

Inulin, oligofructose and mineral metabolism — experimental data and mechanism

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Numerous investigations performed in animal models in the past 10 years have shown repeatedly that non-digestible oligosaccharides (NDO), such as inulin, oligofructose or transgalacto-oligosaccharides (TOS), stimulate mineral absorption, mainly calcium and magnesium. Long-term beneficial effects on bone health have been indicated by accumulation of bone mineral content in growing rats or prevention of bone loss in ovariectomized rats. However, bone mineral content or density are not necessarily associated with bone quality. In recent studies both oligofructose and calcium prevented loss of trabecular bone area induced by oestrogen deficiency, this, however, occurred at different trabecular shapes. The effects of NDO on mineral metabolism may be based on the enhancement of passive and active mineral transport across the intestinal epithelium, mediated by an increase in certain metabolites of the intestinal flora and a reduction of pH. The possible impact of short-chain fatty acids, butyrate in particular, and of polyamines on the stimulation of mineral absorption capacity, and the interaction of oligofructose and antibiotics is discussed.

Prebiotics: Oligofructose: Mineral metabolism: Bone quality

Introduction

Dietary habits have changed in the past 50 years in westernized societies (Ernährungsbericht, 2000) including higher consumption of proteins, fats, sucrose, and sodium chloride, and lower intake of cereals and complex carbohydrates including nondigestible, bulking dietary fibres and non-digestible but fermentable carbohydrates (NDC) like inulin and oligofructose. Since wheat provides 70% of inulin and oligofructose in American diets (Moshfegh *et al.* 1999), it is assumed that the decreased intake of cereals lowered intake of NDC. Some 'modern' diseases, such as coronary heart disease, some cancers or osteoporosis are thought to be associated at least in part with these changes in dietary patterns (Fraser, 1999).

Nondigestible oligosaccharides (NDO), such as inulin, oligofructose, galacto-oligosaccharides (GOS), transgalacto-oligosaccharides (TOS), and soybean-oligosaccharides, or lactulose, but also certain resistant starches, sugar alcohols, fermentable fibre, and gums like guar gum hydrolysate (GGH), attracted attention in the past decade for their physiological and health promoting properties and

thus for their potential to represent candidates for functional food ingredients (Fooks *et al.* 1999). It was observed that NDO had specific effects on gut physiology. Changes herein were reported to be associated with benefits for the host, such as improved gastrointestinal well-being associated with changes in the intestinal flora (van Loo *et al.* 1999). Although fibre substances like pectin or cellulose also pass through the small intestine undigested, there are several differences in physical properties like dispersibility, viscosity, and adsorbing capacity compared to inulin and oligofructose (Schneeman, 1999). These physical differences presumably explain the variation of physiological effects and why different NDO, fibre substances, and sugar alcohols affect mineral metabolism differently. Lactulose for example, increased calcium absorption from 26% to 37% in rats as did other sugars whose digestion was limited, like L- and D-arabinose, raffinose or sugar alcohols like xylitol (Brommage *et al.* 1993). In animal experiments it was shown that inulin and oligofructose improved mineral absorption (for review see Scholz-Ahrens *et al.* 2001) and that this was associated with the production of short-chain fatty acids (SCFA) and a lower pH in the

Abbreviations: 1,25[OH]₂D₃, calcitriol; GGH, guar gum hydrolysate; GOS, galacto-oligosaccharides; NDC, nondigestible but fermentable carbohydrates; NDO, nondigestible oligosaccharides; ODC, ornithine decarboxylase; OVX, ovariectomy; SCFA, short-chain fatty acids; T.Ar, tissue area; Tb.Ar, trabecular area; Tb.Ar/T.Ar, trabecular area as percentage of tissue area; TBPf, trabecular bone pattern factor; Tb.Pm, trabecular perimeter; TOS, transgalacto-oligosaccharides.

Note: For the definition of the terms inulin and oligofructose please refer to the introductory paper (p. S139) and its footnote.

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Table 1. Documented effects of nondigestible carbohydrates and related compounds on bioavailability of different minerals in rats

Substance	% of Diet	Mineral	Animal model — rat	Method	Effect	Authors
Oligofructose	2.5–20%	Ca	● Growing	● Isolated caecum, colon ● Balances ● DEXA ● Femur Ca ● Bone structure	+++++	Brommage <i>et al.</i> 1993; Bouhnik <i>et al.</i> 1997; Chonan <i>et al.</i> 1995; Delzenne <i>et al.</i> 1995; Godá <i>et al.</i> 1995; Hämmäläinen 1994; Hara <i>et al.</i> 1996; Heijnen <i>et al.</i> 1993; Lopez <i>et al.</i> 2000; Mattila <i>et al.</i> 1998; Ohta <i>et al.</i> 1994, 1995a, 1995b, 1998b, 1998c; Sako <i>et al.</i> 1999; Scholz-Ahrens <i>et al.</i> 1998; Scholz-Ahrens <i>et al.</i> 2001; Schulz <i>et al.</i> 1993; Takahara <i>et al.</i> 2000
TOS			● Mg-deficient		+++	
Lactulose			● Fe-deficient		+	
Resistant starch			● Gastrectomized		+++	
Resistant sugar Xylitol, maltitol GGH			● Ovariectomized		+++++	
Oligofructose	5–10%	P	● Growing	● Balances ● Femur P, Humerus P	+ , 0, –	Hämäläinen, 1994; Heijnen <i>et al.</i> 1993; Mattila <i>et al.</i> 1998; Ohta <i>et al.</i> 1994, 1998a; Scholz-Ahrens <i>et al.</i> 2002
Lactulose			● Mg-deficient		+	
Xylitol			● Gastrectomized			
Oligofructose	5–10%	Mg	● Ovariectomized	● Balances ● Isolated caecum, isolated colon	+++++	Baba <i>et al.</i> 1996; Delzenne <i>et al.</i> 1995; Heijnen <i>et al.</i> 1993; Lopez <i>et al.</i> 2000; Ohta <i>et al.</i> 1994, 1995b; Schulz <i>et al.</i> 1993
TOS			● Growing			
Lactulose			● Mg-deficient			
Resistant starch	10%	Cu Fe Zn	● Fe-deficient	● Balances ● Haematology	+++	Delzenne <i>et al.</i> 1995; Lopez <i>et al.</i> 2000; Ohta <i>et al.</i> 1995b, 1998b
Oligofructose			● Growing		+++	
			● Gastrectomized		++	

Negative (–), no (0), or positive effects with increasing degree of markedness (+, +++, +++++) are indicated. TOS = Transgalacto-oligosaccharides, GGH = Guar gum hydrolysates.

intestinal lumen. Compared to inulin, oligofructose stimulated calcium absorption slightly more effectively, while the effect on magnesium was similar (Delzenne *et al.* 1995). The production of total SCFA was not different but lactate was significantly higher following xylo-oligosaccharides and butyrate and was highest in oligofructose (derived both from inulin and sucrose) fed rats (Campbell *et al.* 1997). Moreover, the dose of NDO and the background dietary calcium used in different studies have their own impact on mineral absorption (Scholz-Ahrens *et al.* 2002).

To what extent observations on mineral balance allow assumptions on bone mineralization or bone quality requires information on the persistence of the stimulating effect of inulin and oligofructose on mineral absorption, and on the relevance of improved calcium absorption with respect to bone mineralization, bone density and bone structure. These aspects are discussed in the following review.

Mineral balance

Most experiments on NDO lasted 3–4 weeks and were done in young growing rats (Table 1). In some cases animals were gastrectomized or they were magnesium or iron deficient. In some experiments mineral disappearance from the isolated or ligated caecum or colon was studied or the positive effect on mineral availability was demonstrated by prevention of anaemia, i.e. haematological parameters or by prevention of mineral deficiency. The doses used varied mostly between 2.5% and 10% in the diet. Minerals that were analysed included calcium, phosphorous, magnesium, iron, copper, and zinc. In young growing rats NDO exerted their most prominent effects on calcium and magnesium availability (Table 1). In aged ovariectomized rats 5% oligofructose added to a diet high in calcium (1%) persistently reduced faecal calcium loss after 4 and 8 weeks. This effect became significant after 16 weeks (Fig. 1). Calcium retention was slightly but persistently higher after 4, 8, and 16 weeks. This effect failed significance because increased absorption was associated with increased urinary calcium. Higher urinary calcium however, reflected metabolic reaction on higher calcium influx from the gut and not calcium mobilization from the skeleton because bone mineral content was higher on oligofructose (Scholz-Ahrens *et al.* 2002). Iron absorption was less effectively stimulated compared to calcium or magnesium but more effectively than zinc (Table 1, Delzenne *et al.* 1995). Phosphorous absorption was not affected (Ohta *et al.* 1994) or was significantly lower on a diet with 1% calcium on oligofructose with 76.3 ± 2.82 [mean (pooled SEM)] compared to the group on maize starch with $87.8 (2.82)$ after 4 weeks (Scholz-Ahrens *et al.* 2002). Since phosphorous excretion via urine was significantly lower as well, phosphorous retention was not affected after 4 and 8 weeks (not shown), and after 16 weeks (Fig. 2).

Bone mineralization

Few experiments were reported, in which the effect of

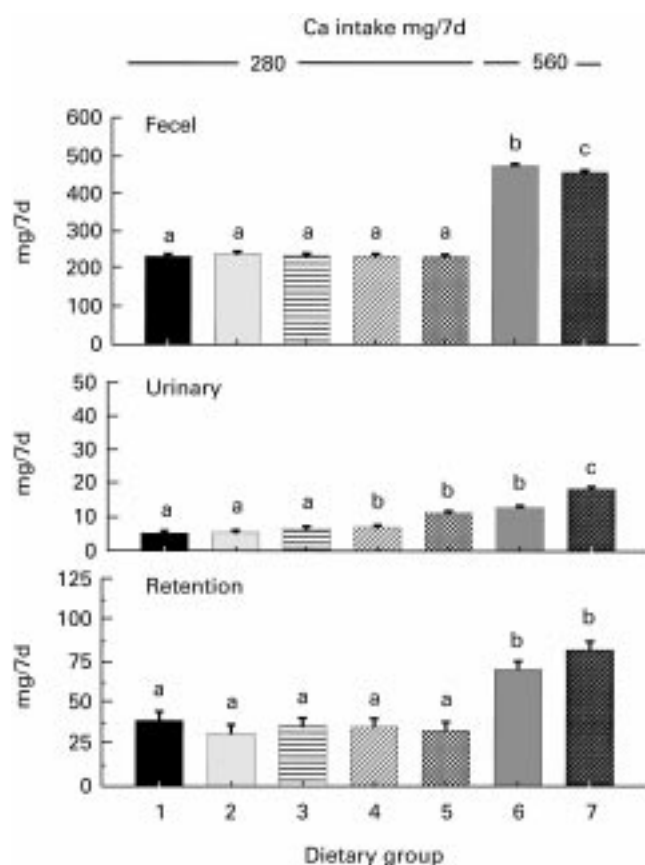


Fig. 1. Effect of oligofructose on 7-day calcium (Ca) balance in ovariectomized rats after 16 weeks on diets containing (g calcium/kg diet) / (g oligofructose/kg diet) as follows: ■, 5/0; □, 5/0; ▨, 5/25; ▩, 5/50; ▪, 5/100; ▫, 10/0; ▬, 10/50. Animals in group 1 were sham operated, animals in groups 2–7 were ovariectomized. Least square means and pooled SEM of MANOVA. a, b, c indicates significant differences between groups with P of at least < 0.05 .

NDO on bone mineral content was investigated (Chonan *et al.* 1995; Ohta *et al.* 1998a; Scholz-Ahrens *et al.* 1998). Ovariectomy (OVX) in the adult or aged rat is an accepted method to simulate human postmenopausal state (Kimmel, 1996). In young (Chonan *et al.* 1995) and adult (Scholz-Ahrens *et al.* 2002) ovariectomized rats galacto-oligosaccharides and oligofructose effectively prevented loss of bone mineral content or density. The preventive effect in the femur was more prominent after 8 weeks than after 16 weeks while in the lumbar vertebra it occurred persistently over 16 weeks but with less magnitude. The addition of 5% oligofructose prevented bone loss significantly in the femur and lumbar vertebra in the presence of high dietary calcium (1%) but not at 0.5% (Fig. 3). At 0.5% calcium 10% oligofructose was needed to significantly increase bone mineralization, and this was only observed in the femur (Scholz-Ahrens *et al.* 2002).

Bone trabecular structure — bone quality

In an experiment by our group, oligofructose was studied for its effect on bone trabecular structure in a long-term study in adult ovariectomized rats. Trabecular structure

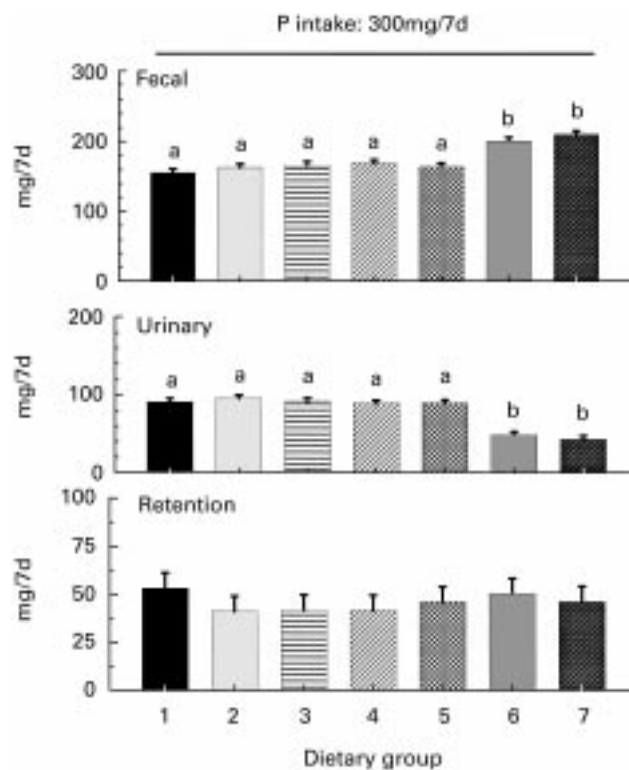


Fig. 2. Effect of oligofructose on 7-day phosphorus (P) balance in ovariectomized rats after 16 weeks on diets containing (g calcium/kg diet) / (g oligofructose/kg diet) as follows: ■, 5/0; □, 5/0; ▨, 5/25; ▩, 5/50; ▪, 5/100; ▫, 10/0; ▬, 10/50. Animals in group 1 were sham operated, animals in groups 2–7 were ovariectomized. Least square means and pooled SEM of MANOVA. a, b, c indicates significant differences between groups with P of at least < 0.05 .

was assessed by computer-supported image analyses of contact microradiographs, a method described in detail elsewhere (Hein, 1997) and applied before to assess bio-availability of calcium from different sources (Scholz-Ahrens *et al.* 1997). Ovariectomy induced loss of tibia trabecular bone, particularly trabecular area (Tb.Ar/T.Ar), and trabecular perimeter (Tb.Pm) was reduced while tissue area (T.Ar) was not affected (Fig. 4a). To prevent ovariectomy-induced bone loss, rats were treated with higher dietary calcium (1.0% v. 0.5%) or by including 5% oligofructose in the diet or by combining both treatments for 8 and 16 weeks. Trabecular structure was affected differently by these treatments: Higher dietary calcium as well as including oligofructose preserved the trabecular area almost to the same extent (Fig. 4b). Higher dietary calcium, however, conserved fewer but thicker trabecules, while oligofructose conserved more trabecules compared to dietary calcium. This was also indicated by the higher values for trabecular perimeter after oligofructose, compared to the control group, 2 (Fig. 4b). The combined intervention, i.e. including 5% oligofructose into a diet containing 1% calcium, preserved a larger Tb.Ar/T.Ar at almost the same number but larger trabecular perimeter (Tb.Pm), thus indicating longer trabecules. Moreover, cortical thickness (C.Th) was significantly higher (Fig. 4c). Based on the bone structure analysis we demonstrated different bone quality following varying diets

(Fig. 4). It may be speculated that a certain bone structure represents bone with more or less elasticity and break force resistance. From civil engineering it is known that a construction made of many thin beams is more stable than one made of a few thick ones. Therefore a certain trabecular architecture may reflect a surrogate for a lower fracture risk. Further data, however, are needed to define an architecture that is advantageous for bone health.

Mechanism

In the first place an enhancement of the passive calcium transport by NDO was favoured and postulated by several authors (Ohta *et al.* 1993; Levrat *et al.* 1991; Chonan *et al.* 1995), because the higher calcium solubility in the large intestine and its absorption was associated with a lower luminal pH, and a selective stimulation of bacterial growth *in vitro* and in the intestinal lumen, mainly bifidobacteria and lactobacilli (Wang & Gibson,1993; Rowland & Tanaka, 1993; Gibson & Roberfroid, 1995). At fermentation of inulin and oligofructose by the microflora the reduction of pH occurs as the result of stimulated production of lactic acid and short-chain fatty acids, mainly propionate and acetate, and at a lower level but at a higher rate, butyrate (Levrat *et al.* 1991). At lower pH more mineral is soluble in the gut lumen and thus is more readily absorbed from the gut mucosa cell (Ohta *et al.* 1995a). Apart from stimulating the passive calcium absorption indirectly by increasing its solubility via lowering the pH (Table 2), SCFA directly stimulated calcium disappearance across the colon in humans more effectively than a solution containing calcium + NaCl, although the pH of the latter was lower (Trinidad *et al.* 1996). Calcium propionate stimulated calcium uptake more effectively than calcium acetate, although the pH of the infusion solutions were not different. It was discussed that a more rapid uptake of propionate compared to acetate was due to its greater lipid-solubility, which is associated with chain length (Trinidad *et al.* 1996).

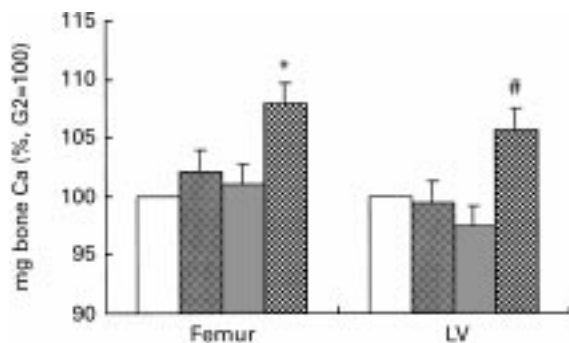


Fig. 3. Effect of oligofructose on calcium content (mg) of femur (left) and 1.+4. lumbar vertebra (LV, right) in ovariectomized rats after 16 wks on diets containing (g calcium/kg diet) / (g oligofructose/kg diet) as follows: □, 5/0; ▨, 5/50; ■, 10/0; ▩, 10/50. Least square means and pooled SEM of MANOVA. Values given are percentages of the mean of 5/0. Significantly different (*P* of at least <0.05) as follows: *different from 5/0 and 10/0, #different from 5/0, 5/50 and 10/0.

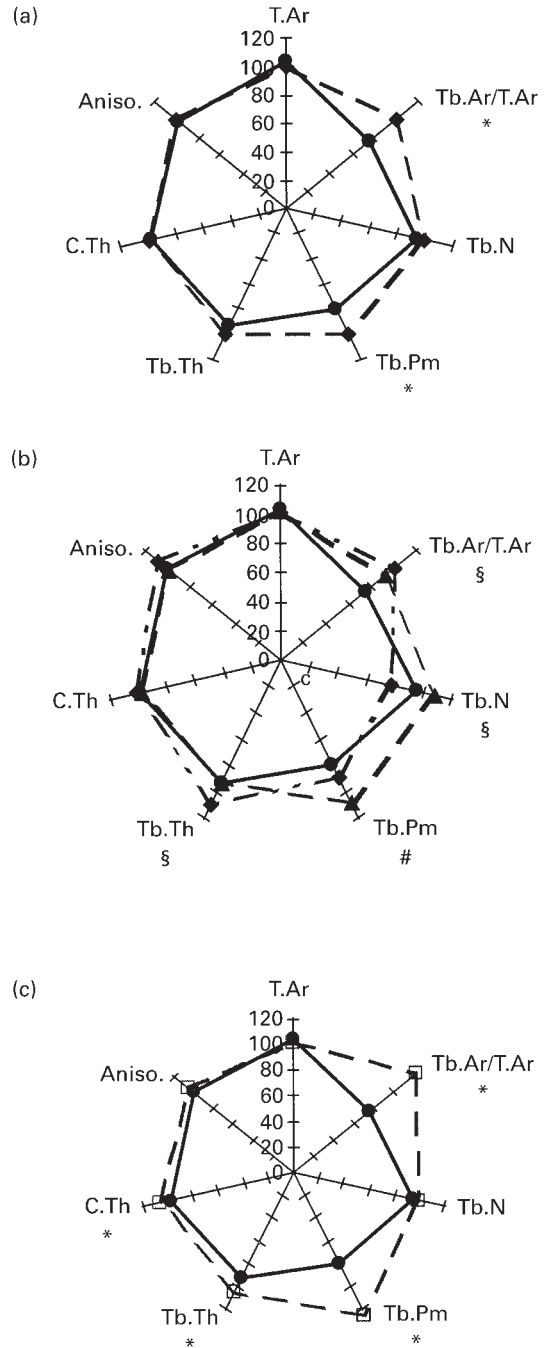


Fig. 4. Parameters of tibia trabecular structure following microradiography and computer-supported image analysis of the proximal tibia of adult ovariectomized rats on different diets. The figures indicate different bone quality depending on whether ovariectomy-induced bone loss (a) was prevented by oligofructose or calcium (b) or a combination of both (c). The experimental groups were fed semipurified diets with 5 g/kg phosphorous and 5 g/kg calcium without oligofructose ((a) —◆—; group 1 and —●—; group 2), 5 g/kg calcium plus 50 g oligofructose /kg (—▲—; group 4) and 10 g/kg calcium either without ((b) —◆—; group 6) or with (—□—; group 7) 50 g oligofructose /kg. Group 1 was sham operated, groups 2, 4, 6, and 7 were ovariectomized. Least-squares means from MANOVA were given. Significant differences with *P* at least < 0.05 as follows: in (a) *1 v. 2; in (b) #4 different from 2, §6 different from 2; in (c) * 7 v. 2.

Table 2. Aspects probably involved in the oligofructose-mediated stimulation of mineral absorption and bone mineralization

Effect of oligofructose on	Mediated via	Effect
Passive transport	↑ ● SCFA production (acetate > propionate > butyrate) > lactate ● Propionate > acetate	↓ pH, ↑ mineral solubility ↑ exchange of cellular proton (H+) for luminal cation (Ca++)
Resorptive surface	↑ ● Polyamines ● SCFA, mainly butyrate	Growth and proliferation factor Growth substrate for colonocytes
Active transport	↑ ● Butyrate ● Polyamines ?	↑ synthesis of CaBP ↑ synthesis of 1,25(OH) ₂ D ₃ receptor ?

Butyrate is given prominence to be a potent candidate for enhancing mineral absorption. Butyrate is a substrate for cell growth and proliferation (Lupton & Kurtz, 1993). By increasing the floral butyrate production, fructo-oligosaccharides (FOS) might indirectly induce cell growth and growth of the gut's absorptive area, another way to contribute to the enhanced mineral absorption (Table 2). Moreover, FOS might be stimulative on the active calcium transport via butyrate production. Sodium butyrate increased the concentration of calbindin and of 1,25[OH]₂D₃ receptor activity in chick primary culture cells (Anita & Anthony, 1992). In rats that were fed a diet containing oligofructose (from sucrose), Ohta *et al.* (1998c) observed a higher expression of calcium binding protein (calbindin-D9k) in the large intestine, while that in the small intestine was decreased. Results reported by Le Blay *et al.* (1999) support the hypothesis that butyrate may have a marked impact on the effect of oligofructose. They observed that oligofructose reduced the pH in the caecal lumen after 2 weeks and this was mainly due to stimulated synthesis of lactate (63.5 μmol/g wet contents at oligofructose and 3.6 μmol/g at basal) > acetate > butyrate. However, after 8 weeks, lactate production came down to concentration of basal diet, while acetate and butyrate were persistently stimulated over 27 weeks. Acetate was 69.5 μmol/g wet contents at oligofructose and 47.6 μmol/g at basal. Butyrate was 15.3 μmol/g wet contents at oligofructose and 4.7 μmol/g at basal. One

might ask whether the low concentration of butyrate in relation to acetate, or lactate could have such a strong effect. Butyrate is very quickly taken up by the enterocytes and makes its detection difficult (Trinidad *et al.* 1996). This circumstance may explain the lack of coincidence of high luminal butyrate concentration and increased mineral uptake across the apical membrane. Inulin and oligofructose mainly stimulate growth of bifidobacteria, but these microbes produce only a little butyrate compared to other strains (Djouzi & Andrieux, 1997). Therefore, data presently available do not support a causal relationship between stimulated mineral absorption by bifidobacteria via butyrate.

Polyamines, e.g. spermine, spermidine, and putrescine, are metabolites generated by several strains of microbes (Noack *et al.* 2000) as well as by higher organisms (Straub *et al.* 1995). Hence they occur almost ubiquitously. Polyamines are potent agents to stimulate proliferation of several organs including intestine (Buts *et al.* 1993; Lupton & Kurtz, 1993; Löser *et al.* 1999) or cells in culture like osteoblasts (Klein & Carlos, 1995). Thus they might at least in part be responsible for cell growth and enlargement of the resorptive surface and by this are further candidates for mineral absorption enhancers. Moreover it was proposed that polyamines have a metabolic effect. The low bone mass in subjects who consume excessive amounts of alcohol was explained by its inhibitory effect on osteoblast proliferation or on the activity of ornithine

Table 3. Effects of exogenous polyamines, oligofructose (as potential initiator of microbial polyamines synthesis), or antibiotics (for eradication of microbial polyamine production) on bone mineralization and intestinal weight and pH in adult ovariectomized rats

	G1	G2	G3	G4	G5	G6	G7	G8	G9	P-value
Femur weight (mg)	494 ^b (12)	448 ^a (12)	457 ^{ab} (10)	445 ^a (12)	479 ^{ab} (9)	500 ^b (10)	486 ^{ab} (7)	476 ^{ab} (10)	489 ^{ab} (9)	0.000
Femur ash (mg)	240 ^{bc} 3.3	235 ^{ab} 4.0	233 ^{ab} 3.2	232 ^{ab} 4.5	242 ^{bc} 3.6	248 ^c 2.8	228 ^{ab} 3.0	223 ^a 2.1	237 ^{bc} 3.3	0.001
Caecum weight (g)	0.57 ^a (0.02)	0.55 ^a (0.01)	0.55 ^a (0.01)	0.55 ^a (0.01)	0.85 ^b (0.03)	1.31 ^c (0.06)	0.86 ^b (0.05)	0.83 ^b (0.05)	1.33 ^c (0.09)	0.001
Caecal contents (g)	2.37 ^a (0.11)	2.56 ^a (0.01)	2.50 ^a (0.01)	2.31 ^a (0.11)	5.58 ^b (0.37)	8.85 ^c (0.57)	4.97 ^b (0.42)	5.17 ^b (0.53)	9.95 ^c (0.99)	0.001
Caecum pH	7.95 ^b (0.03)	7.98 ^{bc} (0.08)	8.15 ^{cd} (0.03)	8.05 ^{bc} (0.05)	7.63 ^a (0.04)	7.62 ^a (0.04)	8.26 ^d (0.04)	8.29 ^d (0.06)	7.91 ^b (0.10)	0.001
Jejunum Ph	7.97 ^{ab} (0.07)	7.75 ^a (0.17)	7.97 ^{ab} (0.08)	8.13 ^b (0.12)	7.75 ^a (0.03)	7.73 ^a (0.06)	8.02 ^{ab} (0.08)	8.20 ^b (0.06)	7.97 ^{ab} (0.04)	0.001

ANOVA, mean and (SEM), N = 12–14, values within a row not sharing a superscript letter are significantly different with P as indicated. The rats were fed semisynthetic diets for 16 weeks that contained 7 g calcium/kg diet and either no additives (G1, G2; G1 = sham operated), or spermidine, spermine and putrescine in physiologic amounts (G3), high amounts (G4, equivalent to 10 × polyamine content of G3), oligofructose (50 g/kg diet, G5), oligofructose + antibiotics (neomycin/metronidazol) (G6), antibiotics alone (G7), antibiotics + high amounts of polyamines (G8) or oligofructose + antibiotics + high amounts of polyamines (G9).

decarboxylase (ODC), the rate-limiting enzyme for polyamine synthesis (Klein & Carlos, 1995). Dietary inulin stimulated ODC activity (Rémésy *et al.* 1993), indicating increased polyamine synthesis by the intestinal microflora. Stimulated intestinal microbial polyamine production was also observed after feeding of guar gum or pectin, two highly fermentable dietary fibres (Noack *et al.* 1998). Since exogenous or microbial polyamines are readily taken up by enterocytes or can pass into the circulation (Bardócz *et al.* 1993), polyamines may also stimulate gene expression of calcium binding proteins in the gut.

When rats were fed oligofructose for 4 weeks, putrescine concentration was higher and spermidine concentration lower, while total polyamine concentration was unchanged in the caecal contents compared to a standard diet. In the caecal tissue putrescine, spermidine and total polyamine concentrations were increased with diets containing oligofructose (Delzenne *et al.* 2000). Higher polyamine concentrations were also observed in rats after feeding of pectin or guar gum (Noack *et al.* 1998).

In vitro studies have demonstrated that fusobacterium synthesised mainly putrescine and bacteroides mainly spermidine, while bifidobacterium did not synthesise any of the polyamines (Noack *et al.* 1998). This observation does not support a causal relation between oligofructose-stimulated mineral absorption and increased polyamine synthesis due to selective stimulation of growth of intestinal bifidobacteria.

To test whether the oligofructose-induced stimulation of mineral absorption is mediated by polyamines, a study with adult ovariectomized Fisher-344 rats was done by our group. The animals were fed semisynthetic diets for 16 weeks that contained 7 g calcium/kg diet and either no additives (G1, G2; G1 = sham operated), or spermidine, spermine and putrescine in physiological amounts (G3), high amounts (G4, equivalent to 10 × polyamine content of G3), oligofructose (50 g/kg diet, G5), oligofructose + antibiotics (neomycin/metronidazol) in order to eradicate the intestinal flora (G6), antibiotics alone (G7), antibiotics + high amounts of polyamines (G8) or oligofructose + antibiotics + high amounts of polyamines (G9). The hypothesis was that oligofructose would increase mineral availability to a more or less comparable degree than exogenous polyamines and that this effect may be diminished in the presence of antibiotics, which eradicate most polyamine producers. We observed a significant loss of femur weight following ovariectomy (Table 3). The highest femur ash was gained in the groups containing oligofructose, either alone (G5) or in combination with antibiotics (G6) or in combination with antibiotics + polyamines (G9). Exogenous polyamines slightly increased the pH of caecal contents and significantly of jejunal contents at the high dose. In contrast to diets containing oligofructose, especially in combination with antibiotics (G6), exogenous polyamines alone did not prevent loss of femur weight and femur ash (Table 3). Antibiotics alone almost prevented loss of femur weight, but not loss of femur ash, which was prevented when antibiotics were combined with oligofructose (G6, Fig. 5). Antibiotics increased caecum weight and weight of caecal contents in a comparable magnitude as oligofructose. However, antibiotics did not reduce the

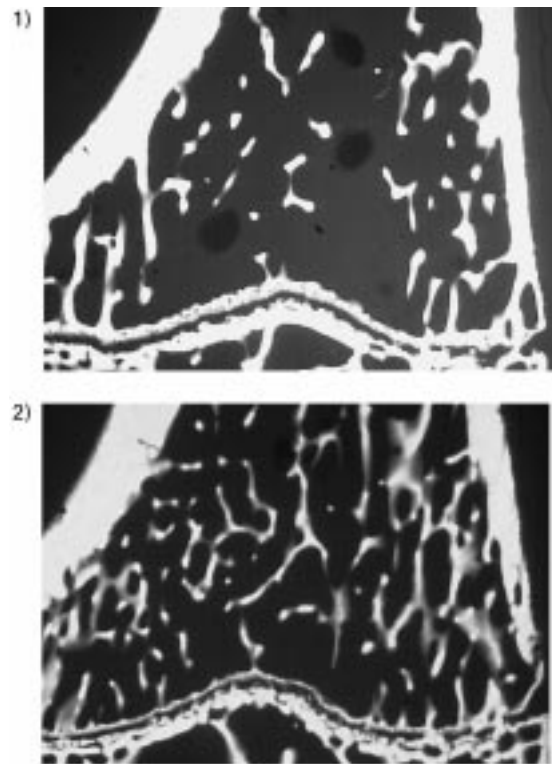


Fig. 5. Microradiograph of the proximal tibia of a 9-month old ovariectomized Fisher-344 rat after treatment with antibiotics (1) or after treatment with antibiotics in the presence of oligofructose (2).

pH in the caecal contents, in contrast to oligofructose, but rather increased it. Data of this experiment revealed that the femur ash, in contrast to femur weight, was highest correlated with pH of the caecal content and to a much less degree to the weight of the caecum or of the caecal contents (Table 4). Femur weight was highest correlated with caecal weight.

We conclude that the potential of oligofructose to prevent ovariectomy-induced loss of bone weight and bone mineral may not be mediated via polyamines, since exogenous polyamines were not protective, possibly because endogenous polyamine synthesis compensated for it. The bone-protecting effect of oligofructose in combination with a reduction of fecal flora was associated with a significant drop of caecal pH. This indicates that oligofructose can prevent not only loss of bone mineral

Table 4. Pearson correlation coefficient (*r*) of means of bone mineral and of caecal pH, caecal weight and weight of caecal content in ovariectomized rats fed semisynthetic diets that contained physiological amounts of polyamines, high amounts of polyamines, oligofructose, antibiotics, oligofructose + antibiotics, polyamines + antibiotics or oligofructose + antibiotics + polyamines to induce different femur ash

Femur	pH	Caecum	
		Content (g)	Organ weight (g)
		<i>r</i>	
Bone weight (g)	-0.35	0.67	0.71
Ash weight (g)	-0.94	0.37	0.39
Calcium (mg)	-0.88	0.37	0.40

content but also the undesirable side effect of raising alkalinity in the large bowel following treatment with antibiotics.

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