	Male gx rats	10	5	6	39	5	75	sp	OF	11
	ns									IN	11
Manganese											
Balance, *μg/d*	ns	Male rats	5	10	3	12	6	10, 30, 50	sp	OF	9
Zinc											
Retention, *%*	↑	Male rats	[100]	10	3.5	73	7	10	sp	OF	14
Absorption, *%*	ns	Male rats	3	8	5	145	8	60	np	IN	10
	↑	Cannulated pigs	[85 kg]	8	3 d						10
Balance, *μg/d*	ns	Male rats	5	10	3	45	6	10, 30, 50	sp	OF	9
Balance, *μg/d*	↑	Male rats	[100]	8	4	70	5	10	sp	Syn1	13
Retention, *% and nmol*	↑	Male rats	2–20 mo	10	4	37	5	75	sp	IN	15

[1] ITF, inulin-type fructan; BW, body weight; gx, gastrectomized; Hb, hemoglobin; Ht, hematocrit; IN, inulin; np, nonpurified; ns, not significant; OF, oligofructose; sp, semipurified; Syn1, Synergy1; (↑), marginally significant.

Calcium. It has been shown repeatedly that inulin-type fructans, mainly as part of a semipurified diet, stimulate the absorption of calcium and enhance the calcium content of bone under certain conditions. These results have been summarized before (for review see 26–29), and were recently confirmed (12,13,16,18, 19,30–34). In some cases no beneficial effect was observed, but this may be explained by the experimental conditions and the parameters that have been analyzed.

For example, in the adult ovariectomized rat, which is a more suitable animal model for postmenopausal osteoporosis than the rapidly growing rat, calcium balances are around zero or only slightly positive (35). This is because these rats have a much lower demand for calcium net uptake by the bone. After 4 wk on a semipurified diet containing 25, 50, or 100 g oligofructose/kg diet in the presence of a recommended amount of dietary calcium for rats (5 g/kg), no stimulating effect on calcium absorption was seen. Surprisingly, the fecal excretion of calcium was higher and the calcium absorption lower compared with the control group, when 25 g oligofructose/kg diet was fed.

After 8 wk and after 16 wk a tendency for lower fecal calcium excretion and thus for higher calcium absorption was observed for 25, 50, and 100 g oligofructose/kg diet compared with control animals. Only with the highest content of oligofructose did this effect become significant after 8 wk. Urinary calcium excretion was higher after 4, 8, and 16 wk when 50 and 100 g oligofructose/kg diet were fed. This was because of the slightly higher intestinal calcium absorption after 8 and 16 wk but not related to increased bone resorption. When rats were fed 50 g oligofructose/kg in the presence of a high dietary calcium content (10 g/kg), calcium absorption tended to be higher after 4 and 8 wk and was significantly higher after 16 wk (35).

The effect of oligofructose on calcium retention for different experimental conditions is summarized in **Figure 1**. At the recommended content of dietary calcium, there was a significant positive effect only when 100 g oligofructose/kg diet was given and calcium absorbtion was measured in a metabolic balance at wk 8 of the intervention. When dietary calcium was raised on its own from 5 g/kg to 10 g/kg diet without oligofructose, at wk 4 of the intervention the calcium retention tended to be higher, but it was significantly higher when the content of calcium was raised in the presence of 50 g oligofructose/kg diet. The calcium retention tended to be higher persistently after 4, 8, and 16 wk compared to its control group, when oligofructose was given in the presence of 10 g/kg calcium.

No significant effects of oligofructose were found in 5-wk-old growing rats that were fed an experimental semipurified diet over a period of 3 wk that contained 6 g calcium and 3.5 g phosphorus/kg diet (9). Neither 10, 30, nor 50 g oligofructose/kg diet stimulated the absorption and retention of calcium significantly. These results are in accordance with our calcium retention data, showing that the stimulation of calcium retention at a certain time period may be small, especially in the short term. This does not imply a lack of significance for a cumulative effect in the long term, as for calcium accretion in the skeleton or for the protection of the integrity of the trabecular structure (35).

As in our study (35), Coudray et al. (36) reported a tendency for a higher calcium absorption and balance in rats that were fed 100 g inulin/kg with 3 different semipurified diets with a background diet that contained 2.5, 5.0, and 7.5 g calcium/kg diet. A significantly higher value was observed only for the calcium absorption and retention ratio (percentage of intake). These results are, however, difficult to interpret because the animals were fed ad libitum in contrast to our animals, which were strictly pair-fed. The authors mentioned that the feed intake (and thus calcium intake) and the growth rate were significantly depressed when inulin was in the diet (36). The higher calcium retention ratio thus may be attributed in part to the lower absolute intake because it is known that intake and retention ratio are negatively correlated. Nevertheless, the calcium absorption was slightly higher despite its lower intake,

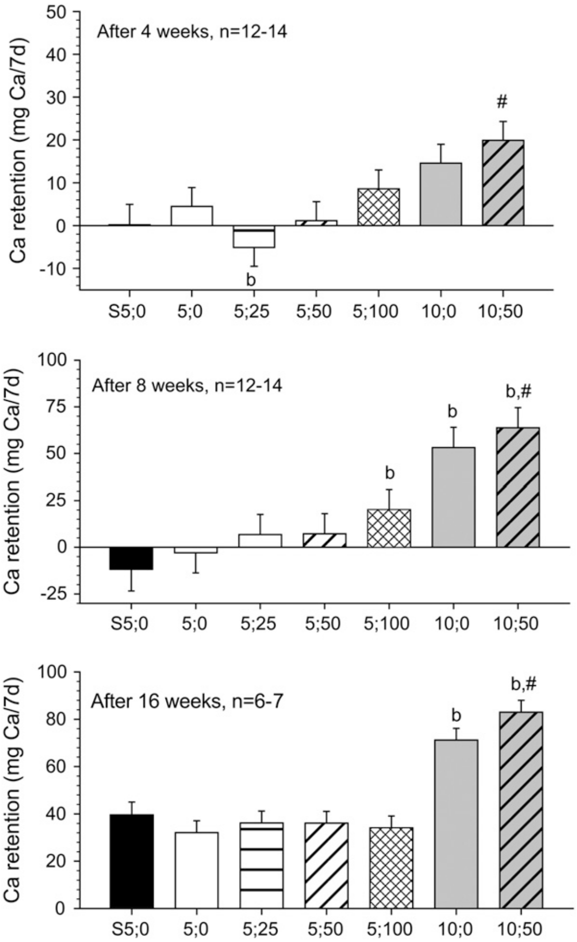

FIGURE 1 Calcium retention after 4 wk (*top*), 8 wk (*middle*), and 16 wk (*bottom*) on diets with varying contents of calcium (5 and 10 g/kg diet) and oligofructose (25, 50, and 100 g/kg diet) compared with the ovariectomized control group with 5 g calcium and 0 g oligofructose/kg diet. S, sham-operated animals. Mean and SEM, n = 12–14, in balances at 4 and 8 wk, and n = 6–7 in balance at 16 wk. Means of a group are significantly different with $P < 0.05$ as follows: different from ovariectomized controls (*b*); from rats on 5 g calcium and 50 g oligofructose/kg diet (#) (35).

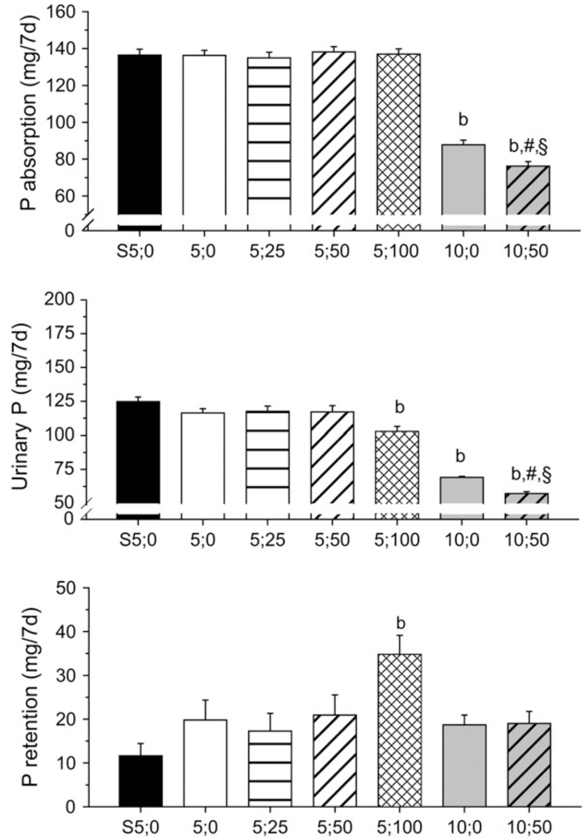

FIGURE 2 Phosphorus absorption (*top*), urinary phosphorus excretion (*middle*), and phosphorus retention (*bottom*) in ovariectomized adult rats after 4 wk on diets with varying contents of calcium (5 and 10 g/kg diet) and oligofructose (25, 50, and 100 g/kg diet) compared with the ovariectomized control group with 5g calcium and 0 g oligofructose/kg diet. S = sham operated animals. Mean and SEM, n = 12–14. Means of a group are significantly different with $P < 0.05$ as follows: different from ovariectomized controls (*b*); from rats on 5 g calcium and 50 g oligofructose/kg diet (#); from rats on 10 g calcium and 0 g oligofructose (*§*) (35).

indicating a stimulating effect on calcium absorption by inulin. In an experiment with stable isotopes, the same authors reported that 35 or 75 g inulin/kg significantly stimulated the calcium absorption and retention in short-term experiments (30 d) with rats at ages varying between 2 and 20 mo, but the numeric effect was higher in aged rats (10 and 20 mo) compared with young and adult rats of 2 and 5 mo (18).

One group directly compared the effect of a combination of short- and long-chain inulin-type fructans (Synergy) on calcium absorption in the presence of a standard rat chow (Exp. 1) with the effect gained in the presence of a semipurified diet (Exp. 2). With both diets, inulin-like fructans significantly depressed the fecal calcium excretion. Although the stimulating effect on calcium absorption (mg/d) was not significant, it was more pronounced in the presence of a semisynthetic than of a standard rat diet (13). The apparent percentage calcium absorption was significantly higher in the presence of a purified diet, but there was no effect in the presence of the standard diet. These differences may be explained in part by the fact that the unsupplemented rat diet contained 15 g/kg naturally occurring fructans, which may have attenuated the effect of supplemented Synergy. However, these results are difficult to discuss because other factors than the basal diet differed as well: the rats used with the purified diet were younger (100 g body weight) than those used with the standard diet (200 g body weight). Moreover, the mineral content of the semipurified diet was lower (5 g/kg calcium and 4 g/kg phosphorus) than that in the standard diet (9 g/kg calcium and 7 g/kg phosphorus). This lower calcium content may have contributed to the outcome because we have shown that oligofructose was more effective in the presence of a higher content of dietary calcium (10 g/kg) than of a lower content (5 g/kg).

In summary, the effect of inulin-type fructans on calcium absorption may be smaller when the background diet or habitual diet already contains considerable amounts of inulin-type fructans or other fermentable fibers or when its content of calcium is low. Another aspect is the age and or physiological stage of the animals: Inulin-type fructans may be more effective when animals have a higher demand for calcium as in the rapidly growing age range (4–10 wk) or in estrogen deficiency. These observations should be taken into consideration in designing experiments and interpreting results. Even a low stimulating effect on calcium absorption of inulin-like fructans may result in a relevant accretion of BMC in the long term, provided that the effect persists. The persistence of the effect was repeatedly found for oligofructose (16,17,32,35), for oligofructose in the presence of antibiotics (27), for a mixture of oligofructose/acacia gum in the presence of probiotic lactic acid bacteria (37,38), for inulin (18,30,32,33), and for the mixture of inulin and oligofructose (Synergy) (13,34).

Phosphorus. In practical nutrition, phosphorus intake is abundant, whereas calcium intake is regarded as too low. Therefore, considerations of bone health frequently focus on dietary calcium. However, phosphorus should not be neglected because bone mineral consists of hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ and thus contains both elements, with a calcium-to-phosphorus ratio of 1.67 (mol:mol), corresponding to 2.15 (wt:wt), which is slightly lower in biological than in stoichiometric hydroxyapatite (39).

The Ca:P ratio of the diet and the content of calcium and phosphorus in absolute terms both affect the calcium and bone metabolism (40,41). This was demonstrated in a trial with vitamin D receptor-knockout mice. A ratio of ≤ 1 was unfavorable for bone compared with a ratio of 2 (40). However, when the Ca:P ratio of 2 was maintained, a diet with 5 g phosphorus/kg diet had a more favorable effect on weight gain, plasma parathyroid hormone (PTH), and BMC than a formulation with 2.5 g phosphorus/kg. These results might indicate that an increase of dietary calcium as a result of strategies for osteoporosis prevention should consider adequate intake of not only calcium but also phosphorus. Recent reports underline that 10% to 15% of older women have intakes of <70% of the recommended daily allowance of phosphorus (42). This point should be given more attention because there is an increasing demand and a large market for calcium supplements, and these are mainly carbonate or citrate salts (42). In contrast to calcium phosphate salts or milk, their use may exceed the dietary Ca:P ratio recommended for adults by the national guidelines of Germany, Austria, and Switzerland of 1.4 g/g or 1.1 mol/mol (43).

It is therefore of interest how inulin-type fructans act on phosphorus absorption and retention. In contrast to their effect on calcium and magnesium metabolism, that on phosphorus metabolism is less clear (see summarized results in **Table 2**). The present evidence is based on studies that were performed under varying experimental conditions and in different animal species, and no clear picture can be obtained at present. For example, in dogs, phosphorus absorption was not affected by 10 g/kg oligofructose over 3 wk (22). In growing pigs, inulin had no effect on phosphorus absorption but reduced urinary phosphorus excretion (44). In rats, we observed for most dietary conditions no significant effect on phosphorus absorption or retention. However, at the recommended amount of calcium (5 g/kg), we found a significantly higher phosphorus retention at the medium dose of oligofructose (50 g/kg) after 8 wk and at the high dose of oligofructose (100 g/kg) after 4 wk (Table 2 and Fig. 2). This effect was mediated by a reduction of urinary phosphorus excretion. In a short-term trial with 5-wk-old rats over 3 wk, there was a decrease of phosphorus absorption, a significant increase of phosphorus retention as a percentage of absorption, but no effect on mg/d phosphorus retention with increasing content of dietary oligofructose (9).

There was a significant stimulating effect of inulin-type fructans on the phosphorus content of bone in several studies (16,23,35,45) but not in all (21) (Table 3). Takahara et al. (16) used young male rats in a short-term experiment of 2 wk. The incorporation of phosphorus into the femur was stimulated by 50 g/kg oligofructose to a ratio of 5–6%, which was similar to the ratio for calcium but much lower than that for magnesium of 20–40%. The stimulation of the absorption of calcium was 25%, and that of magnesium was 27%. Phosphorus balance was not measured. The bone volume and trabecular volume were also increased. Results by Hirama et al. (45) were obtained in 5-wk-old gastrectomized male rats that received diets with 7.5% oligofructose. A higher phosphorus content in the surface of cortical bone at the femoral neck after oligofructose was observed only in intact animals, not in gastrectomized rats. The increasing effect was significant only at the endosteal site, and there was no significance at the periosteal or middle sites.

In ovariectomized adult rats, we observed that 25 and 50 g oligofructose/kg of a purified diet that contained 5 g/kg calcium

TABLE 2 Effect of inulin-type fructans on phosphorus balance in animal models

Phosphorus	Effect	Animals	Age (*wk*) or body weight [*kg*] at start	*n*	Duration, *wk*	Dietary Ca, *g/kg*	Dietary P, *g/kg*	Dietary ITF, *g/kg*	Type of ITF[1]	Literature cited
Absorption	ns	Dogs	36–132	5	3	21	13	10	OF	22
	ns	Growing pigs	[30 kg]	6	2	6.1	7.5	50	IN	44
	ns	ovx rats	20	7–14	4; 8; 16	5	5	25; 50; 100	OF	35
	ns			7–14	8; 16	10		50		35
	↓	ovx rats	20	12–14	4	10		50		35
	↓	Growing rats	5	10	3	6	3.5	10, 30, 50	OF	9
Urinary	ns	ovx rats	20	7	16	5	5	25, 50, 100	OF	35
	ns			7	16	10	5	50		35
	↓	Growing pigs	[30 kg]	6	2	6	7.5	50	IN	44
	↓	ovx rats	20	12–14	4; 8;	5	5	100	OF	35
	↓			12–14	4; 8	10		50		35
Retention	ns	ovx rats	20	12–14	4	5	5	25; 50;	OF	35
	ns			12–14	8			25; 100		35
	ns			7	16			25; 50; 100		35
	ns			7–14	4; 8; 16	10		50		35
	ns	Growing rats	5	10	3	6	3.5	10, 30, 50	OF	9
	↑	ovx rats	20	12–14	4	5	5	100	OF	35
	↑			12–14	8			50		35

[1] IN, inulin; OF, oligofructose.

and 5 g/kg phosphorus had no effect on phosphorus content in the femora (**Tables 3** and **4**) and lumbar vertebrae (**Tables** 3 and 5) after 8 wk and 16 wk ($n = 7$ each), except in the femora when 100 g/kg oligofructose was given. However, when all animals of the short term and the long term were pooled in a multifactorial ANOVA with a correction for age, there was a significant cumulative effect in the lumbar vertebrae and femora (**Fig. 3**). In the presence of a diet with high dietary calcium (10 g/kg), the content of phosphorus in the lumbar vertebrae was significantly lower, whereas the phosphorus content in the femora was slightly higher in rats fed oligofructose (50 g/kg). In the presence of a diet with recommended dietary calcium (5 g/kg), the content of phosphorus in the lumbar vertebrae was slightly higher, and that in the femora significantly higher in rats fed 100 g/kg oligofructose. Ohta et al. (21) found no alteration of the phosphorus content of the femur by 50 g/kg oligofructose in young intact and ovariectomized mice being fed a diet that contained 5 g/kg calcium and 3 g/kg phosphorus. In this case the phosphorus content may have been too low to enable a further improvement of phosphorus absorption.

In summary, the diversity of outcomes with respect to phosphorus metabolism in rats fed diets with inulin-type fructans indicates a less conclusive situation than in calcium metabolism. This may be explained with the different absorption mechanisms. The homeostasis of calcium is tightly controlled, and the regulation occurs at the gut level. Calcium that is not required by the body will hardly be absorbed. The homeostasis of phosphorus is less strictly controlled. Dietary phosphorus is absorbed to a high proportion from inorganic salts and less effectively absorbed if it is part of a diet based on whole grain, which contains phytate. The homeostasis of phosphorus is mainly regulated by the kidney, which allows a wide variation of urinary phosphorus excretion. The absolute amounts of soluble calcium and phosphorus in the gut lumen depend on the amounts ingested but also on their ratio in the diet. Because calcium homeostasis already is regulated at the gut level, a large increase of the calcium content in the diet slightly raises the amount of absorbed calcium but markedly reduces the absorbed proportion. Consequently, the luminal concentration of calcium increases, and insoluble calcium phosphates will be formed. Subsequently the phosphorus absorption will decrease. Up to a certain amount the reduction of the urinary excretion can compensate for this, and the phosphorus retention will not be strongly affected. However, at conditions with less balanced Ca:P ratios of the diet, an adequate rise in the phosphorus content with the diet should be considered when dietary calcium is going to be raised (40,46,47).

BMC, BMD, trabecular structure

The numeric outcome of the effects of inulin-type fructans on mineral absorption and retention and on BMC depends on experimental conditions such as the background diet and its content of minerals or fructans. Moreover the animals' age, sex, or hormonal status is of importance. Balance studies only give a view on calcium metabolism through a short time slot, whereas the analysis of mineral accretion in the skeleton, bone density, and architecture are results of long-term impact. Apart from the duration of the experiment, the analytical approach to assess and the skeletal site to detect bone mineral or structure will affect the outcome. Effects on mineral balance can be shown under optimum conditions after 2–3 wk. However, with respect to bone health, only bone-related parameters are of interest, i.e., BMC or BMD or structure. To be sure that this is a persisting effect, rat experiments should last at least 3–4 mo, and repeated measurements, preferably within the same animal (monitoring of bone mineral) should be performed. The picture might become more complicated when interactions of these factors exist, e.g., the duration had a different impact on the effect of inulin-type fructans on the appendicular compared with the axial skeleton (see Tables 4 and 5).

To improve the BMC, a small but persistent stimulating effect on calcium retention might be more important than a large short-term but vanishing effect. For example, in adult ovariectomized rats, the stimulation of calcium retention by 100 g/kg oligofructose in the presence of 5g/kg calcium was only significant ($P < 0.05$) in 1 of 3 balances (after 8 wk), and retention was only slightly increased at the other times (after 4 or

TABLE 3 Effect of inulin-type fructans on phosphorus content in bone of animal models

Phosphorus	Effect	Animals	Age (*wk*) or body weight [*kg*] at start	*n*	Duration, *wk*	Dietary Ca, *g/kg*	Dietary P, *g/kg*	Dietary ITF, *g/kg*	Type of ITF[1]	Literature cited
Femur Neck	ns	Male rats	6	8	2	5	3	50	OF	16
Diaphysis	↑									16
Metaphyis	↑									16
Femur Endosteal site	↑	Male rats	5	7	4	5	Not given	75	OF	45
Periosteal site	ns									45
Femur	ns	Intact mice	8	8	6	5	3	5	OF	21
	ns	ovx mice								21
Femur	ns	ovx rats	20	7	8	5	5	25; 50; 100	OF	35
	ns					10		50		35
	ns	ovx rats	20	7	16	5	5	25; 50		35
	ns					10		50		35
	↑	ovx rats	20	7	16	5	5	100		35
Lumbar vertebra	ns	ovx rats	20	7	8, 16	5	5	25; 50; 100	OF	35
	ns					10		50		35
	↓		20	14	8, 16[2]	10	5	50		35
Tibia	↑	Laying hens	57	20	4			10	OF	23
	↑							10	IN	23

[1] IN = inulin, OF = oligofructose.
[2] Significance obtained from MANOVA after correcting for age.

TABLE 4 Effect of diets containing different concentrations of oligofructose on length, weight, and content of calcium and phosphorus of femora in ovariectomized rats after 8 and 16 wk

Femur	G1 S[1] 5;0[2]	G2 5;0[2]	G3 5;25[2]	G4 5;50[2]	G5 5;100[2]	G6 10;0[2]	G7 10;50[2]
After 8 wk, *n*	6	7	7	7	7	7	7
Length, *mm*	28.72 ± 0.19	29.04 ± 0.09	28.86 ± 0.11	28.87 ± 0.29	28.86 ± 0.16	28.73 ± 0.17	29.21 ± 0.15
Weight, *mg*	439 ± 3.94	443 ± 5.71	436 ± 11.38	441 ± 9.89	430 ± 10.98	428 ± 7.80	469 ± 5.19*#§
Ca, *mg*	89.12 ± 1.61	87.42 ± 1.30	89.48 ± 2.66	89.85 ± 1.82	91.28 ± 2.01	86.53 ± 2.59	96.81 ± 1.59*#§
P, *mg*	41.31 ± 0.66	40.42 ± 0.63	40.73 ± 1.32	41.97 ± 0.94	42.12 ± 0.98	40.49 ± 1.21	42.85 ± 1.43
After 16 wk, *n*	6	7	7	7	7	7	7
Length, *mm*	28.82 ± 0.25	29.17 ± 0.19	28.99 ± 0.19	28.80 ± 0.18	28.81 ± 0.12	29.17 ± 0.09	28.74 ± 0.17
Weight,$ *mg*	475 ± 18.87	460 ± 35.05	468 ± 13.45	446 ± 28.34	501 ± 19.99‡	472 ± 30.76	451 ± 43.90
Ca,$ *mg*	88.05 ± 1.85	80.86 ± 4.44	86.33 ± 1.35	82.55 ± 2.99‡	89.51 ± 2.09	83.89 ± 2.68	85.47 ± 3.78‡
P,$ *mg*	37.86 ± 0.88‡	34.49 ± 1.48‡	36.32 ± 0.78‡	35.85 ± 1.42‡	38.61 ± 0.40*‡	36.77 ± 1.20‡	36.71 ± 2.00‡

ANOVA, values are least square means ± SEM.

[1] Sham-operated.

[2] Ca g/kg feed; oligofructose g/kg feed.

Symbols indicate significant differences with *P* at least <0.05 as follows: *Different from G2; #different from G4; §different from G6; $different from 8 wk as a global effect over all groups; ‡different from 8 wk.

16 wk). Despite this, the femur calcium content as a cumulative effect measured in 16 animals was significantly higher ($P < 0.05$), reaching 90.4 ± 1.41 mg, compared with the control group with 84.1 ± 2.14 (35). This was not the case for a lower dose of oligofructose. In contrast, the calcium content in the lumbar vertebrae did not increase, showing that the skeletal sites with more weight-bearing load (in quadrupeds this is the appendicular skeleton) might be favored with respect to an adequate supply of calcium. The mineralization of the lumbar vertebrae was increased only when the calcium supply was raised up to 10 g/kg in the presence of oligofructose (50 g/kg) (35). Similar results have been reported by others in rapidly growing Sprague Dawley rats with a body weight of 100 g, namely a comparatively small stimulation of calcium absorption or retention but a significant cumulative effect on femur calcium content after 15 d (13).

In intact and ovariectomized mice, inulin-type fructans alone had no significant effect on the bone ash and the content of single minerals (calcium, magnesium, phosphorus) in the femora. In contrast to assessing bone mineral by ashing, the analysis of BMD at different sites of the femur by dual-energy x-ray absorptiometry (DEXA) was more sensitive to detect higher mineral content in mice that were fed a diet with inulin-type fructans. BMD of the total femur and of its proximal and distal regions were significantly higher than in the control group. This was not the case for BMD at the midshaft (21). These results indicate a preventive effect of inulin-type fructans on estrogen-deficiency-induced loss of mainly trabecular bone but not of cortical bone (21). They also confirm our results in ovariectomized rats (35), namely that the analysis of BMC of total bone is less sensitive than the analysis of trabecular bone, e.g., by microradiography, to detect effects of inulin-type fructans on bone.

Because of its higher turnover activity, trabecular bone is more reactive than cortical bone to external factors such as hormonal or dietary intervention, especially in older animals. This might explain that the lowest amount of oligofructose that we tested (25 g/kg) significantly repressed the ovariectomy-induced loss of trabecular bone as assessed by histomorphometry, and effects of ovariectomy and inulin-type fructans on total

TABLE 5 Effect of diets containing different concentrations of oligofructose on weight and content of calcium and phosphorus of lumbar vertebrae in ovariectomized rats

Lumbar vertebrae	G1 S[1] 5;0[2]	G2 5;0[2]	G3 5;25[2]	G4 5;50[2]	G5 5;100[2]	G6 10;0[2]	G7 10;50[2]
After 8 wk, *n*	6	7	7	7	7	7	7
Weight, *mg*	271 ± 7.71	264 ± 10.51	251 ± 6.73	286 ± 11.68	261 ± 12.59	256 ± 5.30	290 ± 10.66§
Ca, *mg*	41.69 ± 1.37	40.98 ± 0.96	38.95 ± 1.27	42.06 ± 0.93	41.98 ± 1.41	39.29 ± 1.24	42.86 ± 1.2§
P, *mg*	21.13 ± 0.38	21.82 ± 0.76	21.70 ± 1.07	21.47 ± 0.54	18.76 ± 1.51	19.67 ± 0.46*	18.72 ± 0.38*#
After 16 wk, *n*	6	7	7	7	7	7	7
Weight,$ *mg*	247 ± 10.94	253 ± 7.99	234 ± 7.73	252 ± 12.31‡	234 ± 15.51‡	225 ± 7.82*‡	260 ± 10.12§,‡
Ca, *mg*	42.33 ± 0.82	42.22 ± 1.24	40.29 ± 1.06	40.75 ± 0.96	41.81 ± 1.39	41.80 ± 1.54	45.14 ± 0.74#
P, *mg*	20.82 ± 0.59	20.59 ± 0.86	19.92 ± 0.93	20.06 ± 0.76	24.38 ± 2.77	21.38 ± 1.10	19.00 ± 0.72

ANOVA, values are least square means ± SEM.

[1] Sham-operated.

[2] Ca g/kg feed; oligofructose g/kg feed.

Symbols indicate significant differences with $P < 0.05$ as follows: *Different from G2; #different from G4; §different from G6; $different from 8 wk as a global effect over all groups; ‡different from 8 wk.

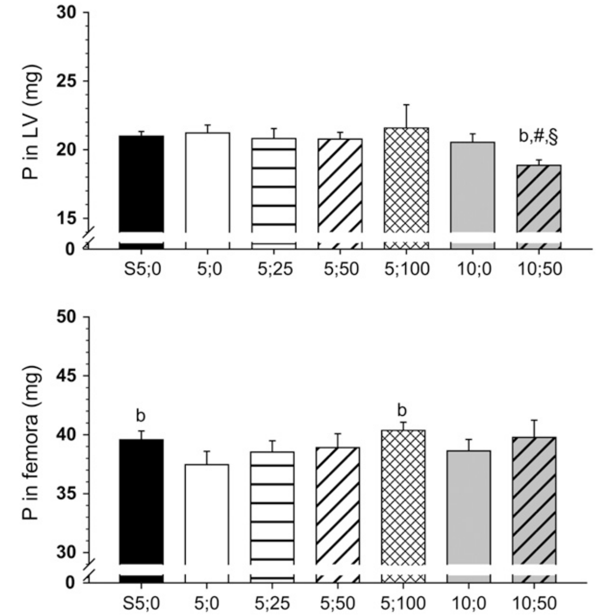

FIGURE 3 Cumulative phosphorus content in the lumbar vertebrae (*top*) and in the femora (*bottom*) of ovariectomized adult rats after 8 and 16 wk (pooled values from MANOVA after correcting for age) on diets with varying contents of calcium (5 and 10 g/kg diet) and oligofructose (25, 50, and 100 g/kg diet) compared with the ovariectomized control group (5 g calcium and 0 g oligofructose/kg diet). S, sham operated animals. Mean and SEM, $n = 12$–14. Means of a group are significantly different ($P < 0.05$) as follows: different from ovariectomized controls = *b*; from rats on 5 g calcium and 50 g oligofructose/kg diet (#); from rats on 10 g calcium and 0 g oligofructose (§) (K. E. Scholz-Ahrens and J. Schrezenmeir, unpublished observation).

bone ash, which includes the cortical bone, failed to reach significance (35). Thus, to detect effects of inulin-type fructans on the mineral content by chemical analysis of the whole bone, more animals are required. In addition, a longer period of time is probably necessary to find cumulative effects by analysis of whole-BMC.

Results by Hirama et al. (45) support this assumption. In 5-wk-old gastrectomized male rats that received diets with 7.5 g/kg oligofructose, they observed higher trabecular bone area in rats that received oligofructose when semiquantitative visualization was performed, but there was no significant quantitative effect on the content of calcium and magnesium of the cortical bone.

In growing rats ($n = 10$–12), inulin in the diet most significantly increased whole-body bone mineral density measured by DEXA but less significantly when whole-body bone mineral content was analyzed (33). The effect of inulin was significant with the introduction of a 50 g/kg diet, but no further effect was achieved when 100 g/kg was given. Whole-body bone area, a measure of bone size, was lower at 100 g/kg inulin compared with 50 g/kg. This effect was explained by the lower body weights that were achieved presumably as a consequence of the lower energy content of inulin compared with digestible carbohydrates.

In a trial with 4-wk-old male rats with ad libitum feeding over 23 d, 50 g oligofructose/kg as part of a purified diet was used. No effect on BMD by DEXA in the femur or tibia was seen, but the authors reported an increased calcium concentration in the femur and tibia per gram dry weight and a higher bone strength as assessed by 3-point bending test (17). The intervention time may have been too short to detect a significant stimulation of BMD. These data indicate again that increments in mineral retention, even if they are small, may contribute to a more stable trabecular network or locally higher mineralization of bone with small effects on BMD but large effects on bone stability.

Impact of polymerization degree of inulin-type fructans

From several trials in animal models it became obvious that the different inulin-type fructans may differ in their potential to stimulate mineral absorption or mineral accretion in the skeleton (see above). This potential may depend on the DP because it is assumed that oligofructose with shorter DP will be fermented more rapidly in the upper large intestine, whereas inulin will be fermented predominantly in the lower part, and a mixture of short-chain and long-chain inulin (Synergy) will be fermented all along the whole gut and thus more effectively.

Younes et al. (20) observed a stimulation of calcium absorption in adult rats by inulin, resistant starch, and inulin plus resistant starch compared with a control diet with wheat starch and 7.5 g/kg calcium, but most significantly if inulin was given together with resistant starch. Because the doses per kilogram diet differed (100 g inulin; 150 g resistant starch, and 50 g inulin plus 75 g resistant starch), it is difficult to attribute the effect exclusively to the substance.

When inulin-type fructans with different chain lengths and types of branching were introduced into a diet progressively at a dose of 25, 50, and 100 g/kg over 4 wk, a significant effect on calcium and magnesium retention solely was observed with the mixture of short-chain and long-chain inulin-type fructans (Synergy) in growing rats (19). Branched-chain fructans and oligofructose had a significant effect on magnesium retention but not on calcium retention.

In 7-wk-old growing rats, a comparison was made with 50 g inulin or oligofructose/kg diet, or a combination of both (92% inulin plus 8% oligofructose) as part of a purified diet in the presence of 5 g/kg calcium (30). After 4 wk a significant increase of femoral BMD or spinal BMC was observed with inulin but not with oligofructose or the combination of both. Collagen cross links, a marker for bone resorption, were depressed in all groups but most marked in the group fed inulin. The group that consumed the combination of inulin plus oligofructose had the lowest values for mineral accretion. This is presumably the consequence of the least amount of calcium retained as indicated by the 3-day calcium balance, although this was not significant. The feed intake and the body weight gain were the lowest, and the fecal weight was the highest, among the 3 different fructans, although not significantly different among groups either. If the initial body weight was not different among the groups, it could be suggested from this short-term experiment that inulin plus oligofructose may have caused the highest luminal osmolarity, which may have increased the luminal and finally the fecal amount of fluid and nonabsorbed nutrients.

Recently it was shown that inulin and oligofructose increased the cecal wall weight in a similar magnitude. However, the stimulation of cancellous BMD in the tibia and lumbar vertebrae was more pronounced, and that of cortical bone became significant only with inulin (32). The greater beneficial effect on bone can be explained by a higher calcium absorption as indicated by a higher expression of the calcium binding protein CaBP-9kDa in the gut and by a lower bone resorption as indicated by a lower excretion of a bone resorption marker compared with oligofructose (32). Butyrate is a potent stimulator of CaBP-9kDa expression and thus of the active calcium absorption pathway mediated via the increases in $1{,}25(OH)_2D_3$ receptor binding activity (48). Because butyrate is the major fermentation product of inulin, whereas mostly acetate and lactate are produced on short-chain inulin-type fructans (49), these findings can explain the advantage of inulin over oligofructose with respect to bone mineralization via this mechanism.

In summary, these data support the hypothesis that an expanded site and a prolonged time of fermentation in the cecum and colon may favor the stimulation of calcium absorption. It was shown in a gut simulation model and discussed that inulin compared with short-chain inulin-type fructans such as oligofructose revealed a higher prebiotic effect with respect to fermentation activity and bacterial composition, i.e., a higher short-chain fatty acid production, a lower proteolytic activity, and a slower but more prolonged fermentation rate (50). This characteristic might be less important for the stimulation of magnesium and iron absorption because it was shown that a stronger recovery from anemia was revealed by short-chain oligofructose than by inulin (10).

Prebiotics in the presence of probiotics: synbiotics

Little information exists on the influence of prebiotics in the presence of probiotics, so-called synbiotics, on mineral and bone metabolism. These studies have been reviewed recently (51). We have observed a significantly higher content of bone ash in the lumbar vertebrae, when a lactobacillus strain was combined with a prebiotic mixture of oligofructose and acacia gum. The effect was not significant when the prebiotic or the probiotic was given on its own (37). This observation indicates that there might be a higher potential to improve mineral accretion in the skeleton if an optimally combined synbiotic is applied. This field opens a wide area of options; however, each specific combination has to be tested to prove a beneficial effect for bone health.

Prebiotics in the presence of antibiotics

We performed a long-term experiment with ovariectomized adult rats, which were fed semisynthetic diets and oligofructose in the presence or absence of antibiotics. This is of interest because the preventive use of antibiotics in livestock production to protect gut health and prevent diarrhea was banned in Europe in 2006 (52) because of the risk of bacterial resistance (53). Probiotics and/or prebiotics may offer an alternative to the preventive use of antibiotics and may help to overcome side effects of the use of antibiotics, such as luminal alkalization and diarrhea as discussed by Brunse et al. (54) and thus impairment of gut health. After 4 mo on a semisynthetic diet containing antibiotics, there was a significant rise of the pH in the cecal chymus in ovariectomized rats. The increase of pH was associated with a low content of ash in the femur. In the presence of oligofructose in the diet, the pH was reduced to the same degree either with or without antibiotics. This effect was associated with significantly higher ash content in the femur and trabecular bone area in the tibia compared with animals on antibiotic treatment without oligofructose (51).

Mechanism

Inulin-type fructans improve BMC by stimulating mineral absorption. This seems to be mediated by the microbial production of short-chain fatty acids in the large intestine, mainly in the cecum, which reduce the luminal pH and consequently increase the soluble fraction of minerals. Furthermore, the increase of cecum weight and of villus height indicate an increase in the absorptive surface, which might be mediated predominantly by butyrate (55). This enhanced mineral transport is also supplied by a pronounced osmotic effect, as discussed by Bongers and van den Heuvel (56). Oligofructose in particular enhance solubility of minerals and consequently increase the paracellular transport but also stimulate the transcellular exchange of protons, when intracellular H^+ is exchanged for luminal Ca^{2+}. It was shown by Lutz and Scharrer (57) that this proton exchange was stimulated by butyrate in the distal rat colon in vivo. A stimulated active calcium transport was also described by others (58) and discussed before (26–28,56).

More recently other factors have been described that presumably contribute to the positive effect of inulin-type fructans on BMC, BMD, and bone structure. These have been reviewed recently by Scholz-Ahrens et al. (51) and are summarized in **Table 6**. Some of the effects are gut-associated factors such as changes in gut morphology and mucus production and composition (59) and stimulation of the gut-associated immune defense (60). These might be regarded as indicators for a "healthier" gut, which is the requirement for an optimum mineral absorption. Together with an enlarged absorption surface, a healthier gut will result in a higher absorption capacity. Whether the increased production of mucins after ingestion of inulin-type fructans is a marker of a more stabilized mucosal barrier (59) or is an indication of mucosal irritation (61) is under ongoing discussion (62,63). It should be considered, however, that the effects reported (61) were dependent on the Ca content of the diet and that in those experiments rats received a diet with very low calcium content.

At least some of the effects of inulin-type fructans are proven to be mediated through the alteration of the microbial flora because the modulation of the mucus production was linked to the presence of innate flora and could not be observed in germ-free animals (59). As a substrate for certain probiotic bacteria, it can be assumed that inulin-type fructans contribute to the probiotic stimulation of calcium uptake by enterocytes (64,65) and to the degradation of mineral-complexing phytic acid by means of phytase (66) in probiotic strains.

The stabilization of the microbial flora by oligofructose may have contributed to the prevention of the loss of BMC and trabecular structure in ovariectomized adult rats on semisynthetic

TABLE 6 More recent findings on the mechanism of effects on mineral metabolism and bone mineral content of inulin-type fructans

Observation	Literature cited
Amelioration of gut health	Kleessen et al., 2003 (59)
Stimulation of immune defense	Roller et al., 2004 (60)
Increased expression of Calbindin-D9k, vitamin D-receptor, caudal-related homeobox transcription factor, osteoprotegerin, other nondefined proteins in the gut	Ariyasu et al., 2002 (67); Fukushima et al., 2005 (68); Ohta et al., 1998 (58); Raschka and Daniel 2005 (69)
Stabilization of the intestinal flora improved BMC in the presence of antibiotics	Brunser et al., 2006 (54) (infants); Scholz-Ahrens and Schrezenmeir, 2002 (27), Scholz-Ahrens and Schrezenmeir 2007 (51)
Release of bone modulating factors (phytoestrogens)	Mathey et al., 2004 (70); Ohta et al., 2002 (21)
Suppression of bone resorption over bone formation	Zafar et al., 2004 (34)
Probiotic degradation of mineral complexing phytic acid	Lan et al., 2002 (66)
Probiotic stimulation of calcium uptake by enterocytes	Gilman and Cashman, 2006 (64); Narva et al., 2004 (65)

diets (27) and in such rats on semisynthetic diets together with antibiotics (51).

The increased expression of a variety of cytokines that are known to be involved, or gene transcripts in mucosal cells that are assumed to play a role in the transcellular and paracellular calcium transport process, have been reported to be affected by inulin-type fructans or by trehalose (58,67–69). Moreover, oligofructose enhanced the protective effects of dietary soy isoflavone on bone resorption in ovariectomized rats, and it is assumed that inulin-type fructans increase the release of bone-modulating factors by enhancement of the isoflavone bioavailability (21,70).

It was shown that inulin-type fructans restrain bone resorption in rats (31,34) and by this contribute to a higher mineral content of bone. The factors mediating this are not known. This might be an indirect effect mediated through the additionally absorbed calcium.

In summary, prebiotic nondigestible oligosaccharides (NDO) are fermented by the microbiota of the host with a stimulation of growth and or activity of certain intestinal bacteria, preferentially bifidobacteria and lactobacilli. Prebiotic NDO increased the absorption of several minerals (calcium, magnesium, in some cases phosphorus) and trace elements (mainly copper, iron, zinc). Inulin-type fructans including oligofructose derived from inulin by hydrolysis, and from sucrose by enzymatic transfructosylation, are the substances that are best investigated. The stimulation of absorption seems to be more pronounced when the demand for calcium is high, i.e., in intact animals in the rapid growing stage and in animals with impaired calcium absorption as a result of ovariectomy or gastrectomy. Even when the stimulation of calcium absorption was small, there was a significantly higher mineral accumulation in the skeleton if the intervention was long enough because the stimulating effect was persistent over several months. Inulin-type fructans stimulated mineral absorption and bone mineral accretion when combined with probiotic lactobacilli and in the presence of antibiotics. Direct comparison of substances has shown that a more pronounced effect was achieved when the prebiotic NDO contained not only short-chain inulin-type fructans. Long-chain inulin or a mixture of inulin and oligofructose were the most effective in rats in the majority of cases. A mechanism by which inulin-type fructans mediate this effect is proposed that includes direct effects such as acidification of the intestinal lumen by short-chain fatty acids, a rise in soluble minerals, and the enlargement of the absorption surface. Indirect effects are discussed as well, including stabilization of the intestinal flora, stimulation of immune defense, amelioration of gut health, increased expression of bone-relevant cytokines, suppression of bone resorption, increase of the bioavailability of phytoestrogens, and, finally, via probiotics, the stimulation of calcium uptake by enterocytes.

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