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Inulin, oligofructose and bone health: experimental approaches and mechanisms

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Inulin-type fructans have been proposed to benefit mineral retention, thereby enhancing bone health. Many, but not all, experimental animal studies have shown increased mineral absorption by feeding non-digestible oligosaccharides. Possible reasons for inconsistencies are explored. A few studies have reported an enhanced bone mineral density or content. Bone health can be evaluated in chronic feeding studies with bone densitometry, bone breaking strength, bone mineral concentration and bone structure. Isotopic Ca tracers can be used to determine the point of metabolism affected by feeding a functional food ingredient. These methods and the effects of feeding inulin-type fructose are reviewed. Inulin-type fructans enhance Mg retention. Chicory long-chain inulin and oligofructose enhance femoral Ca content, bone mineral density and Ca retention through enhanced Ca absorption and suppressed bone turnover rates, but it is not bone-promoting under all conditions.

Inulin: Oligofructose: Bone health

The mineral absorption enhancing ability of various functional ingredients has been a topic of much research recently. Of these, non-digestible carbohydrates, which enhance Ca absorption, have been the most studied. Ca utilization is of primary interest because it is the main mineral in bone and it is the most deficient of the bone nutrients in the diet. Many, but not all, studies show that inulin-type fructans benefit bone. In this review, a number of factors which can influence effects of these ingredients on mineral absorption and retention and bone health will be discussed including life stage, adequacy of oestrogen, dietary composition and acute *v*. chronic effects. In order to increase bone mass, Ca retention must increase. Some recent insights on mechanisms of action will be reviewed.

First, however, various approaches and choice of animal models that have been used to evaluate the effect of inulin-type fructose on Ca metabolism and bone quality will be described. Table 1 summarizes the methods most commonly used to assess bone and Ca metabolism and bone quality, with some indication of merits and weaknesses. The methods chosen determine what type of information can be gained from each study.

Methods for determining Ca and bone metabolism

Ca and bone metabolism are best studied with Ca isotope tracers. Isotopic Ca tracers can be used to determine either Ca or bone metabolism as 99 % of the Ca in the body is in bone. If one tracer is given orally and a second tracer is given intravenously, Ca absorption can be determined. If tracers are administered as part of a metabolic balance study, it is possible to determine the components of Ca metabolism including absorption, excretion, endogenous secretion, bone formation rates and bone resorption rates. Use of isotopic Ca tracers and kinetic modelling for determining the components of metabolism have been described elsewhere (Weaver *et al.* 2003). This approach has much strength and some important weaknesses. Use of isotopic Ca tracers allows precise measurement of mineral transfer throughout the body. Complete kinetic analysis can be used to determine the point of metabolism, i.e. the gut, kidney or bone, perturbed by a dietary constituent such as an inulin-type fructan. Measurement of an isotope in body fluids or tissues avoids confusion as to the origin of the mineral. Orally administered isotopes reflect the diet if the isotope is given in the form of interest. This is important because faecal Ca contains both Ca from the diet and endogenous secretions. Urinary Ca can derive from diet and bone. Isotopes can be used to distinguish the origin of Ca in the urine. Intravenously administered isotopes can be used to determine clearance of endogenous minerals. Weaknesses of this approach include the rather specialized facilities and capacity required for administering and measuring isotopic tracers. Isotopic tracers are generally used to determine whole-body changes over relatively short periods. Thus, they are neither used to determine long-term effects on the whole skeleton nor can they give information on a specific bone site. Tracers are not used to determine bone quality.

Metabolic balance studies can give information on mineral retention and net absorption. Isotopic tracers are required to estimate true absorption, which is higher than the net absorption as it includes the mineral fraction that is absorbed and re-excreted into the gut as part of endogenous secretion. The conduct of metabolic balance studies in human and animal studies and monitoring of compliance has been described elsewhere (Weaver & Liebman, 2002; Weaver *et al.* 2003). Balance studies are labour-intensive. The large variability in faecal mineral excretion means that large errors are associated with balance studies, especially when

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Table 1. Methods for assessing bone/calcium metabolism and quality

Method	Target site	Characteristics
Ca balance	Whole body	Variable
Ca kinetics	Whole body	Sensitive, labour-intensive
Biochemical markers	Whole body	Variable, indirect, available
Histology	Bone-specific	Invasive, quantities bone formation
Bone density	Bone-specific	Long-term, precise
Bone breaking	Bone-specific	Invasive, indicates quality

performed without isotopic tracers. Variability may be less in animal than human studies because a monotonous highly defined diet is used in animal studies, which reduces variability in intake. Furthermore, the mineral can be given in a single form of interest as other dietary constituents can be highly purified for animal studies. On the other hand, too often a functional food ingredient is given at very high levels in an animal diet that would not be sustainable in typical human diets, thereby exaggerating the benefits.

Biochemical markers of bone turnover can also be used to determine bone metabolism. Various kits are commercially available that can be used to determine bone formation rates and bone resorption rates in serum and urine samples. The major advantage of this approach is that they are available to any laboratory. However, they are highly variable and are not in units of either bone or Ca. Generally, they derive from fragments from bone matrix proteins or are enzymes or proteins derived from osteoblastic activity.

Methods for assessing dietary effects on specific bones

Bone characteristics are generally assessed by histology or bone densitometry. In animal models, mineral content can be directly measured in bones and breaking strength can be determined on excised bones. Changes in bone require a chronic feeding period to see an effect of intervention as a modelling–remodelling cycle takes about 30 d in rats and 120 d in man. It may take an intervention of several cycles to see the impact of a dietary intervention. Evaluating specific bone sites is helpful to understand the role of diet in reducing risk of fracture at vulnerable sites, especially the hip and spine.

Both static and dynamic measurements can be determined with bone histology. Static measures characterize bone architecture and can give estimates of bone quality. Dynamic measurements require labelling bones twice a few days apart with markers such as fluorescent calcein. The distance between the two labels determined under a microscope can be used to estimate bone formation rates. This is more accurate and specific to a particular bone site compared to biochemical markers of bone turnover. Histology is labour-intensive and destructive of samples.

Direct mineral analysis of bones gives information similar to bone mineral content by densitometry. Both indicate cumulative mineral retention, but cannot determine whether changes are due to alternations in absorption, excretion or bone turnover. Bone mineral density obtained from bone densitometry adjusts bone mineral content by the two-dimensional area of the scan, thus it is not truly volumetric bone density. Quantitative computer tomography (QCT) can be used to determine volumetric bone density. Microcomputed tomography (μCT) can be used to determine ultrastructure of bone and can give information similar to static measures of histomorphometry without sample destruction. Both of these methods can be used to measure cross-sectional bone size changes that indicate bone strength over any area scanned.

Bone breaking strength is simple to perform and can directly measure loads required to fracture. However, interpretation of benefits of diet may be underestimated. Frequently, breaking is performed on the midshaft of a long bone that is mostly cortical bone. Diet effects are more often seen in trabecular bone that is more abundant at the ends of long bones and in the vertebra.

When do inulin-type fructans stimulate mineral absorption and bone health in animals?

The effect of inulin-type fructans on important bone minerals, i.e. Ca, Mg and P, has been studied in man and in animal models. The effects on Mg, but not P, have been positive and relatively consistent (see review by Beynen *et al.* 2002; Coudray *et al.* 2003). However, the effects on Ca are inconsistent. Some show increased Ca absorption and retention (Coudray *et al.* 1997; van den Heuvel *et al.* 1999; Griffin *et al.* 2002, 2003) and others do not (Martin *et al.* 2002; Tahiri *et al.* 2003). For inulin-type fructans to be a benefit to Ca absorption and bone retention, we must understand the conditions that promote its effect and characteristics of those individuals who respond. Conditions which may affect the ability of inulin-type fructans to promote bone health include life-stage and related oestrogen status, Ca status, composition of the food matrix surrounding ingestion of inulin-type fructans, and acute *v.* chronic feeding of inulin-type fructans.

Life stage could have a strong effect on the role of a Ca absorption enhancer. During growth, bone formation rates exceed bone resorption rates. The endocrine regulators influencing bone are optimized during the pubertal growth spurt (Weaver, 2002). Thus, the hormonal milieu may be so up-regulated that little further increases in Ca absorption may be achieved by adding a functional food ingredient. Evidence shows that feeding a diet supplemented with oligofructose-enriched inulin enhanced Ca absorption in adolescents (Griffin *et al.* 2002) but the benefit is not shown in all subjects, which may depend on genetically programmed Ca absorption efficiency, Ca status and sexual maturity (Griffin *et al.* 2003). Ca absorption enhancers may have a very different effect at other life stages. In later life, bone loss, where bone resorption rates exceed bone formation rates, is common. Bone loss has been strongly related to oestrogen deficiency. Use of hormone therapy to correct oestrogen deficiency in postmenopausal women as a strategy to reduce bone loss has been discouraged since the report of serious side-effects (Writing Group of WHI, 2002). As a consequence, interest in dietary solutions to reducing bone loss is increasing. In a study of twelve postmenopausal women, feeding 10 g oligofructose per day for 5 weeks resulted in no benefit on ^{44}Ca absorption (Tahiri *et al.* 2003). However, there was a positive trend in women over 6 years postmenopausal. A lesser effect in perimenopause than stable menopause would suggest that changing hormone status is a stronger influence than diet than when hormones have stabilized. This has been shown to be true for Ca supplementation. Ca supplementation is less effective during perimenopause than stable menopause (Institute of Medicine, 1997). This may not be true for soya isoflavones that have been

shown to have a positive effect on spine bone mineral density in perimenopausal women (Alekel *et al.* 2000).

The role of diet on the influence of inulin-type fructans on Ca absorption can be separated into three categories.

(1) Historical diet determines Ca status that inversely relates to Ca absorption by the formula: fractional absorption = 0·889 − 0·0964 ln load (Heaney *et al.* 1990).
(2) The composition of the diet can influence Ca absorption. Relevant to the influence of inulin-type fructans is the ratio of inulin-type fructans in the diet and the absolute composition of each.
(3) The acute *v*. chronic effects of feeding inulin-type fructans.

The absolute amount of Ca and inulin-type fructans in the diet or their ratio may influence the effect on Ca absorption. A comparison of a number of rat studies is shown in Table 2. Most studies are in young growing rats, but the Zafar *et al.* (2004*a*,*b*) study used 6-month-old ovariectomized rats. Dietary inulin enhances Ca absorption in a dose–response manner up to 20 % (Levrat *et al.* 1991). Unpublished data from our laboratory suggest that feeding at 1 % is insufficient to enhance Ca absorption. At the other extreme, studies using 10 % inulin in animal diets exceed what is practical for human diets. The effect of inulin-type fructans appeared to be weaker or not effective if Ca intakes were at or below the recommended level for rat diets, i.e. 0·5 % (Chonan & Watanuki, 1996; Scholtz-Ahrens & Schrezenmeir, 2002). However, we recently demonstrated a positive effect of oligofructose-enriched inulin on 0·5 % Ca (Zafar *et al.* 2004*a*,*b*). Dietary Ca intakes of 1 % are not representative of human diets and it would not be practical to double the Ca requirement in a human diet. Overall, the ratio of Ca to inulin-type fructans does not account for the differences seen in Ca absorption. Discrepancies may be related to methodology as most of the studies used metabolic balance to estimate apparent absorption, except for the Zafar *et al.* (2004*a*,*b*) study that used isotopic tracers to determine true absorption. Moreover, whole-body bone mineral content and density were increased by 5 or 10 % inulin at Ca concentrations of 0·2, 0·5 or 1 % (Roberfroid *et al.* 2002).

Several studies have shown that the benefits of inulin-type fructans are effective chronically as well as acutely. For example, a positive Ca balance was observed on diets containing 0·5 % Ca and 5 % oligofructose after 8–10 d and after 18–20 d (Chonan & Watanuki, 1996). However, some adaptation is apparent as the effect on Ca absorption decreases over time in contrast to Mg (Chonan *et al.* 1995; Ohta *et al.* 1998*b*). This reflects the stronger homeostatic control mechanisms of Ca in response to improved Ca status compared to Mg.

Mechanistic aspects of the stimulating effect of inulin-type fructans on bone

Cashman (2003) has reviewed the several theories that have been suggested to explain how inulin-type fructans promote Ca absorption. The most commonly held belief is that fermentation of inulin-type fructans by intestinal microflora in the large intestine lowers the pH through formation of SCFA. The lower pH increases mineral solubility that leads to enhanced Ca absorption or SCFA may directly increase transcellular Ca absorption. The important role for intestinal bacteria has been shown in rats, which exhibited reduced Ca and Mg absorption in response to feeding 5 % galactooligosaccharides after being treated with neomycin (Chonan *et al.* 2001). Alternatively, non-digestible fibres may increase mineral absorption through increasing the surface area of the intestine or through enhanced permeability, mechanisms that would not be restricted to the lower intestine (Kishi *et al.* 1996). Nor would the effects of reduced calbindin D9k in the small intestine after feeding inulin-type fructans be a lower gut effect (Ohta *et al.* 1998*a*).

The effect of oligofructose-enriched inulin on Ca metabolism was studied by isotopic Ca tracers using kinetic modelling as part of a metabolic balance study in 6-month-old virgin ovariectomized rats (for 3 months) as a model for postmenopausal women (Zafar *et al.* 2004*a*,*b*). The study design is shown in Fig. 1. Ca absorption capacity of rats fed with inulin-type fructans was not different than for control rats. However, kinetic modelling showed absorbed Ca increased 46 % by the presence of inulin-type fructans. This favours the hypothesis of lower gut fermentation since pre-feeding inulin-type fructans had no effect on Ca absorption efficiency when not co-ingested. Other effects of inulin-type fructans on Ca metabolism are shown in Fig. 2. Bone formation rates increased 44 % and bone resorption rates were completely suppressed, resulting in an increase in Ca retention of 89 %. Another kinetic study failed to show an effect on Ca metabolism despite improved Ca absorption (Morohashi *et al.* 1998). Differences between the two studies could be due to the difference in animal models as Morohashi *et al.* (1998) used young, male rats, whereas Zafar *et al.* (2004*a*,*b*) used the older, ovariectomized rat model, or it could be due to methodological differences. The time following isotopic tracer administration may have been too short for reliable estimates of bone turnover in the Morohashi *et al.* (1998) study. We have found that isotopic tracers need to be followed for at least 4 d to determine bone resorption. Thus, it would be useful to determine the effect of inulin-type fructans at different life stages, including repeating the young animal model.

The Zafar *et al.* (2004*a*,*b*) study illustrates another lesson in methodology. Femur shaft bone breaking strength was not affected by oligofructose-enriched fructans despite an increase

Table 2. Influence of the dietary calcium:inulin ratio on the increase in calcium absorption: review of experimental data

Ca (% diet)	Inulin (% diet)	Significant ↑ Ca absorption	Reference
1	5	Yes	Scholtz-Ahrens & Schrezenmeir (2002)
0·5	10	Yes (weaker)	Scholtz-Ahrens & Schrezenmeir (2002)
0·5	5	No	Taguchi *et al.* (1994)
0·5	5·5	Yes	Zafar *et al.* (2004*a*,*b*)
0·5	5	Yes	Chonan & Watanuki (1996)
0·05	5	No	Chonan & Watanuki (1996)

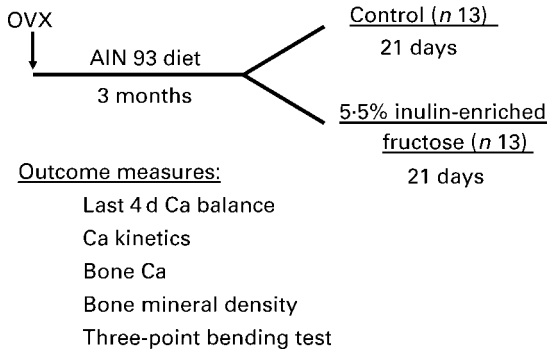

Fig. 1. Study design for testing calcium metabolism in ovariectomized (OVX) rats.

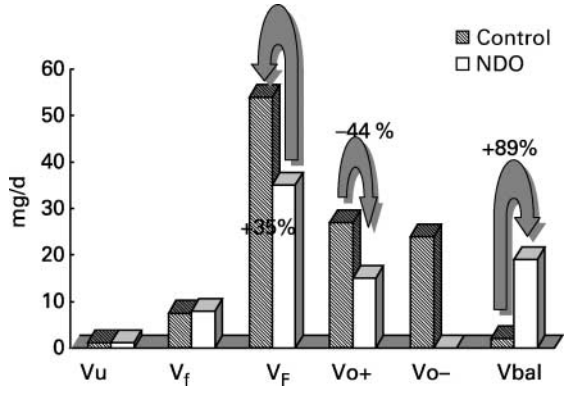

Fig. 2. Effect of feeding non-digestible oligosaccharides (NDO; inulin-enriched fructose) on rates of transport in rats. Values in mg/d: Vu, urinary calcium; V_f, endogenous calcium; V_F, faecal calcium; VO+, bone formation rates; Vo−, bone resorption rates; and Vbal, calcium balance. (From Zafar *et al.* (2004*a*,*b*).

of 89 % in Ca retention. Diet effects are frequently seen in trabecular bone before cortical bone. The midshaft of the long bones is mostly cortical. It would have been more relevant to determine the breaking strength of the spine or distal ends of the long bones. Indeed, distal femur bone mineral density was significantly greater in rats receiving inulin-type fructans than in the control group.

Another life stage difference may be in the interaction between functional food ingredients such as inulin and isoflavones. In young mice, a synergy was observed between dietary inulin and isoflavones on bone mineral density (Ohta *et al.* 2002). In contrast, we found no synergy in ^{45}Ca absorption, but inulin decreased equol production from the daidzein in the soy isoflavones suggesting a modification of colonic bacteria (Zafar *et al.* 2004*a*,*b*). A synergy between inulin and isoflavones is plausible because inulin is a prebiotic for colonic bacteria that assists in the hydrolysis of soya isoflavones preceding absorption. The observation that inulin reduced equol formation suggests a shift in metabolism of the isoflavones.

Conclusions and future direction

The concept that a functional food ingredient can enhance Ca absorption and bone health is provocative. There is much evidence that inulin-type fructans enhance mineral utilization in some situations. Two main areas of needed research are to understand the underlying mechanism and to extend some of the recent findings in animal models to man. Further study in animals to confirm the intestinal site for increased Ca absorption is warranted. Kinetic studies under increasing Ca intakes could address this question. Additional animal work on the effect of inulin-type fructans on bone architecture is needed.

In man, determining whether oligofructose-enriched inulin can reduce bone resorption in postmenopausal women as they do in a rat model would be an exciting possibility. Long-term feeding studies in man to determine the effect of inulin-type fructans on bone density and quality are the ultimate test for a claim on bone health.

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