Prebiotics, Probiotics, Polyunsaturated Fatty Acids, and Bone Health

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Abstract

Improvement of peak bone mass in younger age and reducing bone loss in aging are two strategies to reduce the risk for developing osteoporosis. Modulating intestinal calcium absorption by modifying the diet can contribute to improvement of bone mass, and reduction of inflammation during menopause can help reduce the risk of bone loss. Calcium absorption takes place via an active process in the duodenum, modulated by active vitamin D, or by passive paracellular absorption that can take place throughout the intestine. Prebiotics are nondigestible carbohydrates which promote bacterial growth in the colon. Fermentation by the bacteria results in the production of organic acids which reduce the pH in the large intestine and may improve solubility of minerals increasing passive diffusion via the paracellular pathway. Increased cell proliferation and hypertrophy of the colon wall have also been reported, while some authors also report increased expression of calbindin-D9k, the protein responsible for carrying calcium through the intestinal cell. While the mechanism by which probiotics improve calcium absorption has not been proven, it is possible that the mechanism is similar to that of the prebiotics. Another dietary component that can affect intestinal calcium absorption is long-chain polyunsaturated fatty acids (LCPUFA). These have been shown to improve calcium absorption by modulating the action of vitamin D in the intestine, modulating intestinal membrane composition and thereby increasing activity of the membrane pumps responsible for transport of minerals across the basolateral membranes. The omega 3 LCPUFAs also have

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specific effects on bone cells and reduce inflammation which may be of benefit to bone especially during menopause. In addition, LCPUFAs may have a prebiotic effect, modulating gut microflora. The possible contribution of these dietary components to calcium absorption and bone maintenance in rats and younger as well as older adults is presented.

Keywords
Calcium absorption • Prebiotics • Probiotics • Gut microflora • Long-chain polyunsaturated fatty acids • Bone density • Rats • Humans

Introduction

Maximizing peak bone mass during adolescence, in addition to minimizing bone resorption in old age, may be the key to postponing and possibly preventing bone fractures due to osteoporosis, later in life. A key way to accomplish this is through increased calcium intake [1]. Normally, only about 30 % of the dietary calcium is absorbed by the body and deposited in the bones. Improved calcium absorption in the body could raise the balance of calcium retained in the body and could therefore have important consequences on the occurrence of osteoporosis and bone fractures. Calcium absorption can be manipulated using foods to influence the bacterial population in the large intestine, for example, or change transporters in the epithelial cells. The following chapter provides an overview of the role that prebiotics, probiotics, and long-chain polyunsaturated fatty acids (LCPUFA) may play in optimizing intestinal calcium absorption and improving bone health.

Calcium Absorption

The majority of calcium, up to 90 %, is absorbed in the small intestine. There are two distinct processes involved, transcellular uptake which takes place mainly in the upper part of the small intestine and paracellular uptake which takes place throughout the small intestine and can also take place in the colon [2]. When calcium intake is normal or high, the relative amount of calcium absorbed in the duodenum is low with the largest amount being absorbed in the lower half of the small intestine, particularly in the ileum. Transcellular active absorption is upregulated when calcium intake is low and is also high in growing children. With a low dietary calcium intake, the amount absorbed in the duodenum may be larger than with the paracellular pathway [3].

The transcellular pathway requires metabolic energy, and it is dependent on vitamin D. The process can be divided into three steps: entry, intracellular diffusion, and extrusion. The entry across the brush border utilizes several transport proteins, such as transient receptor potential vanilloid (TRPV6). The entry is down an electrochemical gradient, and the channels are not voltage gated [4]. The rate-limiting step is the diffusion of calcium across the intestinal cell. This process is vitamin D dependent and uses calbindin-D_{9k}, which is induced by the active form of vitamin 1,25 dihydroxyvitamin D (1,25 (OH) D_3). The transport of the calcium across the basolateral membrane is via the calcium-magnesium ATPase (PMCA1b), which is also vitamin D dependent and an active process. There is a sodium-calcium exchanger located in the basolateral membrane, but it plays a small role in calcium absorption [2, 3, 5].

Passive calcium absorption, or paracellular diffusion down a chemical gradient, takes place throughout the small intestine. This transport is responsible for most of the calcium absorption when intake is high due to downregulation or saturation of active absorption [2, 3, 5]. Calcium absorption in the colon could contribute up to 10 % of the total calcium being absorbed. In the rat, up to 11 % of calcium absorbed could be via

the colon [3]. Some active calcium absorption can also take place in the colon, as calbindin-D_{9k} is also found in the rat cecum and large intestine [3].

Several studies in rats and humans have been published over the past decade which support an effect of indigestible fiber (prebiotics), intestinal microflora (probiotics), and long-chain polyunsaturated fatty acids (LCPUFA) on intestinal calcium absorption.

Prebiotics

Prebiotics are nondigestible food ingredients, mostly carbohydrates, and these promote bacterial growth primarily in the colon. The fermentation is thought to provide health benefits which include increased mineral absorption. Chicory inulin and oligofructose are the most studied prebiotics [6]. Inulin belongs to the fructan family, which is an important storage carbohydrate found in noticeable amounts in chicory, artichokes, onions, and asparagus. Inulin-type fructans are composed of beta-D-fructofuranoses attached by beta-2-1 linkages. The first monomer of the chain is either a beta-D-glucopyranosyl or beta-D-fructopyranosyl residue. They constitute a group of oligosaccharides derived from sucrose that are isolated from natural vegetable sources [7]. Generally a product with a degree of polymerization (DP) from 2 to 60+ is labeled as inulin, whereas oligofructose which is produced by partially enzymatic hydrolysis of inulin is defined by a DP < 10 [6]. Fructooligosaccharides (FOS) stimulate the growth of bifidobacteria. The luminal bacteria in the large intestine ferment indigestible carbohydrates, and various authors suggested an important correlation between increases in the absorption of minerals and the fermentation of indigestible carbohydrates in the large intestine [5, 8].

Several hypotheses about the mechanisms of action of the prebiotics have been proposed. Indigestible oligosaccharides reach the large intestine intact and are fermented by bacteria in the intestinal lumen. The result is an increase in the production of organic acids such as acetate, propionate, and butyrate, also known as short-chain fatty acids (SCFA) [9]. Due to the synthesis of these acids, the luminal pH drops which may help dissolve insoluble calcium salts in the luminal content and accelerate the passive diffusion of minerals via the paracellular pathway. It is also possible that these short-chain fatty acids (SCFA) contribute directly to the enhancement of calcium absorption via a cation exchange mechanism, by increased exchange of cellular H^+ for luminal Ca^{2+}. Chonan et al. [10] showed that in rats fed with galacto-oligosaccharides (GOS), cecal pH dropped and this was correlated with the highest uptake and retention of calcium. Raschka and Daniel [9] reported that feeding inulin and short-chain FOS lowered cecal pH and increased total cecal calcium, soluble calcium, and ionized calcium. Secondly, absorption of SCFA is accompanied by absorption of minerals. SCFA, especially butyrate, could serve as a fuel for mucosal cells and stimulate cell proliferation, leading to hypertrophy of the colon wall which can lead to enhanced capacity for absorption of minerals [11]. Perez-Conesa et al. [12] reported increased cell density and crypt depth in the distal colon in rats fed with a diet high in galacto-oligosaccharides (GOS) and showed that these parameters correlated with measured calcium absorption. Raschka and Daniel [9], using the Ussing chamber model, reported similar calcium absorption rates in various intestinal segments from control and inulin + FOS-fed rats, but the prebiotic diet did increase cecal surface and wall weight. More recent work reported increased cecal wall weight and reduced cecal pH in rats fed with varying levels of GOS for 8 weeks [13].

Another suggested mechanism is upregulation of active calcium absorption. Raschka and Daniel [9] reported changes in mRNA levels between control and inulin + FOS-fed rats for genes that may be involved in calcium absorption. Increased transcript levels were reported for calbindin as well as the sodium-calcium exchanger, but no effect was observed on the TRPV6. Ohta et al. [14] showed that in rats, FOS diets increased colorectal and cecal calbindin D_{9k}. Figure 13.1 provides a summary of these suggested mechanisms of action by prebiotics.

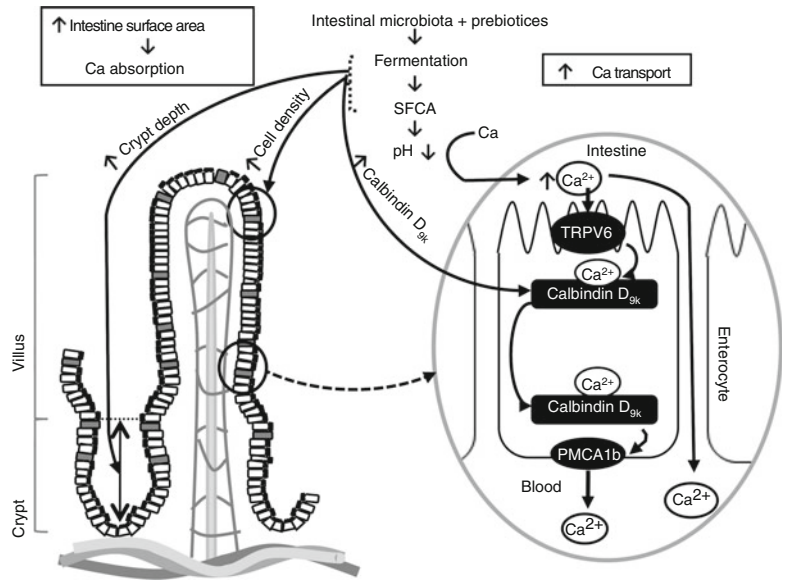

Fig. 13.1 The hypothesized mechanisms of the positive prebiotic effect on calcium absorption. *SCFA* short-chain fatty acid (Reprinted from Parks and Weaver [5]. With permission from Decker Publishing Inc.)

There have been many studies done in rats and in humans investigating the effect of prebiotics on calcium absorption, calcium retention, and bone properties. Some of these are summarized below.

Animal Studies

With regard to calcium balance and absorption, several fibers have been studied: oligofructose [15–21] and several others including inulin [22, 23], galacto-oligosaccharides (GOS) [13, 24, 25], lactulose [26], or resistant starch [27, 28]. In general inulin-type fructans exhibit a dose-dependent effect on calcium absorption. Some studies however have shown a similar increase of about 60 % in apparent calcium absorption in the presence of 5 or 10 % inulin or FOS [26, 29].

Various bone parameters have changed/improved when rats were fed with FOS or inulin. One study in rats showed that the various fructans could have different effects. In this small study, rats were fed with either short-chain FOS (DP2-8), inulin (DP>23), or a mixture of these (92 % inulin/8 % FOS) at 5 % of the diet for 4 weeks. While the fibers did not have a significant effect on calcium balance, ex vivo femur bone density was significantly higher in the group fed with inulin. Urinary excretion of C telopeptide of type I collagen, a marker of bone resorption, was also reduced significantly in the inulin-fed group [23]. Feeding intact rats with 5 % of either GOS or FOS improved bone mineral content (BMC) [25], enhanced bone volume [15], and increased whole-body bone mineral density (BMD) [20]. In ovariectomized rats, FOS prevented osteopenia [15] and improved bone ash weight, calcium content and bone microarchitecture [21].

GOS at varying levels (2–8 % of the diet) significantly decreased cecal pH and increased wall weight and content weight in a dose-dependent manner. Calcium absorption, femur calcium uptake, calcium retention, and femur strength were significantly improved [13]. Feeding GOS increased the relative proportion of bifidobacteria in the large intestine, thereby changing the colonic bacterial community structure. Weaver et al. [30] compared eight different novel fibers to cellulose over 12 weeks in rats. The rats were fed with the fibers at 4–5 % of their diets. Two resistant starches, soluble fiber dextrin and polydextrose, increased bone calcium content, while soluble corn fiber and soluble fiber dextrin improved whole-body BMC, BMD, including having a specific effect on bone structure. Cortical thickness and area, as well as bone strength, were also improved. Soluble fiber dextrin as well as a mixture of inulin and FOS

Table 13.1 The effect of 5 % dietary soluble corn fiber on various bone parameters in the growing rat

Bone parameter	Positive change (%)
Total body BMC (g)	7.6
Total body BMD (g/cm^2)	2.9
Femur	
Volumetric bone density (g/cm^2)	8.3
Cortical area (mm^2)	19.6
Cortical thickness (mm)	22.4
Peak breaking force (N)	8.8

Based on data from Ref. [30]

significantly improved zinc retention, while soluble corn fiber improved magnesium retention (Table 13.1).

Human Studies

Studies in humans at various ages have shown some effects of fermentable carbohydrates on calcium absorption and retention. In adolescents, 5 g of FOS per day in orange juice improved fractional calcium absorption by 10 % [31]. Griffin et al. (2002) supplemented young girls with 8 g/day of FOS or a mixture of FOS and inulin on top of a sufficient calcium intake of 1,200–1,300 mg/day for 3 weeks [32]. The FOS alone did not have a significant effect, but the mixture enhanced calcium absorption by 18 % in comparison to the control group. Urinary calcium did not change, so it may be assumed that the absorbed calcium was retained. A further study by Griffin et al., in girls aged 10–15 years, showed that 8 g of a mixture of FOS and inulin per day over 4 weeks improved calcium absorption from 33 to 36 %. The most significant effect was found in those girls with a lower habitual calcium absorption [33]. Coudray et al. in 1997 showed that 40 g chicory inulin per day stepwise increased for 26 days improved calcium absorption from 21 to 33 %, a 58 % increase [34]. In contrast, a study in young adults by Van den Heuvel et al. [35], where the diets were supplemented with 15 g inulin per day for 21 days, and a study by Martin et al. [36] using 9 g/day of a FOS/inulin mixture had no effect on calcium absorption.

Griffin et al. (2002) speculated that if the additional calcium obtained from the rise in calcium absorption was retained daily over 2 years during maximum bone mineralization as would take place during adolescence, a net increase of 65 g relating to 5.5 % gain in bone mass could take place [32]. In 9- to 13-year-old children, 1 year of supplementation, with 8 g/day of inulin, resulted in a significant increase in whole-body bone mineral content (245 ± 11 vs. 210 ± 10 g) and BMD, compared to the control group [37].

Tahiri et al. [38] investigated the effect of 10 g/day of FOS for 5 weeks on calcium absorption in older women. No significant effect was found, but sub-analysis indicated that this dose may have affected absorption in women more than 6 years past menopause. In another study conducted in older women, more than 5 years past menopause, 10 g/day of lactulose for 9 days and 10 g of a mixture of FOS and inulin for 6 weeks significantly increased mean calcium absorption (by 5 and 7 %, respectively) [39–41]. In women who were a minimum of 10 years past menopause, 10 g/day of a mixture of FOS/inulin for 6 weeks significantly increased true fractional calcium absorption compared to the control group [39].

Data on specific bone effects in older adults are sparse. Bone turnover markers do not seem to respond well to prebiotics. Tahiri et al. [38] failed to measure an effect on osteocalcin, a marker of bone turnover, and urinary excretion of deoxypyridinoline, a marker of bone resorption. Holloway et al. [39] showed a change in biomarkers but only in the women in whom calcium absorption was significantly improved.

Coxam [42] summarized the various human studies and commented on the possible impact of prebiotic supplementation at various ages. Studies by Van der Heuvel et al., Griffin et al., and by Abrams et al. [31–33, 37, 43] indicated that prebiotics could affect peak bone mass accrual and help optimize peak bone mass. Calcium absorption in older adults could be improved, [34] while in women with postmenopausal osteoporosis, several studies were inconclusive. In elderly women, studies by Tahiri et al., Van der Heuvel et al., Kim et al., and Holloway et al. [38–40, 44] reported improved calcium absorption, with only

two reporting an effect on bone metabolism [41, 44]. Long-term effects of prebiotics on bone health and risk of fracture therefore need further investigation.

Probiotics

The endogenous bacterial population can be manipulated by introducing exogenous bacteria into the colonic microflora, and these exogenous bacteria are called probiotics. Probiotics may be defined as viable microorganisms that (when ingested) have a beneficial effect on the health and metabolism of their host. The most popular strains are represented by the following genera: Lactobacillus, Streptococcus, and Bifidobacterium [7, 45, 46].

The mechanisms by which probiotics exert biological effects are not clear, and research is still required to confirm the possible mechanisms. One suggestion is that the indigenous anaerobic flora limits the concentration of potentially pathogenic flora in the digestive tract. Although probiotic bacteria are thought to mediate their effects by using some of the same mechanisms as the native intestinal flora, probiotics may also work through other modes of action such as supplying enzymes or influencing enzyme activity in the gastrointestinal tract or synthesizing vitamins [7, 45–48]. Other suggested mechanisms of action include degradation by probiotics of the mineral complexing phytic acid and stimulation of calcium uptake by the enterocytes [46].

Only few studies have been published on the effect of probiotics on mineral absorption. In 1994 one of the first studies to be done using probiotics reported that feeding *Bifidobacterium longum* BB536 to rats, with or without lactulose, increased breaking strength of the bones [49]. When rats were fed with yogurt containing probiotics, short-chain fatty acid production was increased and calcium absorption improved [50]. Perez-Conesa et al. [51] fed functional follow-on infant formulae containing pre- and/or probiotics to rats and found that calcium and magnesium absorption was enhanced and bone calcium improved in groups fed with synbiotics, a mixture of pre- and probiotics. The formula containing only probiotics improved tibial calcium content significantly compared to the control diet devoid of pre- and probiotics. These results suggested that probiotics even in the absence of prebiotics may affect mineral balance.

Narva et al. [52] studied the effect of a bioactive peptide, valyl-prolyl-proline (VPP), in water or *Lactobacillus helveticus*-fermented milk in the ovariectomized (OVX) female rat. In this model *Lactobacillus helveticus*-fermented milk containing VPP attenuated bone loss due to OVX by 16 % compared to water plus VPP. The fermented milk also significantly increased tibial moment of inertia. These results should be interpreted with caution as the calcium intake between the OVX control group and the OVX group receiving the fermented milk was significantly different. The authors did consider differences in nutritional intakes and concluded that the difference in calcium intake was probably too small to affect bone density significantly. They do point out that other nutrients in the fermented milk also could have had an effect.

Perez-Conesa et al. [53] found that *Bifidobacterium bifidum* and *Bifidobacterium longum* significantly increased femoral and tibial calcium content in weanling rats when fed for 30 days. These authors showed that probiotics increase crypt depth in the colon and lowered colon pH compared to a control diet with no probiotics. Calcium absorption was correlated with the pH of the colonic contents. *Lactobacillus rhamnosus* HN001 improved calcium and magnesium retention in growing male rats, but due to differences in food intake, the results were inconclusive. In the female OVX rat, HN001 reduced the rate of bone loss and improved bone density after 12 weeks of feeding in comparison to the OVX control (Fig. 13.2). HN001 had no effect on the bone resorption marker, CTX-1 [54]. Scholz-Ahrens et al. [46] tested prebiotics alone and in combination with *Lactobacillus acidophilus* NCC90 using the female OVX rat model. In this model the prebiotics lowered cecal pH over 16 weeks of feeding, as reported before, and increased cecal content weight, but the probiotic strain used had no effect on cecal pH or weight.

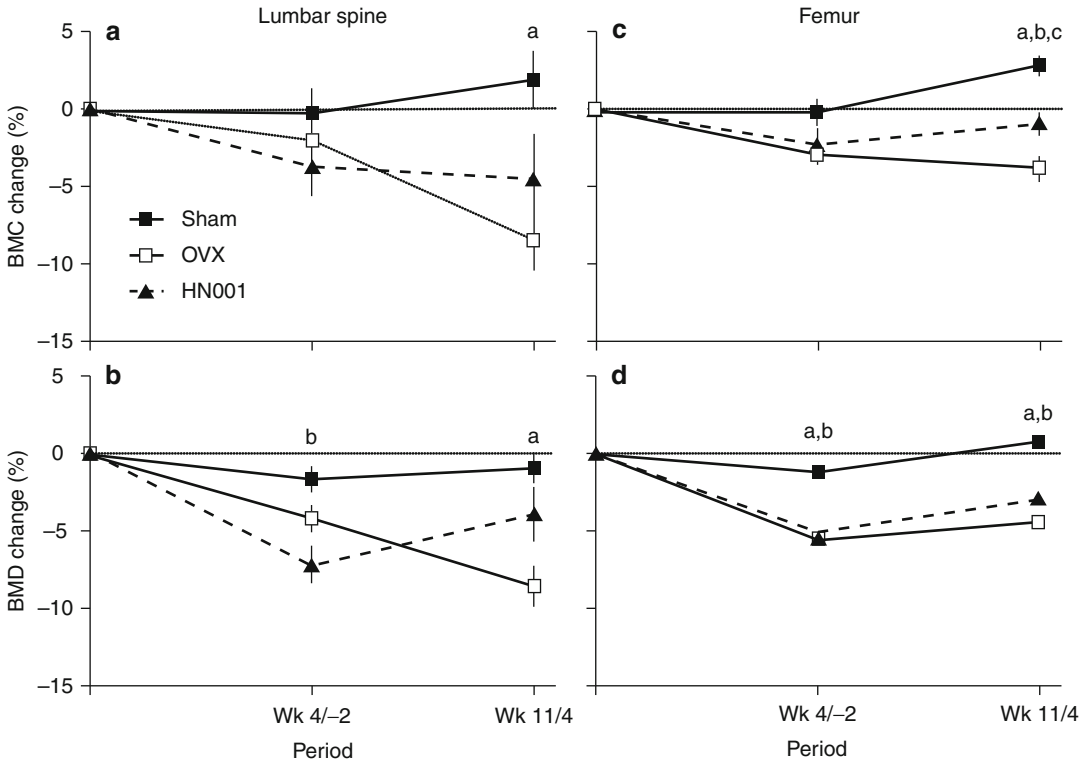

Fig. 13.2 The percentage change of lumbar spine and femur bone mineral content (**a**, **c**) and density (**b**, **d**) of female Sprague Dawley rats between weeks 4 and 2 and weeks 11 and 4. The rats were fed a casein-based diet based on AIN93. The experimental group received 10^9 CFU HN001 per day for 12 weeks. An "a" represents $P<0.05$ for Sham vs. OVX, a "b" for $P<0.05$ for Sham vs. HN001, and a "c" for $P<0.05$ for OVX vs. HN001 (Reprinted from Kruger et al. [54]. With permission from Dairy Science Technology)

While calcium balance was improved by the combination of pre- and probiotics (synbiotics), only the prebiotics improved calcium balance significantly. Bone parameters such as bone calcium content were improved by the synbiotics, but bone structure was only improved by the prebiotic diets.

Narva et al. [55] reported results of a small study where they compared the effect of milk fermented with *Lactobacillus helveticus* compared to a control on acute changes in calcium metabolism in postmenopausal women. He reported a reduction in serum parathyroid hormone and a raise in serum calcium but no effect on a marker of bone resorption. Cheung et al. [56] in a randomized crossover study compared calcium absorption from fortified soy milk in using normal and fermented milk. The fermented milk was inoculated with *Lactobacillus acidophilus.* No difference was observed between groups.

The calcium absorptive and bone-enhancing effects of the pre- and probiotics are affected by the experimental conditions and the physiological characteristics of the target group. Dietary conditions include the calcium level of the diets and the level of pre- or probiotics provided. In humans the physiological age, which will determine the calcium need, higher for growing adolescents, or after menopause, would affect the outcome. The postmenopausal stage is also important, as it seems as if women more than 6 years past menopause may benefit more by adding pre- or probiotics to their diets. And lastly, calcium absorptive capacity will affect the level to which pre- and probiotics could stimulate absorption.

Long-Chain Polyunsaturated Fatty Acids

Dietary lipids influence bone density by altering the efficiency of intestinal calcium absorption as well as by regulating the processes of bone remodeling and mineralization. Several epidemiological studies have demonstrated a relationship between fat intake and bone health. NHANES III (National Health and Nutrition Examination Survey (1988–1994)) is an ongoing nationally representative survey conducted in the United States involving a large number of men and women of different ages. Data from 14,850 participants were analyzed to determine the relationship between diet and bone density. Total fat intake was negatively associated with hip bone mineral content and density. The effect was evident in both sexes but was more pronounced in men than in women and most profound when total saturated fat intake was compared to bone mineral measurements [57]. More recent data from the Women's Health Initiative also reported a negative association between hip fracture risk and saturated fat intake [58]. Total lower fracture risk was associated with higher intakes of monounsaturated fatty acids and PUFAs, whereas a higher total fracture risk was associated with a high intake of omega 3 fats specifically [58]. Results from the Framingham Osteoporosis study found that higher intakes of fish (>3 servings per week) were associated with maintenance of femoral neck BMD in men and women; in women higher intakes of omega 3 fats had a higher baseline femoral neck BMD [59]. Data from the same study also indicated that blood phospholipid PUFA levels may be associated with risk of fracture [60]. In contrast, data from the Cardiovascular Health Study reported no association between intakes of LCPUFA and fracture risk, and only small differences in BMD were detected when data were analyzed based on fish and PUFA intake [61].

Humans lack the ability to synthesize fatty acids with a double bond past the carbon-9 position; therefore, these fatty acids must be obtained from the diet and hence are termed "essential." Essential fatty acids (EFAs) are classified into one of two families designated as omega 3 and omega 6 depending on the location of the first unsaturated carbon from the methyl terminus [62]. Alpha-linolenic acid (ALA) (18:3) is the parent compound for the omega 3 series of fatty acids, and linoleic acid (LA) (18:2) is the parent compound for the omega 6 series. The most common and widely recognized dietary source of omega 3 EFAs is fish oil, although α-linolenic acid is present in the chloroplasts of green leafy vegetables and also in some plant oils such as canola. Omega 6 EFAs are found in many edible oils as linoleic acid is found in the seed oils of most plants [63]. Evening primrose oil and most of the vegetable oils used to make margarine contain omega 6 EFAs. The EFAs are elongated and desaturated by endogenous enzymes to form longer-chain PUFAs. The most important of the longer-chain fatty acids are arachidonic acid (AA) of the omega 6 series and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of the omega 3 series. The latter two fatty acids are found in high levels in oily fish, such as tuna and salmon, and fish oils. The longer-chain PUFAs are precursors for various eicosanoids such as prostaglandins and leukotrienes which have a regulatory role in the body.

EFA deficiency reduces intestinal mucosal mass [64]. Dietary EFAs appear to have a trophic effect on the intestinal mucosa and may aid in promoting mucosal recovery after surgery or injury [65]. The fluidity of intestinal cell membranes also increases as the level of membrane lipid unsaturation increases. Aside from changing the physical structure of the intestinal mucosa, dietary fat can also influence the release of enterotrophic peptides. Bolus dosing of long-chain essential fatty acids has been reported to significantly increase the release of peptide tyrosine-tyrosine and enteroglucagon [66].

Dietary intake of omega 3 and omega 6 EFAs is correlated with increased duodenal ion transport. Several mechanisms have been proposed to explain the effect of EFAs on intestinal calcium transport. One possibility is that enhanced membrane fluidity as a result of increased incorporation of EFAs into phospholipids alters the physical environment of membrane-bound enzymes,

thereby enhancing their activity [67]. Another possibility is that EFAs may mimic, or facilitate, the action of vitamin D in promoting calcium absorption. Ca^{2+}-ATPase is the rate-limiting enzyme in active calcium transport. It is upregulated by the calcium-binding protein calmodulin. Unsaturated fatty acids bind with Ca^{2+}-ATPase mimicking the action of calmodulin and stimulating Ca^{2+}-ATPase activity [68]. DHA (but not EPA or AA) has been reported to increase Ca^{2+}-ATPase activity in the absence of calmodulin [68]. In addition, vitamin D receptor (VDR) availability, which is increased by ovariectomy in female rats, is reduced after EFA supplementation [69]. This is possibly indicative of increased vitamin D binding to the receptor.

Several intervention studies involving feeding rats with different combinations of omega 3 and omega 6 EFAs have been conducted. The majority of these studies have utilized OVX rats. Positive results in terms of decreased levels of bone resorption markers, increased levels of bone formation markers, and/or increased bone density measured by DEXA have generally been obtained. It appears that both omega 3 and omega 6 EFAs are required for maximal inhibition of loss of bone density post-ovariectomy. Synergism may exist between the two EFA families; however, the optimal ratio of omega 3/omega 6 EFAs or the effects of individual omega 3 and omega 6 EFAs are yet to be determined.

Few human studies using LCPUFA supplementation and measuring bone outcomes have been conducted. Fish oil supplementation (4 g/day) or a mixture of fish oil and evening primrose oil for 16 weeks in elderly, osteoporotic women increased levels of serum calcium as well as levels of the bone formation marker osteocalcin [70]. Supplementation of elderly, osteoporotic/osteopenic women who had habitually low dietary calcium intakes with 6 g of high PUFA oil in conjunction with 600 mg calcium carbonate per day for 18 months resulted in maintenance of lumbar spine bone density compared to a 3.2 % decrease in lumbar spine density in the control subjects. LCPUFA supplementation for a further 18-month period resulted in an increase of 3.1 % in lumbar spine bone density [71]. PUFAs, therefore, appear to be beneficial in treating senile osteoporosis which is often caused by low dietary calcium intake, a decreased ability to absorb dietary calcium, and decreased vitamin D status as a result of lifestyle and metabolic factors associated with aging [63, 67]. More recent studies supplementing omega 3 fats reported a significant effect by 900 mg omega 3 PUFAs on a bone resorption marker over 6 months [72], improvement of bone formation markers after supplementing with a PUFA, and vitamin-fortified milk for 1 year [73], but in a shorter study, no effect on the bone resorption marker CTx after 3 months of supplementing with 1.48 g omega 3 fats was observed [74]. Exercise combined with 1,000 mg/day of omega 3 fats reduced inflammatory markers and CTx in postmenopausal women and improved femoral neck BMD over 6 months [75].

A recent study by Järvinen et al. [76] reports a positive relationship between dietary PUFAs and BMD at the lumbar spine in 554 women older than 60 years. These findings were significant only in those women who did not use hormone replacement therapy. These authors conclude that PUFAs may be especially important for bone integrity and maintenance in older women. Poulsen et al. [77] suggested that there may be an increased need in women for LCPUFAs after menopause. The fatty acid composition of adipose tissue changes with age, with an increase in adipose tissue content of AA, docosapentaenoic acid (DPA), and DHA [78]. Serum phospholipid composition as well as fatty acid composition of membranes changes after menopause [79], and higher serum levels of DHA in women of this age were reported compared to men [80]. Estrogen may also increase the synthesis of AA and DHA from their precursors [80].

Aging and menopause may lead to a reduction in the ability of endogenous enzymes to convert ALA and LA into the longer-chain metabolites. In addition, intake of omega 6 EFAs increases PGE_2 which in turn stimulates synthesis of proinflammatory cytokines such as interleukin −1 (IL-1), interleukin 6 (IL-6), and tumor necrosis factor-α (TNF-α) [62]. Estrogen deficiency also results in elevation of the levels of these cytokines,

and all three cytokines are known to promote bone resorption [81]. Omega 3 EFAs downregulate production of all prostaglandins but particularly those derived from omega 6 EFAs such as PGE_2. As a result, omega 3 EFAs act as anti-inflammatory agents, inhibiting cytokine synthesis [82]. Estrogen deficiency results in increased bone resorption as well as formation, although the increase in rate of formation is less than that of resorption. This leads to reduced bone density and bone loss. Modulating the dietary ratio of omega 6:omega 3 EFAs may be a means of at least partially compensating for the effects of estrogen deficiency postmenopause. The evidence to date from cross-sectional as well as intervention studies suggests that during estrogen deficiency, a higher consumption of omega 3 EFAs may be beneficial in reducing bone density losses. A recent review summarizes the suggested mechanisms of action of the long-chain PUFAs on bone cells [83]. These actions involve modulation of fatty acid metabolites such as resolvins and protectins, several signaling pathways, cytokines, and growth factors. Omega 3 PUFAs may be protective as they could reduce synthesis of PGE_2, suppress inflammatory cytokines, and give rise to lipid mediators which are anti-inflammatory and thereby reduce bone resorption [83].

It is feasible that the PUFAs may also act similar to a prebiotic agent and affect intestinal microbiota and calcium absorption. In gnotobiotic pigs, omega 3 supplementation affected adhesion of *Lactobacillus paracasei* to the jejunal mucosa and increased the number by 12 % in comparison to the control group. In this particular study, the authors did not measure any mineral uptake activity but inferred that the improvement in adhesion of the lactobacilli could inhibit digestive tract pathogens [84]. Andersen et al. [85] supplemented infants with 5 mL fish oil or sunflower oil per day from 9 to 18 months of age and collected stool samples. Molecular fingerprints of the bacterial DNA were obtained by terminal restriction fragment length polymorphism (T-RFLP). These profiles indicated a few new T-RFs became more dominant, together with an overall increase in diversity of the microbiota. Profiles were also affected if the children were breast-fed during the oil supplementation. Breastfeeding limited the gut response to the oils. In 10-month-old infants, fish oil supplementation could only affect gut microbiota in children fed with cow's milk and not in those fed with infant formula [86]. Finally, free fatty acids, LA, AA, ALA, and DHA, were added to growth medium in physiological concentrations to assess their effects on growth and adhesion of *Lactobacillus GG, Lactobacillus casei,* and *Lactobacillus bulgaricus* [87]. Concentrations at 10–40 μg PUFA/mL inhibited growth and mucus adhesion of all tested strains. The PUFA also altered adhesion sites on Caco-2 cells, and an improvement in adhesion and growth was not consistently measured. These observations should be investigated further as adhesion to mucosal surfaces is necessary to support the health-promoting effects of the probiotics.

Summary

Evidence is presented that addition of prebiotics or probiotics to the diet may improve calcium absorption by modulating intestinal microbiota, increasing SCFA synthesis, and solubilizing calcium. The intestinal wall may also be affected by the pre- and probiotics increasing absorptive surface. In addition, LCPUFA may modulate intestinal membrane composition and pumps, thereby affecting active calcium absorption. LCPUFA however may also affect intestinal microflora, having a prebiotic effect. No research has been done linking a prebiotic effect by LCPUFAs to calcium metabolism. Further research is required.

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