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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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 calcine. 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 alterations in absorption, excetration 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 vertebral...

### 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 44Ca 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). 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. 1998a).

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. 2004a,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. (2004a,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. (2004a,b) study illustrates another lesson in methodology. Femur shaft bone breaking strength was not affected by oligofructose-enriched fructans despite an increase in influence of the dietary calcium/inulin ratio on the increase in calcium absorption: review of experimental data

<table>
<thead>
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<th>Ca (% diet)</th>
<th>Inulin (% diet)</th>
<th>Significant ↑ Ca absorption</th>
<th>Reference</th>
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<tr>
<td>0</td>
<td>5</td>
<td>Yes (weaker)</td>
<td>Scholtz-Ahrens &amp; Schrezenmeir (2002)</td>
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<tr>
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<td>5</td>
<td>No</td>
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<tr>
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<td>Chonan &amp; Watanuki (1996)</td>
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<td>0.05</td>
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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.

References


