

Inulin-type fructans and bone health: state of the art and perspectives in the management of osteoporosis

Véronique Coxam

Groupe Ostéoporose, U3M, INRA Theix, 63 122 Saint-Genès Champanelle, France

If the primary role of diet is to provide sufficient nutrients to meet the metabolic requirements of an individual, there is an emerging rationale to support the hypothesis that, by modulating specific target functions in the body, it can help achieve optimal health. Regarding osteoporosis prevention, since Ca is most likely to be inadequate in terms of dietary intake, every strategy targeting an improvement in Ca absorption is very interesting. Actually, this process may be susceptible to manipulation by fermentable substrates. In this light, inulin-type fructans are very interesting, even if we need to gather more data targeting bone metabolism before health professionals can actively advocate their consumption to prevent senile osteoporosis. Besides targeting the prevention of postmenopausal osteoporosis, inulin-type fructans still remain a source for putative innovative dietary health intervention. Indeed, given in combination with isoflavones, they may have a potential for maintaining or improving the bone mass of human subjects, by modulating the bioavailability of phyto-oestrogens.

Inulin: Oligofructose: Osteoporosis: Prevention

With the human race experiencing a progressive increase in life expectancy, all industrialised countries face a continuous increase in the number of the elderly and, consequently, a growing prevalence of chronic diseases. Since women experience a longer life expectancy, they are more likely to require increased medical care leading to high overall acute and chronic health care cost. This is why osteoporosis, a generalised condition of the bone whose hallmark is increased bone fragility, has become a major health and socio-economic problem worldwide.

It has become commonplace to refer to osteoporosis as multifactorial and two main divisions were suggested many years ago: postmenopausal and senile osteoporosis. Actually, there is an overwhelming body of evidence emphasising that gonadal failure at the time of menopause causes osteopenia and that the administration of oestrogens in postmenopausal women prevents this loss, while in the elderly, because bone is mainly built up with Ca for which deficiency is common, the primary goal of a nutritional strategy is to provide a sufficient bioavailable amount of this nutrient to optimise the genetic potential (Coxam & Horcajada, 2004).

Basically, research in nutrition has led to an exciting emerging rationale supporting the hypothesis that, by modulating specific target functions in the body, diet can help achieve optimal health. Furthermore, it is commonly believed that nutrients interact, thereby altering one another's requirements. In fact, what may not have been expected is the considerable magnitude of these interactions with regard to critical bone nutrients. The effect of non-digestible carbohydrates on nutrients involved in bone health is a good example of such modulations and, in this light, they may provide a useful tool to prevent bone loss in both postmenopausal and ageing women.

The problem of osteoporosis

Osteoporosis has been defined as a systemic disease characterised by low bone mass and microarchitectural impairment of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture (Consensus Development Conference, 1993).

The current lifetime risk of osteoporotic fracture for 50-year-old women is estimated at nearly 40%, higher than the total risk of having breast, endometrial and ovarian cancer (Cummings *et al.* 1985). This is why this health problem is reaching near-epidemic proportions worldwide. Indeed, the disease affects around 75 million people in Europe, the United States and Japan combined, including one in three postmenopausal women, the majority of elderly people and a substantial number of men (Consensus Development Statement, 1997). On a global scale, an average of 407 000 hip, 270 000 clinically diagnosed vertebral and 340 000 forearm fractures are recorded each year in Europe (Cooper *et al.* 1992). Furthermore, if the current trends continue, confident projections in the next two decades support the hypothesis that the magnitude of this disorder will worsen by 60%, unless a suitable intervention can be devised, leading to about 650 000 hip fractures in Europe (Cooper *et al.* 1992). Although the disorder is only clinically relevant once a fracture has occurred, it results in a considerable fiscal drain in terms of health care, the corresponding hospital expenditure being higher than that for other pathologies of the elderly, i.e. more than €10 million (Report from the European Community, 1999). Moreover, approximately 50% of elderly hip-fracture patients do not regain full independence and more than 20% die within 6 months, through

complications connected with the fracture. Thus efforts are needed to improve prevention measures and integrate complementary alternative therapies of proven value.

As currently available treatments never restore the micro-architecture of the bone, and because bone mineral status in later life is the net outcome of lifelong influences on skeletal mineral accretion and loss, there is now an emerging rationale for early intervention, which may actually be best achieved by initiating sound health behaviours early in life and continuing them throughout life.

Prevention through dietary means is especially challenging in technologically advanced societies. For this purpose, a large number of nutrients have been considered as possible determinants of bone health. However, because among the bone-building nutrients Ca is the most likely to be inadequate in terms of dietary intake (Weaver, 2000), functional foods designed to prevent osteoporosis could work by providing Ca, a key nutrient for bone development and maintenance, or by enhancing its absorption or retention to build peak bone mass or to suppress bone turnover (Weaver & Liebman, 2002).

The rationale for calcium

Ca is critical to achieve optimal peak bone mass and modulate the rate of bone loss associated with ageing. Therefore, if it is inadequate during growth, the full genetic programme for skeletal mass cannot be achieved then if Ca intake is too low to offset obligatory losses, acquired skeletal mass cannot be maintained (Heaney, 2003).

The adult body contains about 1 kg Ca and all but 1–2% (as a messenger in a variety of neurological and other cellular signalling events) is in bone. As a result, bone mineralisation is coupled to Ca intake and absorption and any factor that affects the amount retained by the body will alter total bone mineral mass. Furthermore, given the small metabolic pool of Ca, circulating concentration is mainly maintained at the expense of the skeletal compartment, i.e. from an increased bone resorption (Cashman, 2002). Consequently, the size of the bony reserve for Ca will depend on the daily balance between absorbed intake and excretory loss.

Calcium and bone health (Fig. 1)

During childhood. The demand for Ca during this critical period of skeletal growth is probably greater than at any other time of the life cycle. Results from clinical trials are relatively consistent and the emerging picture is that any increase in Ca intake may result in higher bone mineral acquisition rates in children and adolescents, and the improvement is more pronounced when baseline Ca intake is low.

Indeed, Lee *et al.* (1993), recording the Ca intake from birth up to 5 years, found a positive correlation with bone mineral density (BMD) at the wrist. In a 3-year supplementation study (718 mg Ca, baseline being at 894 mg) carried out in twins, an increased BMD was reported at the spine and radius (Johnston *et al.* 1992). Similar data were observed in adolescent girls given either extra dairy products (Chan *et al.* 1995); or 500 mg supplemental Ca for 18 months (in addition to dietary levels of 960 mg/d), a 4% gain in total skeletal Ca being demonstrated (Lloyd *et al.* 1993). These results are consistent with the bone retention of approximately 1–5% (depending on the skeletal site) associated with an

increased Ca intake in the following trials: Andon *et al.* (1994), Lee *et al.* (1994). However, this relationship is much less robust during pubertal growth spurt than during prepuberty, the growth pattern being mainly dominated by sex hormones (Anderson *et al.* 1996). Furthermore, even if the skeletal benefits are not maintained when the Ca supplementation is withdrawn (Slemenda *et al.* 1993; probably because of the 'bone remodelling transient' phenomenon), the gain in bone density throughout the first several decades translates to lower risk of fracture later in life (Matkovic *et al.* 1979, 1990). It is thus likely that variations in Ca nutrition early in life can account for as much as a 5–10% difference in peak bone mass and contribute more than 50% to the hip-fracture rates later in life. Indeed, there is evidence that a high past intake of milk is beneficial to bone health later in life (Reid & New, 1997).

In adults/premenopausal women. BMD is relatively stable between the ages of twenty and fifty; this is probably why trials examining the effect of Ca in premenopausal women have shown conflicting results. For example, a 4-year study of 500 mg supplementation had no significant effect on BMD (Smith *et al.* 1989), while a 3-year study, increasing the Ca intake by 610 mg/d, resulted in less bone loss than in the placebo group (Baran *et al.* 1990). In the same way, total body BMD was increased after 1 year of supplementation with 1000 mg/d in the trial published by Rico *et al.* (1994).

To summarise, a meta-analysis performed on thirty-three eligible trials in young adults found that 1000 mg Ca/d could prevent the loss of 1% bone per year at all sites except the ulna (Welten *et al.* 1995).

In early postmenopausal women. Menopausal status is the overriding factor determining bone loss in women in their early fifties. Thus, given the tremendous impact of gonadal hormones on bone health, a high Ca intake will not offset osteopenia that occurs immediately following menopause.

Indeed, according to supplemental studies, an increase in Ca consumption during early menopause is not effective in retarding bone loss from trabecular regions of the skeleton, although it appeared to slow cortical bone loss (Polley *et al.* 1987; Riis *et al.* 1987; Smith *et al.* 1989; Dawson-Hughes *et al.* 1990; Aloia *et al.* 1994; Elders *et al.* 1994; MacDonald *et al.* 2004). However, these changes are very small and tend not to be sustained.

Nevertheless, if the effect of Ca is small, it is still important because it is quite clear that the bone loss observed in untreated postmenopausal women is exacerbated by a Ca deficiency. Thus, in a Yugoslavian population, the bone mass was found to be 5–6% greater in the district with high Ca, compared to the area with low intake (Matkovic *et al.* 1979).

In the elderly. The rationale for recommending a high Ca intake in the elderly lies in the decrease in Ca absorption efficiency with age. Several studies have shown Ca supplementation to be effective in reducing bone loss at various sites, especially the hip (Dawson-Hughes *et al.* 1990; Nelson *et al.* 1991; Reid *et al.* 1993; Chevalley *et al.* 1994; Devine *et al.* 1995; Prince *et al.* 1995; Reid *et al.* 1995).

To summarise, in the meta-analysis published by Heaney (1996), twenty-three out of twenty-eight Ca intervention studies that excluded early postmenopausal women were positive, and this is verified in all the twelve that controlled for Ca intake. Another meta-analysis yielded similar results, i.e. a Ca supplementation of 1000 mg/d could prevent loss of approximately 1% of the bone mass per year in postmenopausal women not on oestrogen (Cumming, 1990).

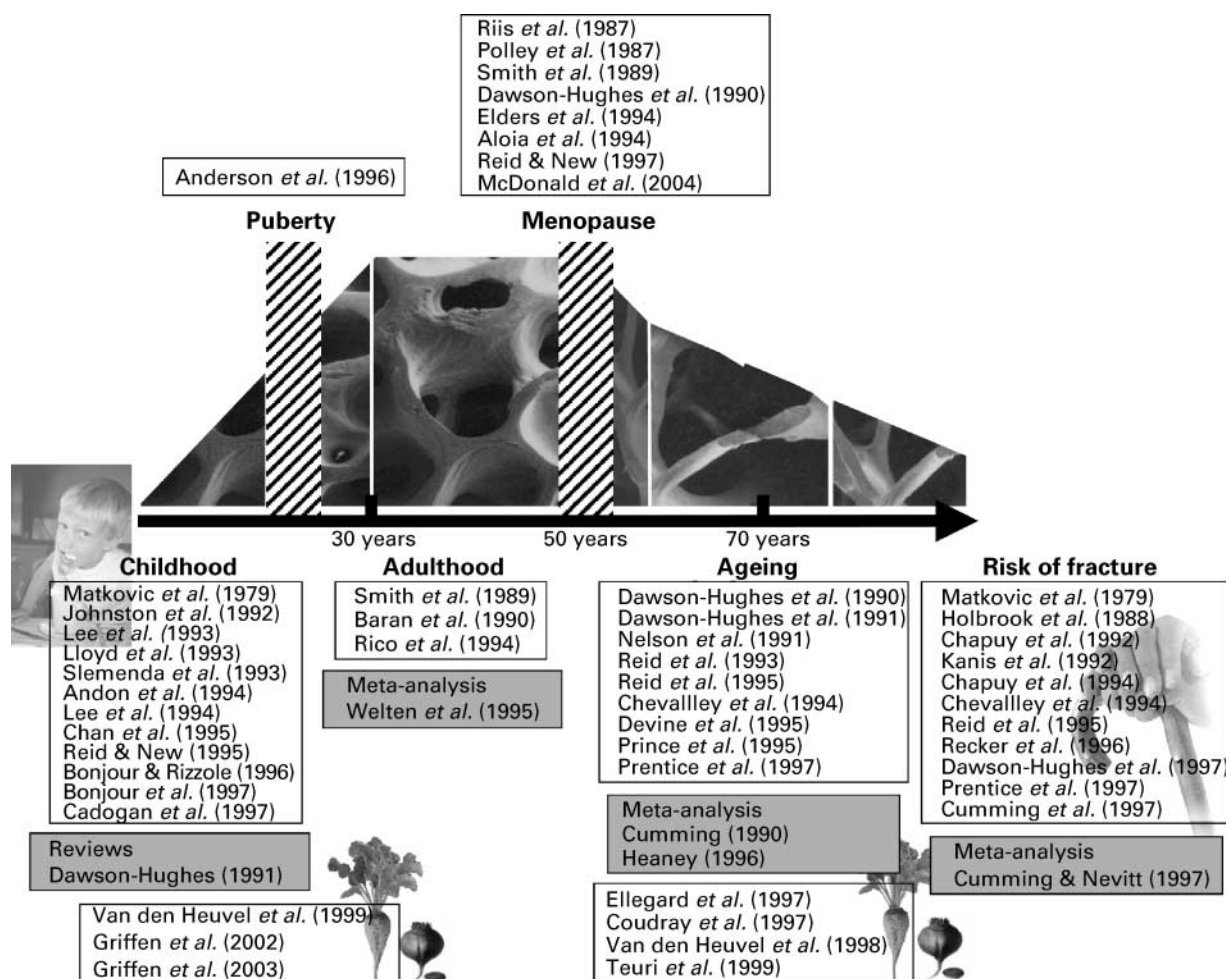


Fig. 1. (A) Main clinical trials (□) and reviews or meta-analyses (■) reporting the effect of Ca supplementation on bone health. (B) Supplementation studies with inulin-type fructans (☞).

Risk of fracture. Although the increase in BMD from additional Ca intake is encouraging, the most important outcome variables are bone fractures, and the body of evidence suggests that Ca supplementation may be effective in preventing them.

These data are supported by the retrospective study by Matkovic *et al.* (1979). Moreover, Reid *et al.* (1995) found, after 4 years of supplementation, nine fractures in seven women in the placebo group and only two in the Ca group. In the same way, in the study by Chevallley *et al.* (1994) six of the fifty-four subjects on Ca developed a vertebral fracture, a much lower figure than that observed in the placebo group. Impressive reductions in hip fractures have also been achieved in elderly women, living in nursing homes, who take a combination of Ca and vitamin D (Chapuy *et al.* 1992, 1994), with protection becoming apparent after 6–12 months of treatment and by 18 months fractures being reduced by more than 30%. Again, individuals on high Ca intake were found to have fewer fractures in the 14-year longitudinal study by Holbrook *et al.* (1988). Finally, a significant reduction in the relative risk of fracture was also demonstrated in the large case–control study by Kanis *et al.* (1992).

In conclusion, most studies are consistent and support the public health policy regarding Ca intake. This was confirmed by the meta-analysis of sixteen observational studies that reported

a small but consistent reduction of fractures (1 g/d being associated with a 24% reduction in the risk of hip fracture; Cumming & Nevitt, 1997).

Calcium dietary intakes

Because, in an adult, the bone mass present at any time in life is a function of the amount achieved at maturity and lost with ageing, and given the fact that the body cannot store Ca in bone above the level of current need, the Ca requirement remains high throughout life. This is reflected by a long history of Ca intake recommendations, even if controversy continues concerning the levels to be recommended (Mertz, 2000).

Current exposure in contemporary people is often only a small fraction of what their primitive ancestors experienced (Heaney, 1996). Indeed, the plant food eaten by hunter-gatherers used to provide a Ca intake that, adjusted for differences in the body size, would have been in the range of 2000–4000 mg/d in adults (Eaton & Nelson, 1991). This is why mechanisms to protect the organism from getting too much of this nutrient have been developed, while the obligatory Ca losses in sedentary adults on typical diets, through shed skin, nails, hair, sweat, urine and digestive secretions, are generally in the range of 160–240 mg/d (Heaney, 1999). Therefore, every strategy

targeting an improvement in the Ca balance is very interesting. However, despite educational efforts and the increased availability of an array of Ca-fortified foods and supplements, intake of Ca remains inadequate. According to the results from the Third National Health and Nutrition Examination Survey in the USA (National Center for Health Statistics, 1994), Ca intakes among females are below the recommended levels after childhood, even when taking into account Ca from supplements (Looker, 2003). This is consistent with the data from the Continuing Survey of Food Intakes by Individuals, which reported that more than 70% of adult men and women in the United States have low dietary Ca intakes, the average being 657 mg/d (and 925 mg/d in males) after the age of nine (US Department of Agriculture, 1999). This is why increasing Ca intake was a primary objective in the Healthy People 2000 campaign and is still included in Healthy People 2010 recommendations (US Department of Health and Human Services, 2000). It has even been estimated that \$2.6 billion in direct medical cost would be spared if individuals over the age of fifty would consume about 1200 mg of supplemental Ca (Bendich *et al.* 1999). Improving Ca intake is also highly recommended in Europe (Report from the European Community, 1999). Indeed, for example, in Italy, according to the Total Diet Study, daily Ca intake is only 76% of the average recommendation (Lombardi-Boccia *et al.* 2003). Again, over 50% of females aged 12–18 years in Ireland fail to achieve the recommended Ca intake, and in the UK 13–18% of 14–34-year-olds and 8–15% of those over 65 years have habitual intakes below the reference (Van Dokkum, 1995). In the same way, in France, according to the INCA (2000) survey, Ca consumption is less than two-thirds of the recommended dietary allowance in 20% of men, 30% of adolescents, 50% of female adolescents and 50% of men after the age of 65 years, and 75% of women after 55 years.

It is thus worthwhile to consider using a diet providing adequate amounts of Ca to decrease the rate of bone attrition. The dietary Ca deficiency may be addressed in different ways. The requirement for Ca relates to the size of its reserve, in other words, to the total bone mass, and this pool is limited (by the genetic and mechanical factors and the level of intestinal absorption). This means that the skeletal response will occur only when Ca is increased from the deficiency level to a threshold value above which there will be no further benefit from additional increases in intake. In this regard, Ca functions as a threshold nutrient. Furthermore, Ca intake may be inadequate for the straightforward reason that it is low; however even when intake is normal, it may still be inadequate because of subnormal absorption or greater than normal excretory loss. Indeed Ca bioavailability is frequently equated to its absorption, i.e. the first barrier.

In fact, given this low absorbability, the prospect of finding substances that might improve its bioavailability has enticed many scientists. However, ageing is associated with progressive reductions in the functional reserve that reflects a lack of successful response or adaptation to physiologic and physical perturbations. Thus, it seems highly likely that both age-related bone loss and the fracture burden of the elderly is partly due to the decline in Ca and vitamin D status. Among the main causes leading to impaired vitamin D status are reduced mucosal mass, low circulating 25(OH)D levels, partial intestinal resistance to 1,25(OH)₂D and impaired renal conversion of 25(OH)D to 1,25(OH)₂D. This is why it is important to provide a wide array of options targeting an improvement in Ca retention. Few Ca

absorption enhancers have been identified and the emerging evidence has shown that some non-digestible oligosaccharides could be an interesting tool.

Inulin-type fructans, a way to improve bone health?

The process of Ca absorption

Adequate consumption of Ca in conjunction with the optimisation of its absorption is likely to optimise bone mass. Actually, the major site of mineral absorption in man is the proximal small bowel through a saturable, transcellular pathway. During this process, luminal Ca²⁺ crosses the microvillar membrane and then translocates to the basolateral membrane of the enterocytes. It is then released by active transport into the general circulation. Intracellular diffusion is subject to physiological and nutritional regulation via vitamin D that is involved in the biosynthesis of a cytosolic Ca-binding protein, calbindin D_{9k}.

Intestinal Ca absorption can also occur via a passive paracellular route, through the tight junctions between mucosal cells. It is non-saturable, concentration-dependent and occurs over the whole length of the small and large intestine. In fact, increasing paracellular absorption is promising because it is not limited by saturation; it is vitamin D-independent and occurs throughout the length of the intestine (Weaver & Liebman, 2002). Indeed, there is increasing evidence that the colon can absorb nutritionally significant quantities of inorganic micronutrients (Trinidad *et al.* 1996; Younes *et al.* 1996) and this process may be susceptible to manipulation by fermentable substrates. In fact, these compounds help to achieve Ca homeostasis by shifting the major site of mineral absorption towards the large intestine (for a review, see Scholz-Arhens *et al.* 2001).

Conceptual effects of inulin-type fructans on Ca absorption

Fermentable substrates such as prebiotics can alter Ca absorption and thus modify its retention. According to the definition given by Gibson & Roberfruid (1995), a prebiotic is 'a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health'.

Inulin and oligofructose-type products have been investigated, so far, most extensively for their nutritional properties (for review, see Cashman, 2003). Classified as dietary fibres, they are natural constituents of many common foods including fruits, vegetables (artichoke contains 20–65% fructans on solid matter, asparagus 30%, onions up to 50%) and cereals (1–4% fructans in wheat flour). Their average daily consumption in the human diet reaches several grams (3–11 g in Europe; Van Loo *et al.* 1995). Inulin can also be industrially produced from chicory roots by hot water extraction, followed by refining and spray drying. The molecule consists of one moiety of glucose (G) to which a number (*n*; the degree of polymerisation being 10 on average, ranging between 3 and 60) of fructose units (F) are bound by a β bond, according to the G–F_{*n*} chemical structure (Franck, 1998). Oligofructose (G–F_{*n*} and F_{*n*} chains) is a subset of inulin treated by a partial enzymatic hydrolysis, with a degree of polymerisation of about 4 (ranging from 2 to 8; Franck, 1998). This degree of polymerisation, as well as the solubility and the structural arrangement of the carbohydrates, are important for the rate of fermentation.

Basically, it has been speculated that the stimulatory effect of inulin/oligofructose on mineral absorption is mainly due to their prebiotic character. Indeed, they are resistant to hydrolysis by small intestinal digestive enzymes, which are specific for α bonds, because of the β configuration of the anomeric C2 in their fructose monomers that form β 2-1 glycosidic linkages (Roberfroid, 2002). This is why they are selectively fermented by the microflora in the human colon leading to a modulation of the composition of the natural ecosystem (dominated by bifidobacteria, while clostridia are decreased; Roberfroid, 1993; Roberfroid *et al.* 1998), a perceived health-promoting genus (Franck, 1998). They also exhibit dietary fibre effects by increasing the colonic biomass and, consequently, the stool weight and frequency (Roberfroid, 1997).

Their colonic fermentation produces SCFA (essentially acetate, propionate and butyrate) and other organic acids (e.g. lactate) that contribute to a lower luminal pH in the large intestine. Actually, Ca occurs in food as salts or is associated with other dietary constituents and it must be released in a soluble and probably ionised form before it can be absorbed. Consequently, the reduction in pH simply leads to a modification of its speciation and hence solubility in the luminal phase so that the bioavailable amount is increased (Rémésy *et al.* 1993; Ohta *et al.* 1995a). Passive diffusion of Ca in the intestinal part of the small intestine and the beginning of the large intestine is thus improved. It is also possible that SCFA contribute directly to the enhancement of Ca absorption via a cation exchange mechanism (increased exchange of cellular H^+ for luminal Ca^{2+} ; Lutz & Scharrer, 1991). In fact, it has been shown that SCFA directly stimulated Ca disappearance across the colon in man more effectively than a solution containing Ca + NaCl, although the pH of the latter was lower (Trinidad *et al.* 1996).

Further, inulin and oligofructose may also modulate transcellular active Ca transport by altering the activity of vitamin D receptor and increasing calbindin D_{9k} (the intracellular ferry protein involved in the translocation of Ca to the basolateral membrane of mucosal epithelial cells; Ohta *et al.* 1998a).

Another way to contribute to the enhanced mineral absorption is the increased production of butyrate and/or certain polyamines by which inulin and oligofructose might indirectly induce histological (cell growth) and functional (enhancement of the gut's absorptive area) changes in the intestinal epithelium (Roberfroid, 1993; Scholz-Ahrens *et al.* 2001). However, according to data published by Scholz-Ahrens & Schrezenmeier (2002), polyamines appear not to mediate this effect, although Rémésy *et al.* (1993) have shown that inulin is able to stimulate ornithine decarboxylase, the rate-limiting enzyme of polyamine synthesis.

Regarding the safety aspects of these molecules, no evidence of treatment-related toxicity, carcinogenicity or genotoxicity have been observed from the standard toxicity tests conducted at doses far above that anticipated for human exposure (the real issue being not that of safety, but rather that of gastro-intestinal intolerance, signs being demonstrated with intakes above 20–30 g; Hidaka *et al.* 1990).

Inulin-type fructans and bone health: animal experiments

Data on Ca balance

Molecules of interest. Various molecules such as oligofructose (Ohta *et al.* 1993, 1994, 1995b, 1998a,b,c; Taguchi *et al.* 1994;

Delzenne *et al.* 1995; Morohashi *et al.* 1998; Lopez *et al.* 2000; Takahara *et al.* 2000; Roberfroid *et al.* 2002; Scholz-Ahrens *et al.* 2002), inulin (Levrat *et al.* 1991), galactooligosaccharides (Chonan *et al.* 1995; Chonan & Watanuki, 1996), lactulose (Brommage *et al.* 1993) or resistant starch (Schulz *et al.* 1993; Younes *et al.* 2001) have been studied.

Experimental models. As far as the experimental model is concerned, the stimulation of Ca absorption by inulin-type fructans was shown in young growing rats (Delzenne *et al.* 1995; Ohta *et al.* 1995a), or in animal models of disease or altered physiological status such as ovariectomy (Chonan *et al.* 1995), postgastrectomy anaemia (Ohta *et al.* 1998d), caecectomy (Brommage *et al.* 1993; Ohta *et al.* 1994b), or in Ca-, Mg- or Fe-deficient animals (Ohta *et al.* 1994a). Oligofructose has also been assessed in healthy dogs (Beynen *et al.* 2002).

Arising points. Inulin-type fructans exhibit a dose-dependent effect on Ca absorption. Levrat *et al.* (1991) showed that dietary inulin given in the range of 0–20% in the diet stimulated intestinal Ca absorption in a dose-dependent manner, coinciding with a progressive decrease in caecal or ileal pH, hypertrophy of caecal walls and a rise in caecal pool of SCFA. In the same way, in the study carried out by Brommage *et al.* (1993), a near linear increase in Ca absorption was demonstrated in rats fed on 5 and 10% lactulose-containing diets. However, it has been shown that a diet supplemented with either 10% of inulin or oligofructose (Delzenne *et al.* 1995) or 5% of oligofructose or other non-digestible carbohydrates (Brommage *et al.* 1993) leads to a similar increase (about 60–65%) in the apparent absorption of Ca. Moreover, raising the content of oligofructose in the diet from 2.5 to 10% in ovariectomised rats showed a bone-sparing effect independent of the dose (Scholz-Ahrens *et al.* 2002).

The issue that dietary Ca content modulates the effect of inulin-type fructans on Ca absorption has also been raised (Rémésy *et al.* 1993). In young growing rats, lactulose induced an increase in Ca absorption only if the diet contained 5 g Ca/kg. No effect was seen when the dietary Ca content was 2 g/kg (Brommage *et al.* 1993). In the same way, Chonan & Watanuki (1996) reported a positive effect of the galactooligosaccharides on Ca absorption and bone content with a diet providing 5 g Ca/kg, but not with a 0.5 g Ca/kg diet. Furthermore, Scholz-Ahrens *et al.* (2001) demonstrated a more prominent effect of oligofructose on Ca balance and bone trabecular structure in ovariectomised rats when Ca intake reached levels higher (10 g Ca/kg diet) than the dietary recommended levels of 5 g Ca/kg diet.

Finally, the issue of persistence of the effect is important to consider. The duration of most experimental studies is relatively short (between 14 and 28 d; Scholz-Ahrens *et al.* 2001; Scholz-Ahrens & Schrezenmeier, 2002). Indeed, Brommage *et al.* (1993) reported a significant increase in Ca absorption on the first day of consumption of a diet providing 5% lactulose that disappeared after 7 d, probably because of a down-regulation of the active transcellular route. On the other hand, apparent Ca absorption was still maintained over 4 weeks in the study performed by Ohta *et al.* (1998d) in gastrectomised rats. Similar results, in ovariectomised rats given a 5% oligofructose enriched food, were reported by Scholz-Ahrens *et al.* (2002).

Consequently, studies are necessary to prove that the benefits of these ingredients on Ca absorption persist in the longer-term. Nevertheless, the question of whether these effects can be translated into benefits to bone health must be highlighted.

Effects on bone health

Parameters of bone turnover. Zafar *et al.* (2003) reported a slower bone turnover (both bone formation and resorption were decreased), together with an improved Ca balance in ovariectomised rats fed an inulin-type fructan-rich diet.

Bone mineral content/bone mineral density. In intact rats, supplementation with 5% of either galactooligosaccharide or oligofructose was shown to improve bone mineral content (BMC) (Chonan & Watanuki, 1996). In the same way, in healthy rats, a 5% oligofructose diet was shown to enhance bone volume at the femoral metaphysis (Takahara *et al.* 2000). In addition, in growing male rats, consumption of 5 or 10% chicory inulin increased whole body BMC and BMD (Roberfroid *et al.* 2002). Finally, Kruger *et al.* (2003) have shown in the growing rat that various fructans, with different chain lengths, do not have the same impact on BMD.

In ovariectomised rats, an experimental model for postmenopausal osteoporosis, Taguchi *et al.* (1994) provided evidence for the prevention of osteopenia following surgery when 2.5, 2 or 5% oligofructose was given. An improvement in bone ash weight and Ca content was highlighted in the study performed by Chonan *et al.* (1995) in ovariectomised animals fed a 5% galactooligosaccharides diet for 4 weeks. Still in ovariectomised rats, Scholz-Arhens *et al.* (2002) reported that, at the recommended Ca intake, a diet providing 10% oligofructose increased femur mineral levels, while the 5% oligofructose diet did so only on high Ca consumption.

In gastrectomised rats (and to a lesser extent in sham-operated animals), BMD was significantly higher in the femur and the tibia after treatment with oligofructose (Ohta *et al.* 1998d).

Bone microarchitecture. Scholz-Ahrens *et al.* (2002) demonstrated that in aged ovariectomised rats, oligofructose added to the diet for 8 weeks prevented the loss of trabecular bone induced by oestrogen deficiency (the effect becoming significant when the diet provided 1% Ca).

Bone strength. Zafar *et al.* (2003) failed to elicit a significant modulation of biomechanical femoral properties in ovariectomised rats exposed to a mixture of inulin + oligofructose, as shown by the lack of any change in the resistance to breaking forces, despite of a 5% increase in femoral Ca content.

In conclusion, dietary supplementation with inulin-type fructans enhances the uptake of Ca, improves BMC in growing rats and alleviates the reduction in BMC and BMD that follows ovariectomy or gastrectomy in rats.

Inulin-type fructans and bone health: clinical data

Effect on Ca absorption. Man lacks a large self-contained caecum, a characteristic of the rat. However, limited information on the effects of fermentable polysaccharides on mineral absorption in human subjects suggests that fermentable carbohydrates may also stimulate Ca absorption.

Adolescents. Van den Heuvel *et al.* (1999a) fed twelve healthy adolescents (aged 14–16 years) orange juice supplemented with either 5g oligofructose or 5g sucrose three times daily over a period of 9d, in addition to their usual diet. They measured true fractional Ca absorption by a dual stable isotope technique on urine samples collected for 36h. A significant relative increase (+10.8%) in the fractional Ca absorption was observed upon ingestion of oligofructose.

In the study by Griffin *et al.* (2002), sixty girls at or near menarche, with consumption of Ca approximating the

recommended dietary intake (1200–1300mg/d), were given either a relatively low amount of oligofructose (8g/d) or a mixture of oligofructose and inulin (oligofructose-enriched inulin) over 3 weeks. A 48h urine collection was carried out after isotope administration so as to detect any modulatory effect on Ca absorption. No difference was seen with the oligofructose. On the other hand, while taking the oligofructose-enriched inulin, Ca absorption increased by 18% to an average of 494mg (v. 416mg in the placebo group), i.e. an absolute daily increase in Ca absorption of 90mg. Moreover, because no significant difference in urinary Ca excretion was demonstrated, it was suggested that the additional Ca that was absorbed was retained within the body.

To identify which subjects are most likely to benefit from consumption of inulin, a further study was carried out in fifty-four girls, aged 10–15 years, who were studied twice in random order after 4 weeks of adaptation to 8g of oligofructose-enriched inulin or 8g placebo, separated by a 2-week wash-out period. Again, the experimental diet significantly increased Ca absorption from 33.1% (SD 9.2%) to 36.1% (SD 9.8%). The most consistent identifiable determinant of a beneficial effect on Ca absorption was the fractional Ca absorption at baseline, with those individuals with lower absorption during placebo period showing the greatest benefit (Griffin *et al.* 2003).

Consequently, inulin-type fructans are able to improve Ca absorption in adolescents. If the absorbed extra Ca is deposited in bones, those molecules may help to optimise peak bone mass. Further long-term studies are thus necessary to assess this possibility.

Adults. In a pivotal study, Coudray *et al.* (1997) performed a balance study with nine healthy volunteers (mean age 25.5 years), who were given up to 40g chicory inulin/d for a period of 26d, according to a crossover design (2d of control diet followed by 14d of progressive increase in inulin amount and then 12d at maximal inulin consumption). Using the metabolic balance methodology, they found that upon inulin ingestion, apparent Ca absorption increased significantly from 21.3±12.5 to 33.7±12.1% (i.e. a 58% increase).

However, opposing this, a study carried out by Van den Heuvel *et al.* (1998) in healthy young adults found no significant differences in mineral absorption, irrespective of the treatment (which consisted of a constant basal diet supplemented for 21d with 15g inulin, oligofructose or galactooligosaccharides daily, or not supplemented). It was hypothesised that a 24h period of urine collection, as used by Van den Heuvel *et al.* (1998), was too short to include the colonic component of Ca absorption and thus to make up a complete balance necessary to detect the effect of fructans.

Finally, in a very short-term study, fifteen young healthy women were given 210mg of Ca as cheese or as a supplement. In each case, the food was either enriched with 15g inulin or without inulin (Teuri *et al.* 1999). Neither intact parathyroid hormone (PTH) nor ionised Ca and total Ca differed over 8h, whether inulin was consumed or not. However, measuring serum PTH and ionised Ca does not provide direct information about Ca absorption, as do isotope techniques. In addition, the length of the trial was probably too short.

Postmenopausal women. In a randomised, double-blind, crossover design, Tahiri *et al.* (2003) investigated the medium-term effect (5 weeks separated by a 3-week washout period) of a moderate daily dose (10g) of oligofructose

in twelve postmenopausal women. Oligofructose failed to show an effect on Ca absorption. However, the results from the subgroup of women who had been menopausal for more than 6 years suggest that this non-digestible carbohydrate may influence Ca absorption in the late postmenopausal phase.

In twelve older healthy women (of at least 5 years past the onset of menopause), Van den Heuvel *et al.* (1999b) investigated the effect of two doses of lactulose (5 and 10 g/d) given at breakfast for 9 d in a crossover design. Mean Ca absorption values during the treatments with 5 and 10 g lactulose were $30.0 \pm 7.6\%$ and $32.2 \pm 7.0\%$, respectively (against $27.7 \pm 7.7\%$ in controls). The difference in absorption between the reference and the 10 g lactulose was significant.

In a similarly designed, double-blind, randomised, 9 d crossover trial in which postmenopausal women were given twice daily yoghurt drinks containing either transgalactooligosaccharides (20 g/d) or sucrose, true Ca absorption was significantly increased by 16% ($20.6 \pm 7.0\%$ to $23.9 \pm 6.9\%$; Van den Heuvel *et al.* 2000).

Finally, in a recent study, fifteen women (who were a minimum of 10 years past the onset of menopause and had taken no hormone replacement therapy for the past years) were treated with 10 g of oligofructose-enriched inulin daily for 6 weeks, according to a double-blind, placebo-controlled, crossover design (Holloway *et al.* 2004). True fractional Ca absorption, measured by dual isotopes before and after treatment, was significantly increased (+7%).

These preliminary intervention studies have shown an improvement in Ca absorption in adolescents or young adults. In the same way, a positive effect has been reported in older women. Nevertheless, it remains to be demonstrated that the benefits of these ingredients on Ca absorption persist and can be translated into benefits to bone health.

Effects on bone health. In a clinical trial carried out on healthy young girls by Griffin *et al.* (2002), a significant increase in fractional and absolute Ca absorption was established after supplementation, while taking daily 1500 mg of Ca, in conditions where a further increase in Ca uptake is difficult to achieve. Assuming that this additional Ca would be retained daily during the 2 years of maximal adolescent bone mineral accumulation, the authors speculate that the net increase in bone mineral mass could be 65 g, an average gain of 5.5%. However, no change in the bone resorption was reported.

Tahiri *et al.* (2003) showed that plasma concentrations of total Ca and PTH, as well as ^{44}Ca plasma enrichment remained identical during both the placebo and the oligofructose periods. Nevertheless, $1,25(\text{OH})_2\text{D}$ showed a tendency to decrease during oligofructose treatment and significantly decreased in the early menopause subgroup. Bone turnover was not modified.

On the other hand, according to Holloway *et al.* (L Holloway, S Moynihan and AL Friedlander, unpublished results), oligofructose-enriched inulin increased Ca absorption in volunteers who were at a minimum 10 years past the onset of menopause before starting the intervention nutrition trial. This 6-week treatment was able to affect markers of bone turnover (urinary deoxypyridinoline: +1.1 nmol/mmol creatinine; plasma osteocalcin: +4.7 ng/ml), even though this effect is difficult to interpret (Fig. 2). The results were even more pronounced (urinary deoxypyridinoline: +1.7 nmol/mmol creatinine; plasma osteocalcin: +6 ng/ml) if the analysis was limited to volunteers who had an increased Ca and Mg absorption rate following intake of the experimental diet. Further the efficacy of treatment

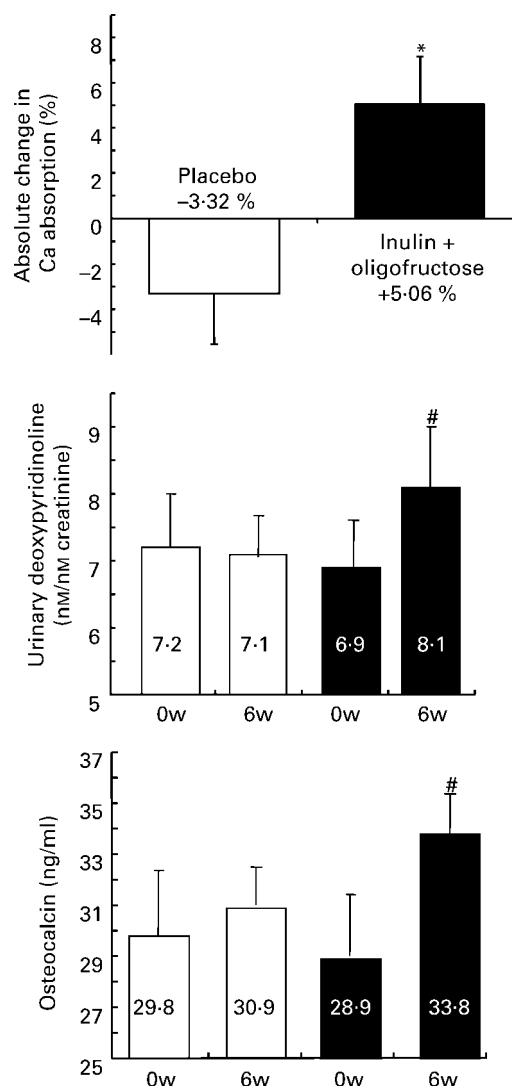


Fig. 2. Influence of a mixture of inulin + oligofructose (oligofructose-enriched inulin) on Ca absorption and markers of bone metabolism (urinary deoxypyridinoline and plasma osteocalcin) in postmenopausal women at baseline (0w) and after 6 weeks of supplementation (6w). *Significantly different from placebo; #significantly different from baseline. Adapted from Holloway *et al.* (L Holloway, S Moynihan and AL Friedlander, unpublished results).

seems to be higher in women with lower initial bone density of the spine.

To summarise, the modulation of Ca absorption in human subjects is quite consensual, although the parameters must be carefully chosen. Indeed plasma PTH or ionised Ca is not accurate enough to assess Ca absorption (Teuri *et al.* 1999). Moreover, the length of the period of urine collection is important. Van den Heuvel *et al.* (1998) failed to show any effect, probably because of experimental bias (it was hypothesised that a 24 h period of urine collection was too short to include the colonic component of Ca absorption). In the same way, Ellegård *et al.* (1997) have measured the mineral balance in ileostomy volunteers, who were administered 15 g of either inulin or oligofructose daily. Data show that the intake of the fructans did not alter the Ca excretion at the end of the small intestine, probably because

the modulation of mineral absorption by fructans must take place mainly in the colon.

As far as the question of whether the extra Ca absorbed is deposited in bones is concerned, there is no clear evidence in man. Long-term studies are needed. However, the impacted bone turnover recently reported by Holloway *et al.* (2004), when long-chain inulin and oligofructose were given together, opens a new area of investigation. Indeed, this mixture outperformed the traditional inulin and oligofructose with regard to Ca absorption, as well. In the same way, in adolescent girls, it increased the true Ca absorption by almost 20%, while oligofructose alone had no significant effect (Griffin *et al.* 2002). Furthermore, in rats fed inulin-type fructans with different degrees of polymerisation (DP; oligofructose, DP 2–8; inulin, DP > 23; and a mixture of 92% inulin and 8% oligofructose), Kruger *et al.* (2003) assessed the retention of Ca, femoral bone density, bone Ca content and excretion of collagen degradation products in the urine. These authors clearly show that the various inulin-type fructans do not have the same effect. Again, Coudray *et al.* (2003) compared different inulin-type fructans that differ in the sugar chain length and in chain branching and found a synergistic effect with the combination of inulin-type fructans with different chain lengths in adult male rats. A potential mechanism for the improved efficiency could be the distribution of the chain lengths resulting in different fermentation profiles of the mixture (especially oligofructose-enriched inulin) that might be critical to obtain maximum efficacy at low daily doses. Actually, the short-chain components such as oligofructose are likely to be most active in the proximal part of the colon (where they can serve as a starter for selective bifidogenic fermentation), while the long-chain molecules could have their effect in the distal part (where they can maintain the metabolic activity of the improved flora). Combining both types of molecules in one product offers a synergistic effect on Ca absorption because

the fermentation process takes place over the full length of the colon, thus maximising the mucosal surface through which the extra-solubilised Ca can migrate (Fig. 3).

Finally, inulin-type fructans have failed to modulate Ca absorption during the first 5 years after the onset of menopause. Indeed, this period is predominantly characterised by hormonal disturbances and it is apparently difficult to modulate Ca metabolism variables during that period (increased Ca intake is not effective in retarding bone loss in early postmenopausal women). However, inulin-type fructans still remain as candidates for putative innovative dietary health intervention to prevent postmenopausal osteoporosis.

The case for postmenopausal osteoporosis prevention. Albright & Reifenstein (1948) have underlined the importance of sex steroids in maintenance of skeletal integrity. Today, an overwhelming body of evidence emphasises that gonadal failure at the time of menopause is the dominating pathogenic factor for osteoporosis and that the administration of oestrogens in postmenopausal women prevents this loss, as long as use is continued. In fact, it has been established that acute ovarian deficiency would account for the loss of about 20% of bone mass in the first 5–7 years of menopause (Avioli & Lindsay, 1990). This is why hormone replacement therapy was the mainstay for the prevention of bone loss in postmenopausal women. However, prophylaxis is currently limited to only a very small minority of eligible women, due to a number of factors that are unlikely to be resolved in the near future (mainly, associated side-effects). This is the reason why scientists have targeted phyto-oestrogen consumption as a possible way to achieve this goal, substantiating their importance based on emerging data which support a potential preventive effect for a range of hormone-dependent conditions (Setchell & Lydeking-Olsen, 2003).

Phyto-oestrogens belong to a broad group of plant-derived compounds called polyphenols that can behave as oestrogen

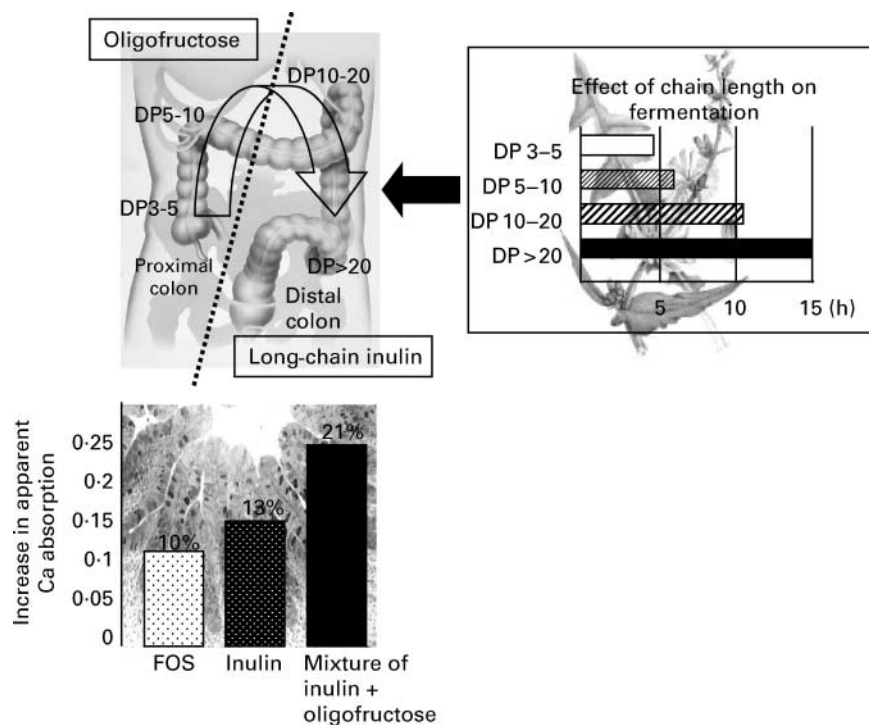


Fig. 3. Influence of different types of fructans (FOS, fructooligosaccharides) that differ in sugar chain length (DP, degree of polymerisation) and in chain branching on Ca absorption in adult rats (adapted from Coudray *et al.* 2003) and hypothesis of the mechanisms of action.

mimics. The most studied molecules are the isoflavones, mainly found in soya or soya products. Conspicuous features of their non-steroidal chemical structure are the phenolic ring which is theoretically a prerequisite for oestrogenic activity (binding to the oestrogen receptor; Setchell, 1998) and a pair of hydroxyl groups, one being a phenol function on an aromatic A ring, while the second lies at the opposite end (Miksicek, 1993). These molecules are receiving great scrutiny as food supplements for the purpose of preventing osteoporosis because, although this condition universally affects all races and nationalities, conspicuous differences may be encountered in the severity of its manifestations, with a weaker effect in Asian countries (Cooper *et al.* 1992; Ling *et al.* 1996). Moreover, most observational studies have provided evidence that postmenopausal women consuming

the highest amounts of soya foods (and hence isoflavones) have the highest BMD, measured either on the femur or on the spine (for review, see Setchell & Lydeking-Olsen, 2003). Some dietary intervention trials targeting BMD as the main outcome have also shown a bone-sparing effect (Dalais *et al.* 1998; Potter *et al.* 1998; Alekel *et al.* 2000; Lydeking-Olsen *et al.* 2002; Morabito *et al.* 2002).

More interestingly, Setchell *et al.* (2002) have hypothesised that intestinal metabolism of isoflavones could be the most important clue to the clinical efficacy of soya foods in preventing osteopenia. In fact, inulin-type fructans may have a beneficial effect on isoflavone metabolism because, as phyto-oestrogens mainly occur in plants as glycosides, they have to be hydrolysed (the glycosidic bonds have to be broken down) before intestinal

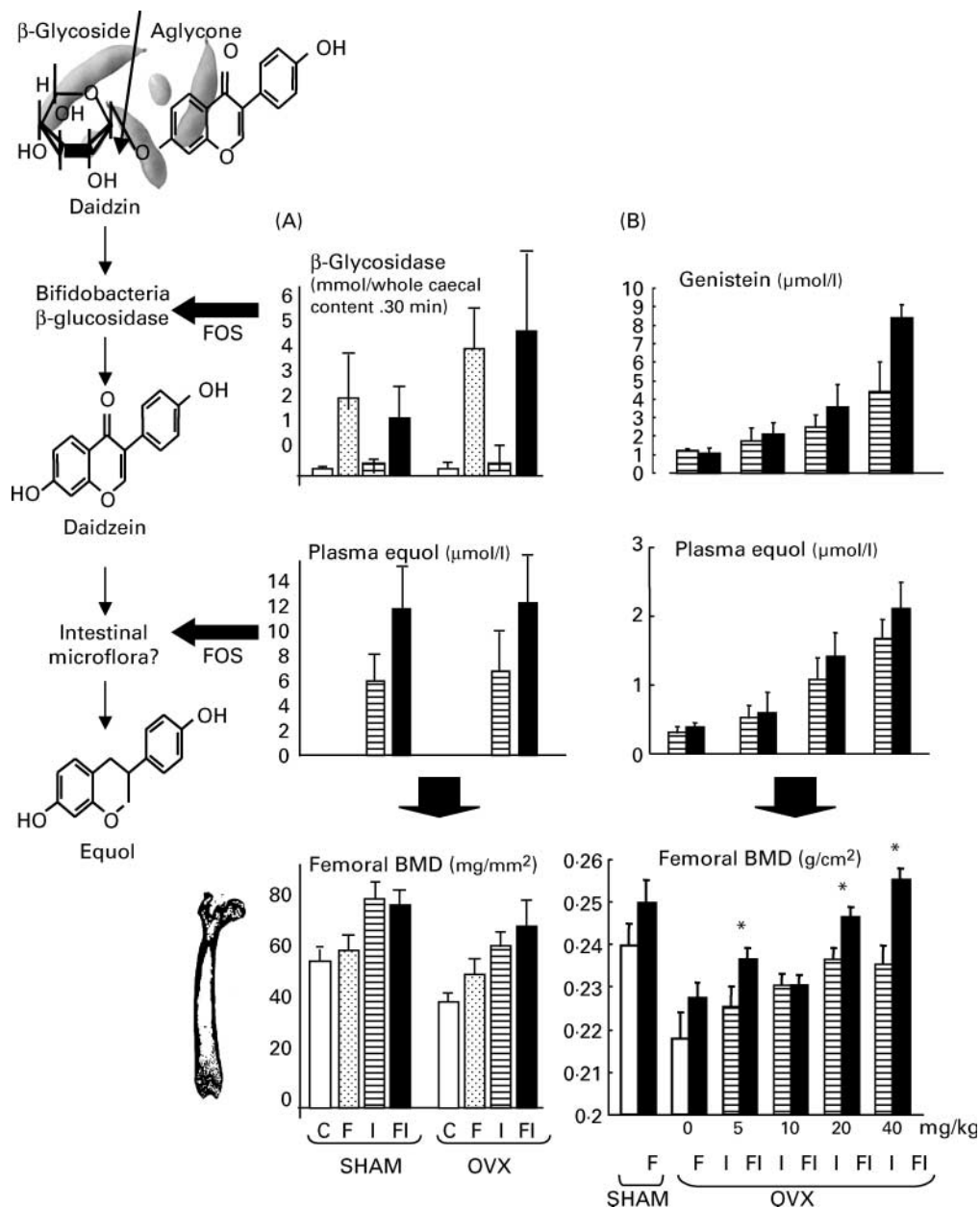


Fig. 4. Influence of isoflavones (I) given alone (at 0, 5, 10, 20 or 40 mg/kg) or together with oligofructose (F; FI) on markers for isoflavones metabolism and on bone mineral density (BMD). FOS, fructooligosaccharides; C, control; OVX, ovariectomised. (A) Adapted from Ohta *et al.* (2002) for mice; (B) adapted from Mathey *et al.* (2003) for the rat.

absorption, glucosidases of intestinal bacteria such as lactobacilli, bacteroides and bifidobacteria being involved in this process. It has been shown that dietary oligofructose may increase β -glucosidase activity in the large intestine, leading to an enhancement of the large intestinal absorption of these compounds (Uehara *et al.* 2001). The clearance of isoflavones is thus prolonged. Besides, phyto-oestrogens (and more especially daidzein) can be further metabolised to be converted into a more potent molecule, equol. However, on average, this bacterial metabolite is found in only 45% of the postmenopausal women studied (Axelson *et al.* 1982). Equol has a longer half-life and a much higher affinity for the oestrogen receptor than its precursor (daidzein) and has the highest antioxidation capacity among isoflavones (Setchell *et al.* 2002). This could explain the preliminary results from a 2-year study carried out in postmenopausal women randomised to consume 500 ml of soyamilk either with or without isoflavones. Greater effects on bone health were elicited when volunteers were able to produce equol, a 2.4% increase in the lumbar spine BMD being demonstrated, compared to the control group, while no significant difference was shown in the non-producers (Lydeking-Olsen *et al.* 2002).

Thus, because a greater efficacy of phyto-oestrogens can be expected if converted into equol by the intestinal microflora, there is a good rationale for considering non-digestible carbohydrates as a tool to target an increase in isoflavones bioavailability. Indeed, it has been established that concurrent dietary intake, in particular high dietary fibre, exerts a major influence on isoflavone metabolism (Xu *et al.* 1994).

In fact, in ovariectomised mice (Ohta *et al.* 2002) or rats (Mathey *et al.* 2003), two experimental models for postmenopausal osteoporosis, oligofructose consumption has been shown to increase the bone-sparing effect of isoflavones by improving equol production (Fig. 4). The lower response regarding the impact on equol production in the rat experiment could probably be explained by the fact that this animal is a spontaneously good producer of equol. Thus, even though these results are too few to draw final conclusions, they appear very provocative.

Conclusion

In conclusion, programmes for the prevention of osteoporosis need to be implemented. A focus on behaviour modification must be adopted by societies to effectively lower incidence rates of hip fractures in the future generations.

Adequate Ca intake from childhood until the end of life is critical for the formation and retention of a healthy skeleton. Beyond Ca intake, Ca bioavailability is also an important parameter in terms of bone health. Because inulin-type fructans can modulate Ca absorption, it is thus of great interest to still ascertain their impact on bone physiology in adolescents and the elderly. Indeed, studies are necessary to prove that the benefits of these ingredients to Ca absorption persist in the longer-term and that they can be translated into benefits to bone health.

Furthermore, because a dietary combination of oligofructose and isoflavones may have a potential promise for maintaining or improving bone mass in experimental animal models for postmenopausal osteoporosis, we also need to gather more data targeting bone metabolism in postmenopausal women before health professionals can actively advocate the increased consumption of inulin-type fructans.

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