# Prebiotics, Probiotics, and Synbiotics Affect Mineral Absorption, Bone Mineral Content, and Bone Structure<sup>1–3</sup>

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#### Abstract

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Several studies in animals and humans have shown positive effects of nondigestible oligosaccharides (NDO) on mineral absorption and metabolism and bone composition and architecture. These include inulin, oligofructose, fructooligosaccharides, galactooligosaccharides, soybean oligosaccharide, and also resistant starches, sugar alcohols, and difructose anhydride. A positive outcome of dietary prebiotics is promoted by a high dietary calcium content up to a threshold level and an optimum amount and composition of supplemented prebiotics. There might be an optimum composition of fructooligosaccharides with different chain lengths (synergy products). The efficacy of dietary prebiotics depends on chronological age, physiological age, menopausal status, and calcium absorption capacity. There is evidence for an independent probiotic effect on facilitating mineral absorption. Synbiotics, i.e., a combination of probiotics and prebiotics, can induce additional effects. Whether a low content of habitual NDO would augment the effect of dietary prebiotics or synbiotics remains to be studied. The underlying mechanisms are manifold: increased solubility of minerals because of increased bacterial production of short-chain fatty acids, which is promoted by the greater supply of substrate; an enlargement of the absorption surface by promoting proliferation of enterocytes mediated by bacterial fermentation products, predominantly lactate and butyrate; increased expression of calcium-binding proteins; improvement of gut health; degradation of mineral complexing phytic acid; release of bone-modulating factors such as phytoestrogens from foods; stabilization of the intestinal flora and ecology, also in the presence of antibiotics; stabilization of the intestinal mucus; and impact of modulating growth factors such as polyamines. In conclusion, prebiotics are the most promising but also best investigated substances with respect to a bone-health-promoting potential, compared with probiotics and synbiotics. The results are more prominent in animal models, where more studies have been performed, than in human studies, where experimental conditions are more difficult to control. J. Nutr. 137: 838S-846S, 2007.

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Accumulating knowledge prompted the scientific community to continually adapt the definitions of probiotics, prebiotics, and synbiotics. Probiotics are "viable, defined microorganisms in sufficient numbers, which alter the microflora in a compartment of the host and by that exert beneficial health effects in this host" (1). Recently it was demonstrated that live and nonviable microorganisms were equally effective to ameliorate experimentally induced colitis because the effect was mediated via the probiotics' DNA rather than by their metabolites or ability to colonize the colon (2). This demonstrated that viability might be a prerequisite in the prevention or amelioration of some but not all disabilities (3). Prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of 1 or a limited number of bacteria (probiotics) in the gastrointestinal tract and thereby exert a health-promoting effect (4). A synbiotic is a product containing prebiotics and probiotics and in which the prebiotic compound selectively favors the probiotic compound (1). The following

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examples may illustrate some prebiotic and synbiotic potentials: Bifidobacterium lactis Lafti B94 is able to utilize a range of prebiotics including inulin, fructo-, galacto-, soybean-, and xylooligosaccharides and resistant starch (5). Whether this has health implications has to be proved. S. thermophilus and bifidobacteria produce folate (6). Because erytrocyte folate content is associated with a greater bone mineral density  $(BMD)^7$  and bone mineral content (BMC) in postmenopausal women (7), this finding may indicate a bone health-improving potential by certain probiotics via the provision of folate. However, it remains to be established whether the amounts provided by probiotics are physiologically relevant under specific conditions. Synbiotics may improve the survival of the bacteria crossing the upper part of the gastrointestinal tract, thereby enhancing their effects in the large bowel. These combined effects can be additive or even synergistic (4). On top of the complementing effect in providing the substrate, resistant starch is regarded as a prebiotic and synbiotic because it can assist in promoting colonization and thus may offer a selective advantage in the host's intestine through adhesion of the bacteria to the granule surface. It may even prolong its viability in adverse food environments (8,9).

Synergy effects are also postulated for the combination of inulin and fructooligosaccharides (FOS) or FOS with various degrees of polymerization (DP). The mixture of such nondigestible carbohydrates of different chain lengths is assumed to be fermented sequentially and thus over a wider range of the large intestine and with improved efficacy.

The following review is restricted to reporting the current knowledge on the effects of prebiotics, probiotics, and synbiotics on mineral absorption, bone mineral accretion, and parameters of bone structure.

# Prebiotics in animal studies: mineral absorption and bone mineral

Inulin, oligofructose, and galactooligosaccharides are the most intensively investigated prebiotics with regard to mineral absorption and retention. In addition, resistant starches and sugar alcohols have been shown to significantly increase mineral absorption and BMC (for reviews see 10-13). Recently it was shown that difructose anhydride prevented parameters of iron deficiency more effectively than FOS (14). Most of the studies on the effects of prebiotics, especially those with respect to bone development, have been performed in rats. It was shown that prebiotics stimulated the absorption of iron and of bone-relevant minerals such as calcium, magnesium, and zinc in short-term experiments and improved BMC in the long-term perspective (10-13). The stimulation of calcium and magnesium absorption by oligofructose was also confirmed for dogs (15). Although phosphorus accretion in the femur might be higher with FOS feeding under special conditions (16), phosphorus balance or retention was not affected in rats or dogs (15,17,18).

In rats results on synergy effects were equivocal (**Table 1**). In adult animals absorption of calcium and magnesium was stimulated in groups receiving inulin or resistant starch. However, the effect on calcium absorption was more significant if a combination of the 2 was given (19). In growing rats only inulin alone significantly increased BMC and density and decreased urinary excretion of collagen crosslinks, a marker of bone resorption, but not oligofructose alone or oligofructose combined with inulin (20). Several FOS with different DP stimulated

#### TABLE 1 Effects of synergetic NDO on mineral retention and BMC in rats

NDO	Effect	Species	Author
Inulin <sup>1,2,11</sup>	* ↑ Ca retention	Adult rats	Younes et al. (19)
	* ↑ Mg retention		
Resistant starch <sup>3</sup>	* ↑ Ca retention		
	* ↑ Mg retention		
Inulin + resistant starch <sup>4</sup>	** ↑ Ca retention		
	* ↑ Mg retention		
Oligofructose <sup>5,12</sup>	ns Bone mineral	Growing rat	Kruger et al. (20)
Inulin	* ↑ Spine BMC		
	* ↑ Femur BMD		
	*↓ Crosslinks		
Inulin + oligofructose <sup>6</sup>	ns Bone mineral		
	ns Ca retention	Growing rats	Coudray et al. (21)
Oligofuctose <sup>1,7,11</sup>	** ↑ Mg retention		
HP-inulin <sup>8</sup>	ns Ca retention		
	* ↑ Mg retention		
Synergy19	** ↑ Ca retention		
	* ↑ Mg retention		
BC-inulin <sup>10</sup>	ns Ca retention		
	* ↑ Mg retention		

<sup>1</sup> Compared with wheat starch; <sup>2</sup> 100 g/kg; <sup>3</sup> 150 g/kg; <sup>4</sup> 50 g/kg inulin + 75 g/kg RS; <sup>5</sup> 50 g/kg; <sup>6</sup> 92% inulin + 8% oligofructose; <sup>7</sup> progressively over 4 wk of 25, 50, and 100 g/kg; <sup>8</sup> high-performance inulin with DP 10–65; <sup>9</sup> 1:1 mixture of oligofructose and HP-inulin; <sup>10</sup> branched-chain inulin; <sup>11</sup> 7.5 g/kg calcium; <sup>12</sup> 5 g/kg calcium. Significant at \**P* < 0.05, \*\**P* < 0.01.

magnesium absorption and retention, but magnesium retention was stimulated more significantly with oligofructose and calcium retention was stimulated only with a blend of oligofructose and an inulin with a high DP ("synergy1") than with 2 different types of inulin. The effect on calcium retention was less distinct: Only the synergy product stimulated calcium retention significantly (21). More studies are needed to elucidate the circumstances under which long-chain FOS, short-chain FOS, or a synergistic combination is best to improve calcium balance or bone mineral accretion.

### Prebiotics in animal studies: bone structure

Oligofructose prevented ovariectomy-induced loss of BMC as indicated by lower bone volume/tissue volume (BV/TV). Beyond this observation bone trabecular structure was differently affected depending on the supplemented amount of oligofructose or the habitual calcium content or a combination of the 2 (11,18): In ovariectomized rats doubling the recommended level of dietary calcium for rats (10.0 vs. 5.0 g/kg diet) was associated with thicker but fewer trabeculae. In contrast, the addition of oligofructose at the recommended level of dietary calcium mostly prevented the loss of trabecular area without reducing the trabecular number. In a different manner oligofructose (50 g/kg diet) given at a high level of dietary calcium (10.0 g/kg diet) preserved bone trabecular area without reduction of trabecular number. Trabecular perimeter and cortical thickness were highest, and the loss of trabecular connectivity (trabecular bone pattern factor) was lowest compared with all other groups. Therefore, in ovariectomized rats a diet containing oligofructose in the presence of a high content of dietary calcium is regarded as most favorable with respect to bone strength and reduced fracture risk.

How could oligofructose affect the trabecular structure? Magnesium, boron, manganese, copper, and zinc are essential

<sup>&</sup>lt;sup>7</sup> Abbreviations used: BMC, bone mineral content; BMD, bone mineral density; DP, degrees of polymerization; FOS, fructooligosaccharides; NDO, nondigestible oligosaccharides.

cofactors for enzymes involved in collagen synthesis and other bone matrix constituents that are required to build up the organic bone matrix (22–25), the precondition for mineral accretion. Thus, it is not surprising that low serum values of magnesium, copper, and zinc were associated with osteoporosis in postmenopausal women (26). An increase of dietary calcium alone impeded the absorption of magnesium (27) and may impede that of other minerals and trace elements that are important for bone matrix formation. Oligofructose not only stimulated calcium absorption but also that of zinc (28) and magnesium in rats with and without suppressed magnesium absorption as a result of high calcium supplementation (17,21). These observations can explain why calcium or oligofructose alone or the 2 in combination may generate different trabecular structures even at similar bone densities.

In growing rats, total and cortical but not trabecular bone volume was lower 4 wk after gastrectomy. Feeding FOS did not prevent these changes significantly but had a strong tendency to affect total and cortical bone volume of sham-operated rats (29). When intact growing male rats were fed 50 g/kg diet, trabecular bone volume was higher than that in control animals in the short term (30).

It has to be stressed that a short-term stimulating effect on mineral absorption does not necessarily allow us to draw conclusions on outcomes for bone mineral or even less for bone trabecular structure or bone quality. Studies in human volunteers on prebiotics and bone mineral accretion are scarce.

#### **Prebiotics in human studies**

Several studies have investigated the effect of prebiotics [inulin, oligofructose, or other nondigestible oligosaccharide (NDO)] on mineral absorption in humans, but with opposing outcomes. Some of the studies showed no significant effect (31–34), whereas others found that prebiotics stimulated the absorption of calcium (35–40) (Table 2) or magnesium (41). Ingestion of 40 g/d of inulin stimulated the calcium absorption in young men as assessed by metabolic balance (35). In other studies the stable isotope technique was applied: In adolescents 15 g/d of oligofructose (36) stimulated calcium absorption, as did 8 g/d of a

combination of oligofructose and inulin in girls (39). When postmenopausal women received 10 g/d of lactulose or 20 g/d of transgalactooligosaccharides, the absorbed calcium was higher than that in controls (37,38).

The lack of significance in some studies may have been caused by differences in the experimental designs. Ellegård et al. (31) analyzed the calcium content in the effluent of ileostomy patients. It is not surprising that the calcium absorption was not affected because the main site of action of FOS is situated in the large bowel. When stable isotope techniques are applied, a sufficient period of collection time of urine is essential, and 24 h is presumably too short to account for the colonic processes (32). This fact may also explain that inulin did not affect circulating parathyroid hormone and ionized calcium 8 h postprandially (42). Therefore, it might be possible that oligofructose, transgalactooligofructose, or inulin would have been effective in young men if the collection time had been long enough, i.e., at least 36 h, as in the studies performed later by Van den Heuvel et al., when they observed a positive effect of 15 g of oligofructose in adolescents (36).

Tahiri et al. (33) applied the metabolic balance technique and the stable isotope technique in parallel and found no significant effect of short-chain oligofructose on calcium absorption in postmenopausal women. It might be that the 10 g given was too low. Another explanation for the lack of significance is presumably the menopausal stage. The calcium balance in the perimenopausal and early postmenopausal period is known to be mainly affected by hormonal changes, and dietary interventions are less obvious (43). When the women were stratified by time of postmenopause, a trend was observed (P < 0.1) for a beneficial effect of short-chain oligofructose on calcium balance in women >6 y postmenopause but not in women <6 y postmenopause (33). There was no significant effect in postmenopausal women when 5 g lactulose was consumed, obviously an insufficient amount, in contrast to the 10 g lactulose (37).

In young girls oligofructose was not effective despite a sufficient collection time of urine (39). The administered dose of 8 g given for 1 wk in the presence of 1500 mg of dietary calcium may have been insufficient. However, when the same amount of a

TABLE 2 Human studies on the effects of prebiotics on calcium absorption

Substance	Amount, g/d	Ca absorption	Subjects, <i>n</i>	Comment	Method	Authors
With no effect						
Inulin	7	ns	Adult	Small intestine	lleostomy effluent	Ellegård et al. (31)
OF	17	ns				
OF	15	ns	Young men (12)	24-h urine coll.	Stable isotope	Van den Heuvel et al. (32)
TOS	15	ns				
Inulin	15	ns				
sc FOS	10	ns	POM (12)	All women	Stable isotope and balance	Tahiri et al. (33)
		ns (P < 0.1)		W. > 6  y POM		
FOS	1	ns (P = 0.055)	Young adults (15)		Stable isotope	López-Huertas et al. (34)
With a significant effect	t					
Inulin	40	*	Young men (9)		Balance	Coudray et al. (35)
OF	15	*	Adolescents (12)	36-h Urine coll.	Stable isotope	Van den Heuvel et al. (36)
Lactulose	5	ns	POM (12)	36-h Urine coll.	Stable isotope	Van den Heuvel et al. (37)
	10	*				
TOS	20	*	POM (12)	36-h Urine coll.	Stable isotope	Van den Heuvel et al. (38)
OF	8	ns	Girls (30)	48-h Urine coll.	Stable isotope	Griffin et al. (39)
OF + inulin	8	*				
sc and Ic FOS mix	8	*	Boys and girls (48)	48-h Urine coll.	Stable isotope	Abrams et al. (40)

OF, oligofructose; TOS, trans-galactooligosaccharides; sc, short chain; lc, long chain; FOS, fructooligosaccharides; POM, postmenopausal; \* P < 0.05; ns P ≥ 0.05

mixture of oligofructose plus inulin was consumed, the effect was significant (39) (Table 2). This observation could be explained by a "synergy effect." The combination of different NDOs, or of NDO with different DP, e.g., oligofructose with a DP of 2–9 plus inulin with a DP > 23, may be called "synergy" in that it enables a serial fermentation of the NDO and thus leads to an extension of the sites of fermentation from the proximal to the distal colon. Generally the flora of an individual becomes more complex with age (44), and it might be that children possess a flora that is less skilled compared with that of adults to ferment FOS with a higher DP, i.e., inulin alone. From this point of view the positive effect of 8 g of synergy, but not of oligofructose alone, is somewhat unexpected, but inulin alone was not tested.

Recently, Abrams et al. (40) studied the effect of 8 g of a synergy product per day in 9- to 13-y-old girls for 1 y. Compared with the control group, the calcium absorption was higher after 8 wk and still higher after 1 y. The significance depended on a polymorphism of the Fok1 vitamin D receptor gene. Stimulation of absorption was not seen in girls with the ff polymorphism but was significant in those with the Ff and more pronounced with the FF polymorphism. Accretion of whole-body BMC and density was higher after 1 y in girls consuming the combination of short- and long-chain inulin-type fructans compared with the control group (40).

When oligofructose was given to infants in either the presence or absence of supplemental zinc, no effect was observed on plasma zinc concentrations. It has to be considered that no parameter of zinc balance or metabolism was reported because the study outcome was not focused on mineral absorption or status but was directed toward the prevalence of diarrhea (45).

#### Probiotics and synbiotics in animals and humans

Probiotics could have a potential effect on bone accretion independent of that of prebiotics. This could occur via microbial production of metabolites or enzymes or synthesis of vitamins (6,46,47) because several vitamins are also involved in calcium metabolism and are required for bone matrix formation and bone accretion as are vitamin D, C, or K (48) or folate (7). One may expect that the optimum combination of probiotics and prebiotics would gain the best results, and this optimum depends on the disease or risk that is aimed to be prevented or reduced. Few studies have investigated the effects of probiotics or synbiotics on the metabolism of minerals or trace elements or on bone health.

When infant formula containing *Lactobacillus reuteri* supplement  $(9 \times 10^6 \text{ cfu})$  was given to infant rhesus monkeys, hematocrit was improved, but no effect on the retention of calcium, iron, and zinc was observed (49). This experiment, however, was not designed to investigate the effect of this microorganism on mineral balance. The primary outcomes of this experiment were nutritional status, gut colonization, and the ability to resist gastrointestinal infection.

Synbiotics stimulated the fecal bacterial counts of lactobacilli and bifdobacteria in human subjects, but little effect was seen when only the probiotic (*Bifidobacterium lactis* HN019) or the prebiotic (galactoologosaccharides) was given (50). Such observation may allow one to assume a more effective stimulation of mineral absorption by synbiotics compared with prebiotics or probiotics alone. The effect of an oral application of *Bifidobacterium longum*, either alone or in combination with lactulose, on the breaking force was tested in rats (51). *Bifidobacterium longum* alone induced a significant rise in bacterial counts but only tended to increase the breaking force. Only when *Bifidobacterium longum* was given together with lactulose was the breaking force significantly higher compared with that in the control group. In that experiment, the effect of lactulose alone was not tested.

It was reported that probiotic yogurt containing strains of *Lactobacillus casei*, *Lactobacillus reuteri*, and *Lactobacillus gasseri* increased apparent calcium absorption and BMC in growing rats (52). However, bone weight was 35% higher than that of the control group, and no information on food consumption and body weight was given.

Scholz-Ahrens et al. (53) and Marten et al. (54) performed an experiment in 5 groups of Fisher-344 rats that were either sham operated (G1, positive control) or ovariectomized (G2-G5) at the age of 6 mo. During the following feeding experiment over 16 wk, all rats received a purified diet providing all nutrients for rats on a restricted feeding regimen that guaranteed the same and complete feed consumption by all animals. In addition the animals received 1 g/d of acidified milk. In 2 groups probiotic lactobacilli (Lactobacillus acidophilus NCC90) were added (G3-probiotic and G5-synbiotic), and in 2 groups 2.5% prebiotics (a mixture of short-chain fructooligosaccharide and acacia gum) were added (G4 and G5). Rats that received prebiotics (G4 and G5) had significantly lower cecal pH and higher cecal contents compared with all other groups. The probiotic strain used in this experiment did not decrease cecal pH or raise cecal contents as an indicator for increased luminal fermentation (53). Compared with that in ovariectomized control rats, urinary phosphorus excretion was significantly lower when prebiotics or synbiotics were given, but phosphorus retention was not different (54). Calcium absorption as assessed by metabolic balance after 15 wk tended to be higher in the synbiotic group and was significantly higher in the prebiotic group compared with ovariectomized controls (54).

The absorption of calcium assessed by 7-d balance within a long-term experiment only allows the view through a narrow time window on available calcium. Therefore, we also analyzed BMC, a parameter of cumulative calcium retention after 16 wk. Femur ash content was lower (-5.8%, P < 0.05) in ovariectomized rats (231.2  $\pm$  5.1 mg) compared with sham controls  $(245.4 \pm 2.5 \text{ mg})$ . There was a trend to prevent this ovariectomyinduced loss of ash in the femur by the supplements. The preventive effect by synbiotics was most pronounced (241.1  $\pm$ 2.4 mg, P < 0.10), that by prebiotics less pronounced (238.7 ± 3.2 mg, P < 0.22), and that by probiotics marginal (236.6 ± 2.4 mg, P < 0.35). Ash content in 2 lumbar vertebrae in ovariectomized rats (116.2  $\pm$  3.3 mg) was lower (-6.6%, P = 0.05) compared with that in sham-operated controls (124.3  $\pm$  2.1 mg). Compared with the ovariectomized control group, lumbar vertebrae ash content was slightly higher after probiotics with  $120.3 \pm 2.4$  mg, more pronounced after prebiotics with  $124.1 \pm 2.2$  mg, and significantly higher when synbiotics were fed with  $127.2 \pm 1.8 \text{ mg} (53)$ .

Bone structure may affect bone strength independent of BMC. Therefore, trabecular bone area of the proximal tibia was assessed, which was done by contact microradiography followed by computer-supported image analyses of digitized radiographs; for details see Scholz-Ahrens et al. (18). A significant loss of trabecular area of 20.1% was induced by ovariectomy ( $12.25 \pm 0.7\%$  vs.  $15.44 \pm 0.7\%$  in sham-operated controls). This loss could not be prevented completely by any of the 3 treatments, but it was smallest in the 2 groups that received prebiotics (9.1% in rats on prebiotics, 12.8% in rats on synbiotics) compared with 16.9% in rats on probiotics (50). We conclude that the specific lactobacilli tested (*L.acidophilus* NCC90) alone are not promising to prevent ovariectomy-induced loss of bone mineral and trabecular area. Other strains (*Lactobacillus helveticus*)

might be more effective (55). Furthermore, the tested mixture of short-chain FOS plus acacia gum appeared to have a lower potential to stimulate mineral absorption than oligofructose (18). With regard to BMC, the synbiotic combination tested was more effective in the axial than in the appendicular bone. Further studies are needed to confirm these findings.

## Mechanisms

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Several factors that contribute to the mechanism by which prebiotics stimulate mineral absorption and improve BMC and structure have been described and reviewed (10–13): The positive effects have been attributed to increased solubility of minerals because of increased bacterial production of shortchain fatty acids through increased supply with substrate; enlargement of the absorption surface by promoting proliferation of enterocytes mediated by bacterial fermentation products, predominantly lactate and butyrate; and increased expression of calcium binding proteins.

Besides these steps involved in prebiotics-induced facilitation of mineral absorption and bone mineral accretion, new findings suggest a more broadened mechanism on the action of prebiotics and, in addition, include direct effects of probiotic bacteria. Potential synbiotic effects are also possible. The following hypotheses are supported by experimental outcomes and contribute to the understanding of how bone health could be supported by prebiotics, probiotics, and synbiotics (see **Table 3**).

Melioration of gut health and stimulation of immune defense. The alterations of intestinal mucosa introduced by dietary FOS are mediated via the gut flora and not via an effect of the substrate per se because only bacteria-associated but not germ-free rats had higher villi and deeper crypts after feeding with a mixture of oligofructose and inulin (56). Furthermore the oligofructose-inulin mixture increased the number of goblet cells and the thickness and composition of the colonic epithelial mucus layer. This composition shifted toward more acidic mucins, predominantly sulfomucins, i.e., changes that indicate a more stabilized mucosa. All of these effects are regarded as beneficial for the health maintenance of the gut (56) because they improve its absorptive function. A stabilized flora contributes to prevent gastrointestinal infections and oxidative damage to the enterocytes. Certain strains of lactic acid bacteria, most effectively Streptococcus thermophilus YIT 2001, showed a high inhibitory activity against experimentally induced lipid peroxidation in enterocyte liposomes and thus against mucosal damage (57). Prebiotics, probiotics, and synbiotics also have

**TABLE 3** Mechanisms that may contribute to the bone health-supporting effect by prebiotics, probiotics, and synbiotics

Effects by prebiotics and synbiotics	Effects by probiotics
Increased solubility of minerals	Degradation of mineral complexing phytic acid
Enlargement of the absorption surface	Stimulation of calcium uptake by enterocytes
Increased expression of calcium	Probiotic antiarthritic effect
binding proteins	
Melioration of gut health	
Stimulation of immune defense	
Release of bone modulating factors	
Stabilization of the intestinal flora	
Impact of modulating growth factors	
Suppression of bone resorption	
relative to bone formation	

been reported to affect the immune system in rats. Prebiotics mainly improved the gut-associated immune defense, and synbiotics additionally developed systemic immunomodulatory capacity (58). Such effects could contribute to a more effective function of the gut and absorption process.

Release of bone-modulating factors. It was shown that FOS increased the bone-preserving effect of phytoestrogens in ovariectomized mice (59) and rats (60). This effect was proposed to be based on the improvement of isoflavone bioavailability. Flavonoids are more rapidly absorbed in their free aglycone form than as the intact glycoside (61). Stimulation of bifidobacteria and lactobacilli by FOS increases the luminal bacterial  $\beta$ -glycosidase activity, an enzyme that hydrolyzes the glycosidic bond of isoflavone conjugates. It converts daidzein to equol, an isoform with a higher bone-preserving potential (62).

Stabilization of the intestinal flora. When antibiotics (neomycin, metronidazol) were added to a control diet to study the effect of prebiotics in the absence of potential polyamine producers, rats tended to have less femur ash compared with shamoperated controls, ovariectomized controls, and oligofructose-fed rats. When oligofructose was added to the antibiotics-containing diet, femur ash was significantly higher than that of ovariectomized controls. At the same time rats treated with antibiotics had the highest pH in the cecal contents with  $8.26 \pm 0.04$ , which was significantly higher (P < 0.004) than that of sham-operated controls with  $7.95 \pm 0.03$ , ovariectomized controls with  $7.98 \pm$ 0.08, oligofructose-fed rats with  $7.63 \pm 0.04$ , and rats on oligofructose plus antibiotics with  $7.62 \pm 0.04$  (11).

Ovariectomy induced a significant loss of bone trabecular area as a percentage of tissue area with  $10.3 \pm 0.6\%$  compared with  $13.3 \pm 0.6\%$  in sham-operated controls (P < 0.002) without affecting trabecular number or thickness. This loss tended to be smaller in rats on oligofructose with  $12.1 \pm 1.1\%$ , and in rats on oligofructose in the presence of antibiotics with  $12.5 \pm 0.6\%$ , compared with sham-operated controls. Antibiotics alone resulted in the lowest trabecular area ( $9.09 \pm 0.76\%$ ), which was significantly lower than that in rats on oligofructose (P < 0.05) and even more pronounced compared with rats on oligofructose plus antibiotics (P < 0.002).

The low trabecular area was also reflected in the trabecular perimeter (Fig. 1). Compared with rats fed oligofructose, the lower trabecular area and perimeter in antibiotics-fed rats were mainly a result of significantly fewer trabecular endpoints, whereas if



**Figure 1** Oligofructose with and without antibiotic treatment and the effect on tibia trabecular perimeter in adult ovariectomized rats. Mean  $\pm$  SEM, n = 14 per group. G1 = sham operated, G2-G5 = ovariectomized. Diets contained 7 g calcium/kg diet and no additives (*sham op and OVX*), 50 g/kg diet oligofructose (*OF* and *OF*+*AB*), and antibiotics (neomycin/metronidazol; *OF*+*AB* and *AB*). Groups were significantly different from OVX with P < 0.05 (*a*), P < 0.01 (*b*); from OF with P < 0.05 (*x*); and from OF+AB with P < 0.01 (*b*).

compared with rats on oligofructose plus antibiotics it was caused by a reduction in trabecular number and trabecular endpoints. Trabecular thickness was not significantly affected, although antibiotic treatment caused the thinnest trabecules ( $61.4 \pm 1.6 \mu m$ ) compared with the ovariectomized control ( $65.2 \pm 1.5 \mu m$ ) or rats on oligofructose alone ( $65.6 \pm 2.5 \mu m$ ).

The different effects of treatments on parameters of trabecular structure by ovariectomy, oligofructose alone or in combination with antibiotics, or antibiotics alone indicate different trabecular shape and network. The different patterns of the structure of the trabecular network in different experimental groups are schematically depicted in Figure 2.

These data demonstrate that prebiotics may overcome some of the negative metabolic effects of antibiotic treatment such as increased cecal pH (11), a result of reduced production of shortchain fatty acids because of the modification of the gut flora with a consecutive depressed mineral solubility, absorbability, and deposition in the skeleton. The supposed lower absorption of trace elements that are involved in bone matrix formation at higher luminal pH may be responsible for an inadequate bone matrix and, consequently, bone structure, an effect that may be overcome by oligofructose (11,18). Furthermore a lower substrate delivery for enterocytes following antibiotic treatment can be assumed together with other possible side effects, but these are not under consideration here. However, these results may contribute to considerations in how far the inclusion of probiotics, prebiotics, or synbiotics into the diet, functional food, or feed may help overcome side effects of antibiotics treatment or prove an alternative to the preventive use of antibiotics in animal nutrition. This item is of particular interest because the WHO advises a ban on the preventive use of antibiotics (63), and the European Union has decided to completely banish its use in livestock production from 2006 on (64).

*Impact of modulating growth factors.* Because polyamines are known to act as luminal mucosal growth factors (65), and because some bacteria were shown to produce polyamines (66),



**Figure 2** Cartoons of presumed bone structure based on the results of selected trabecular structure parameters of rats that had undergone sham operation (*sham op*) or ovariectomy (*OVX*) or ovariectomized rats after supplementation with oligofructose (*OF*) in the presence and absence of antibiotics (*AB*).

Scholz-Ahrens and Schrezenmeir (11) tested whether the increase of BMC in oligofructose-fed rats might be mediated via the stimulation of polyamine-producing bacteria that benefited from oligofructose in the diet. An experiment in 6-mo-old ovariectomized rats was performed. After 16 wk no stimulatory effect of a supplemented mixture of spermine, spermidine, and putrescine on BMC, cecum weight, or pH in cecal contents was observed (11).

However, parameters of bone structure, which were assessed by microcomputed tomography followed by interactive steps on a computer-supported image analysis, were affected: In ovariectomized rats, animals on the medium dose of dietary polyamines (equivalent to 10 times the polyamine content of the semipurified control diet, which was 310 mg polyamines/kg diet) had significantly lower trabecular number  $(57.2 \pm 3.1)$  compared with rats on the control diet (67.0  $\pm$  3.2, P < 0.05), although the trabecular area as a percentage of tissue area of  $10.08 \pm 0.82\%$  was not different from that of rats on control diets of  $10.30 \pm 0.57\%$ , indicating that larger trabecules are preserved. On the other hand, in rats on the high-dose polyamines (equivalent to 10 times the polyamine content of the medium dose), the trabecular number  $(72.1 \pm 5.0)$  was significantly higher, and trabecular area  $(11.19 \pm 0.82\%)$  tended to be higher compared with the group receiving the medium-dose polyamines. These results indicate that ovariectomy-induced changes of bone structure are modulated by polyamines in a dose-independent manner. An optimum amount of luminally or metabolically available polyamines may be required to preserve a high degree of tibia trabecular bone structure, particularly in estrogen deficiency.

Suppression of the bone resorption rate relative to the bone formation rate. A mixture of inulin and FOS was studied in ovariectomized rats, using stable istotope technique. Calcium absorption and bone balance were significantly increased, as were femoral calcium content and mineral density. The bone resorption rate relative to the bone formation rate was significantly depressed. No effect was observed on breaking strength (67).

Probiotic degradation of mineral-complexing phytic acid. In cereal based foods, whole-grain products in particular, minerals are complexed by phytate. In phytate-rich diets the availability of trace elements such as copper, zinc, and iron is significantly depressed in man and animals (68-70). Phytate did not depress the absorption of calcium in adolescent but in young and adult rats, an effect that could be counterbalanced by dietary casein depending on the age of the animals and calcium content of the diet (71). Monogastric animals and humans are lacking endogenous phytase. Therefore, adding phytase of either plant or microbial origin to the diet could help to overcome this problem, as it is practiced in farm animals (72). The addition of phytase-producing bacteria (Mitsuokella jalaludinii, a culture gained from rumens of cattle in Malaysia) was investigated in broiler chicken (73). Four different amounts of culture with increasing phytase activity were added to phytate-rich broiler feed, and these animals were compared with a control group without added culture (negative control) and a phytate-poor diet (positive control). The mean tibia ash of the chicken on phytaterich diets was significantly depressed compared with phytate-poor diets. The depression was prevented with cultures equivalent to 250 U activity and more effectively with 500 U. With even higher doses, no further improvement was achieved.

# *Probiotic stimulation of calcium uptake by enterocytes.* It has been shown that milk fermented with *Lactobacillus helveticus* had some effects on calcium metabolism in postmenopausal

women (74). In the short term it reduced plasma parathyroid hormone and increased serum calcium but had no effect on carboxy-terminal telopeptide of type I collagen. This modulating effect on calcium metabolism may result from facilitated calcium uptake by enterocytes. An increase of calcium uptake was demonstrated in Caco-2 cells for some probiotic bacteria such as *Lactobacillus salivarius* (UCC 118) but not for such others as *Bifidobacterium infantis* (UCC 35624) (75).

**Probiotic antiarthritic effect.** In addition, other probiotic bacteria (*Enterococcus faecium*) are shown to have a bone-preserving effect through their antiarthritic potential. The loss of whole-body BMD in this model was reduced with methotrexate treatment. This effect was more pronounced when *Enterococcus faecium* was added to the treatment but not when it was given alone (76). This observation may be caused by the observed antiinflammatory effect of *Enterococcus faecium* in a rat model for adjuvant arthritis, leading to a healthier gut and thus mineral-absorbing surface and to less bone resorption.

It was observed that *Lactobacillus helveticus* reduced parathyroid hormone levels in postmenopausal women consuming *Lactobacillus helveticus*-fermented milk (74), and it was shown in vitro that *Lactobacillus helveticus*-fermented milk increased osteoblast bone formation in bone marrow-derived osteoblast precursor cells (77). These positive effects on parameters of bone health may not be a probiotic effect mediated by the microorganisms themselves but may be mediated by encrypted bioactive peptides that are released from milk proteins during the fermentation process (77).

*Conclusion.* Several studies in animals and humans have shown positive effects of NDO on mineral metabolism, bone composition, and bone architecture. These effects were not in all cases uniform. Certain experimental or dietary conditions and the physiological characteristics of the target group studied might favor a positive outcome of a study.

Dietary conditions include a high content of calcium up to a threshold level and optimum content of supplemented prebiotics, i.e., high as possible and low enough not to induce side effects such as bloating, abdominal pain, or diarrhea. Doses of 2.5% up to 15% of NDO in rats and of 8 g/d up to 40 g/d in humans were effective, depending on the potency of the NDO. The amount of habitual intake of NDO, which can easily be controlled in animal studies, may be a confounding factor in human studies and may in part explain the inconsistent results in humans. However, this remains to be investigated. The optimum combination of different NDOs should be used. In adult rats inulin combined with resistant starch was more effective than one or the other alone, whereas in growing rats the results are less conclusive.

The characteristics of an individual that affect bone metabolism include the following:

*Physiological age.* Subjects with a high demand for calcium, i.e., at puberty or after menopause, are more predestined to benefit from prebiotics than healthy adults. In young individuals synergy products may be favorably compared with inulin or long-chain NDO because of a less developed diversity of the flora.

*Postmenopausal stage.* Women who are more than 6 y postmenopausal may benefit more than women around postmenopause, whose bone loss is more affected by the decline in circulating estrogens.

*Calcium absorption capacity.* Individuals with a low absorption capacity because of filled calcium stores, i.e., at low

circulating concentrations of calcitriol and low mucosal expression of calcium-binding proteins, may benefit less than individuals with chronically low calcium intakes and an up-regulated absorption capacity. Such individuals may benefit even more when they are supplemented simultaneously with oligofructose and calcium.

The potential for bone health of a synbiotic preparation is affected by the same aspects mentioned above for prebiotics. In addition, the prebiotic has to be combined with the best suitable probiotic. Moreover, a certain synbiotic may be effective in 1 species and not in another because species-dependent susceptibilities to different microorganisms can be assumed to be related to their diverging innate flora. However, little is known in this field, and future research is required, which should be directed toward the development of functional foods for defined target groups at specific risks.

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