Inulin: A Review of its Functional Properties in Relation to Calcium Absorption in Humans

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ABSTRACT

Inulin is a term applied to a heterogeneous blend of fructose polymers found widely distributed in nature as plant storage carbohydrates. Recent evidence suggests a role for inulin in enhancing Ca absorption and thereby promoting bone health. Numerous investigations performed using animal models during the past 10 years have consistently shown that inulin stimulates calcium absorption and increases bone mineral content. Short and long term human studies have indicated that inulin increases Ca absorption in adolescents. This article reviews current research studies (both animal and human) that have been conducted to determine inulin's role in enhancing Ca metabolism. Animal studies appear to confirm inulin-type fructans' positive role in enhancing Calcium absorption from the gut while several short-term human studies indicate a similar trend. However, the evidence for the positive effects of inulin on Ca metabolism in man, in particular adults and post-menopausal women, is currently inadequate and awaits well planned long-term human trials before any firm conclusions can be reached.

Keywords:: Inulin: Inulin-type fructans: Calcium Absorption: Bone Health

INTRODUCTION

Inulin was first identified by Rose in the early 1800s as a carbohydrate isolated from the plant *inula helenum* (Coussment, 1999). Inulin is a natural food ingredient commonly found in varying percentages in dietary foods (Niness, 1999). It is a heterogeneous blend of fructose polymers found commonly in nature as plant storage sugars. Oligofructose is a subgroup of inulin, consisting of polymers with a degree of polymerization (DP) < 10 whereas standard inulin has an average DP of 12 (Franck, 2002). They are essentially polymers of fructose and the degree of polymerization may range between two and sixty with an average of ten (Roberfroid, 2002). Chemically, inulin is defined as a polydisperse linear β(2-1) fructan (Niness, 1999; Roberfroid, 2002). The fructose units in this mixture of linear fructose polymers and oligomers are each linked by β(2-1) bonds. A glucose molecule typically resides at the end of each fructose chain and is linked by an α(1-2) bond as in sucrose. The unique aspect of the structure of inulin is its β(2-1) bonds (Roberfroid and Slavin, 2000). These linkages prevent inulin from being digested like a typical carbohydrate and are responsible for its reduced caloric value and dietary fibre effects. Because of the beta configuration of the anomeric C2 in their fructose polymers that form β(2-1) glycosidic bonds, inulin is resistant to hydrolysis by human small intestine digestive enzymes which are specific for alpha glycosidic linkages (α-amylase) such as those found in starch and sucrose (Niness,1999). The indigestible nature of inulin has resulted in its classification as a dietary fibre (Cherbut, 2002). It has been included in the dietary fibre complex of food because inulin is a part of edible plants, is a type of carbohydrate, cannot be hydrolysed by digestive enzymes, is not absorbed in the small intestine and is fermented by colonic microflora (Roberfroid, 2005). As a result it escapes digestion in the upper gastrointestinal tract and reaches the large intestine virtually intact where it is quantitatively fermented by *Bifidobacteria* mainly and to a lesser extent by *Lactobacilli*. Fermentation of inulin in the colon is an anaerobic process producing gasses (hydrogen, carbon dioxide and methane) and organic acids among which Lactic acid and short chain fatty acids (SCFA) have been found in

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colonic contents (Roberfroid, 2002). Gasses are utilized by bacteria, absorbed and expelled with breath or excreted with stools. The main SCFA synthesized are propionic and butyric acids (Nyman, 2002)

It is estimated that Americans consume on average 1-4 g of inulin per day and Europeans average 3-10 g/d (Van Loo *et al.* 1995). Inulin is widely found in nature in a variety of plants and in some bacteria and fungi (Franck, 2002). Plants containing it include leek, onion, garlic, asparagus, jerusalem artichokes, dahlia tubers, yacon, chicory, cereals such as wheat rye and barley and fruits such as banana (Watzl *et al*, 2005). The most common sources of inulin in the western diet are from wheat, onion, banana, garlic and leek (Roberfroid, 2002). Therefore inulin is present naturally in the diets of the majority of people in the world. The calorific value has been reported to be 1.5 kcal/g and therefore does not significantly contribute to the energy intake (Roberfroid, 1999). The quantity ingested per day is not in milligrams but grams, a fact that strengthens its claim to safety (Coussement,1999). Inulin has now been officially accepted as a food ingredient rather than a food additive (Cummings and McFarlane, 2002). Due to the fermentability of inulin, unusually high doses may cause flatulence and osmotic pressure leading to intestinal discomfort even though it has not been recorded (Coussment, 1999). Historically there is no literature doubting the safety of inulin or inulin containing foods. On the contrary many of these foods have been hailed as promoting good health and may be attributed to the beneficial effects of inulin.

Commercially, inulin is extracted from Chicory (*Cichorium intybus*) and has an average chain length of 12 (Franck, 2002). Table 1 summarises the key properties of commercial inulins used in the food industry.

FUNCTIONAL AND NUTRITIONAL PROPERTIES OF INULIN

Due to the long chain length, inulin is less soluble and has the ability to form microcrystals when sheared in water or milk. It has therefore been used successfully as a fat replacement (Niness,

Table 1: Physico-chemical characteristics of chicory inulin and High Performance inulin

Physico chemical characters	Chicory Inulin	High Performance (HP) Inulin
Chemical Structure	GFn (2<n<60)	GFn (10<n<60)
Average degree of Polymerization	12	25
Dry Matter (%)	95	95
Inulin/Oligofructose content (% on d.m.)	92	99.5
Sugars content (% on d.m.)	8	0.5
PH (10% w/w)	5-7	5-7
Sulphated ash (% on d.m.)	<0.2	<0.2
Heavy metals (ppm on d.m.)	<0.2	<0.2
Appearance	White Powder	White Powder
Taste	Neutral	Neutral
Sweetness (v sucrose=100%)	10%	None
Solubility in water at 25^0C (g/l)	120	25
Viscosity in water (5%) at 10^0C (mPa.s)	1.6	2.4

(F- Fructosyl units, G- Glucosyl units, d.m.- Dry matter)
Source: Franck, 2002

1999). Since inulin has a neutral odour and does not contribute to the viscosity of the food, it is used extensively in the production of high fibre foods that look and taste like standard food formulations (Niness, 1999). Inulin has now been proven to improve the health of the gut by increasing stool output, improving integrity of gut epithelium, increasing absorption of minerals, significantly reducing the incidence of diarrhoea, strengthening the immune system and stimulating growth of beneficial gut microbes (Cherbut, 2002, Nyman, 2002, Marteau, 2002, Watzl *et al.*, 2005).

A large number of studies have indicated that almost all of the inulin ingested enters the colon where it is fermented by the colonic microflora (Kolida *et al.,* 2002). This is perhaps the best known nutritional effect of inulin, namely, as a prebiotic. Both *in vivo* and *in vitro* studies have confirmed that inulin is selectively fermented by *Bifidobacteria* and to a lesser extent *Lactobacilli* (Kolida, *et al*, 2002). This not only establishes a dominant gut flora in which beneficial bacteria are dominant and accounts for most of the positive effects of inulin in the digestive system mentioned earlier. One effect of inulin in the diet that has provoked much interest and research is its apparent ability to influence Ca absorption.

The addition of cereal fibre to human and animal diets has been found to consistently depress absorption and retention of Ca, Mg and Zn and Fe (Greger, 1999). This depression of mineral absorption by cereal products was related more to the phytates than to the fibre content of the foods. The size of the fibre particles and the amount of Ca in the food also affects the rate of absorption. The effect of soluble carbohydrates that are indigestible in the gut on mineral absorption was less consistent. In recent years, there has been increased understanding of the role of disaccharides, oligosaccharides, soluble fibre and other carbohydrates as enhancers of Ca absorption (Griffin and Abrams, 2005). Of these the most widely studied in man, have been the inulin type fructans.

INULIN AND CALCIUM ABSORPTION

Importance of Calcium to the human body

Calcium accounts for 1-2% of the human body by weight which amounts to approximately 1200 grams (Cashman, 2002). Of this, 99% is found in bones and teeth where it is found as Calcium Phosphate providing rigidity and structure. The remaining 1% is found in blood, extra-cellular fluid, muscle and other organs where it participates in physiological activities such as vasodilation, muscle contraction, nerve transmission and glandular secretion. Ca is required for the growth and development of the skeleton (Nordin, 1997). During skeletal growth and maturation which is until the early twenties in humans, Ca accumulates in the skeleton at a rate of 150mg per day. During maturity, the body and therefore the skeleton is in Ca equilibrium. From about the age of 50 in men and after menopause in women Ca balance becomes negative and bone mass is lost from all skeletal sites. Adequate Ca intake is critical to maintaining peak bone mass and also modifies the rate of bone loss in the latter years. In the recent years, convincing evidence has emerged with respect to the importance of dietary Ca and bone health for all age groups (Cashman, 2002). Intervention and cross sectional studies have reported a positive effect of Ca in bone mass of children and adolescents. Valimaki *et al.,* (1994) reported that Ca intake during childhood and adolescence positively affects the bone density of young women and that there was a definite association between Ca intake and bone mineral density.

The Calcium requirement of an individual depends on many factors such as age, sex, pregnancy and ethnicity (Cashman, 2002). Table 2 presents the recommended intakes in the UK and by the European Union.

Table 2: Recommended Ca intakes in the UK and EU

EU PRI (1993)		UK RNI (1991 and 1998)	
Age group (years)	mg/d	age group (years)	mg/d
0.5-1	400	0-1	525
1-3	400	1-3	350
4-6	450	4-6	450
7-10	550	7-10	550
11-14 M	1000	11-14 M	1000
15-17 M	1000	15-18 M	1000
>18 M	700	11-14 F	700
11-14 F	800	15-18 F	800
15-17 F	800	19-50	800
>18 F	700	>50	700

(PRI-Population Reference Intake; RNI-Reference Nutrient Intake)
Estimates of Ca intakes refer to both sexes unless otherwise stated
(Source: British Nutrition Foundation (www.nutrition.org.uk/home verified 16/02/08); DOH, 1991; Cashman, 2002)

Besides the amount of Ca in the diet, its absorption is another critical factor determining the availability of Ca for the body. In addition to dietary increments, the consumption of functional foods which contain ingredients that promote Ca absorption (Lactulose, Inulin, Oligofructose) may ensure that the bioavailability of Ca is maximal (Cashman, 2002).

INULIN'S ROLE IN THE POSSIBLE ENHANCEMENT OF Ca ABSORPTION

Inulin has attracted much attention during the past decade for its physiological and health promoting properties and thus for its potential as a functional food ingredient (Scholz-Arens and Schrezenmeir, 2002). It was observed that inulin has special effects on the gut physiology. The effects were attributed to physical characteristics of inulin such as dispersibility, viscosity and adsorbing capacity. Cashman (2003) has reviewed the several theories that have been proposed to explain how inulin might promote Ca absorption. The most commonly held belief is that fermentation of inulin by intestinal microflora in the large intestine lowers the pH through the formation of short chain fatty acids (SCFA) and lactic acid. The lower pH increases mineral solubility that leads to enhanced Ca absorption or SCFA may directly increase transcellular Ca absorption. Griffin *et al.* (2002) suggests that inulin may have trophic effects in the gastrointestinal tract which may increase Ca absorption either in the colon or throughout the entire gut. 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. Another possibility is that they may have effects on tight junctions leading to increased paracellular absorption (Griffin and Abrams (2005). Paracellular Ca transport occurs via the enterocyte tight junctions and is increased as the tightness of the junctions fall. It is also believed that Ca/H exchange in the distal part of the colon is activated by the absorption of

SCFA (Roberfroid *et al*., 2002). The same author states that another possibility is an increase in the caecocolonic concentration of the calbindin D9k protein known to be involved in the absorption of Ca. Further research is clearly required to positively identify the mechanism(s) by which inulin affects Ca absorption and confirm these hypotheses.

REVIEW OF CURRENT RESEARCH REGARDING INULIN AND Ca METABOLISM

Animal Studies

A large number of investigations have demonstrated that rats fed with inulin absorb more Ca than the control rats (Scholz-Ahrens, 2007; Roberfroid, 2005). Except for one study which reported that oligofructose was more efficient than inulin in promoting recovery in postgastrectomy anaemia in rats, all types of inulin are generally considered more effective in modulating mineral absorption (Roberfroid, 2005). The author reports of a study where the effects of oligofructose, inulin HP and inulin were compared. The latter proved to be the most efficient in enhancing Ca absorption.

In a study conducted by Roberfroid *et al.* (2002), it was demonstrated that chicory inulin not only increased Ca absorption but also increased mineral parameters in whole body bones. The effect of inulin on the whole body bone mineral content (WBBMC), whole body bone area (WBBA) and whole body bone mineral density (WBBMD) was studied using male Wistar rats. The rats were fed three diets containing different Ca levels (0.2,0.5 and 1.0g/100g feed) and inulin levels (0, 5 and 10g/100g feed). WBBMC was measured using dual energy X-ray absorptiometry every four weeks up to 22 weeks. The study indicated that inulin increased WBBMC and WBBMD significantly but not WBBA at all ages and dietary Ca concentrations.

The effect of inulin on Ca metabolism was studied by Ca tracers using kinetic modelling as part of a metabolic balance study using 6 month old ovariectomized rats as a model for postmenopausal women (Weaver, 2005). Even though Ca absorption capacity of inulin fed rats was not different to the controls, kinetic modelling showed a 46% increase in Ca absorption in the presence of inulin. Bone formation rates increased 44% and bone resorption rates were completely suppressed resulting in an 89% increase in Ca retention. The author reports in the same paper of another kinetic study which failed to show an effect on Ca metabolism despite improved Ca absorption. The author reports that the difference in the two results may be due to the disparity of animal models where one used young male rats while the other used older female ovariectomized rats.

The effect of inulin has been demonstrated in a wide variety of experimental protocols in addition to healthy rats (Roberfroid, 2005). Caecectomized rats as well as rats that received Ca directly by stomach gavage or directly by caecal intubation have been used to test the hypothesis that the effect of inulin might be mediated through large bowel fermentation. The conclusion of all these studies is that inulin significantly increases mineral, especially Ca absorption, helps rats to overcome osteopenia and restores a close to normal Ca balance in adult virgin ovariectomized rats. Roberfroid (2005) goes on to state that in young growing rats, two studies have shown that the effects of inulin might decrease with time indicating a possible adaptation and/or a possible feedback down regulation of the active absorption. These findings are not conclusive and require further investigation.

Younes *et al* (2001) conducted a study to determine the effect on Ca balance in rats when given inulin combined with another complex carbohydrate, resistant starch. Thirty two male Wistar rats were distributed into four groups and each group was fed either a fibre free purified diet, diet with 100g inulin, diet with 150g resistant starch or a

diet with a blend of 50g inulin and 75g resistant starch. Results indicated that the addition of complex carbohydrates lead to significant caecal fermentation when compared to the control. Moreover both carbohydrates significantly increased Ca absorption whilst not affecting the plasma levels. Interestingly the combination of the two carbohydrates significantly increased the caecal soluble Ca concentration and the apparent intestinal absorption and balance of Ca. Therefore the authors concluded that inulin combined with other complex carbohydrates show synergistic effects on Ca absorption and balance in rats.

Scholz-Ahrens and Schrezenmeir (2007) summarise in an excellent review that the effect of inulin-type fructans on calcium absorption may be smaller when the background diet or habitual diet already contains considerable amounts of inulin-type fructans or other fermentable fibres or when its content of calcium is low. The authors also suggest that the age and or physiological stage of the animals also affect the role inulin plays in Calcium metabolism. Inulin type fructans may be more effective when animals have a higher demand for calcium as in the rapidly growing age range or in oestrogen deficiency.

Human Studies

While many studies have shown that inulin type fructans significantly increase Ca absorption in rats, human studies are limited (Cashman, 2006). There is however increasing evidence that inulin has the ability to increase Ca absorption in humans as well. Studies conducted on this aspect have been carried out to determine long term as well as short term effects of inulin ingestion.

Coudray *et al* (1997) investigated the effect of chicory inulin and sugar beet fibre on calcium absorption in a crossover design study. They fed nine healthy young adult males (mean age, 25.5 yr) a control diet (containing 18 g/d of dietary fibre) or the same diet supplemented with 40 g/d of either chicory inulin or sugar beet fibre for a period of 28 days, and determined the apparent absorption of calcium by using the mass balance approach. They found that upon inulin ingestion, apparent calcium absorption increased significantly from 21.3% to 33.7% (an increase of 58%); ingestion of sugar beet fibre had no effect.

A number of inulin intervention studies have used stable isotopes of calcium as a more sensitive measure of calcium absorption. For example, in a randomized, double-blind, crossover study, van den Heuvel *et al* (1999) fed 12 healthy male adolescents (14 –16 years of age) either orange juice supplemented with oligofructose (15 g/d) or sucrose (control treatment) three times daily for 9 days, after which they measured true fractional calcium absorption by a dual-stable calcium isotope technique. An increase of 26% in true fractional calcium absorption (47.8% with placebo to 60.1% with oligofructose) was observed upon ingestion of the daily 15 g supplement of oligofructose. In an earlier randomized, crossover study (van den Heuval *et al.*,1998), a daily supplement of 15 g of oligofructose, inulin, or galacto-oligosaccharides for 21 d had no effect on true calcium absorption (measured by a dual stable-isotope technique) in healthy adult men (20 to 30 years of age). The author states the reason for this result may be because urine sampling was carried out for only 24 hours whereas it should be collected for at least 36 hours in order to take into account any late colonic effects on Ca absorption.

Using a balanced, randomized, cross-over design Griffin *et al.*, (2002) conducted a study to assess the effect of oligofructose or a mixture of inulin and oligofructose on Ca absorption in girls at or near menarche. They received in random order a placebo (sucrose), oligofructose or a mixture of inulin+oligofructose for three weeks. Throughout the study the subjects were given 1500mg/d of Ca by providing two glasses of Ca fortified orange juice. At the end of each three week period, Ca

absorption was measured using the dual stable isotope technique. Consumption of the oligofructose+inulin mixture resulted in an 18% increase in Ca absorption (38.2±9.8% v. 32.3±9.8%) although no significant difference was seen between the oligofructose and placebo groups. The study concluded that modest intakes of inulin+oligofructose mixture increases Ca absorption in girls at or near menarche and appears to further strengthen the claims on the synergistic effect of inulin with other sugars observed in animal studies. .

Another similar study was carried out by the same chief author (Griffin *et al.*, 2003) to determine what subject characteristics are associated with this beneficial effect. Data from the original cohort of girls were combined with those of an additional 25 newly recruited subjects. The study was carried out as before. An overall increase in Ca absorption was seen from 33.1% (SD 9.2%) to 36.1 (SD 9.8%) when given an oligofructose+inulin mixture. The authors reported that the most consistent identifiable determinant of a beneficial effect of inulin on Ca absorption was fractional Ca absorption during the placebo period. The individuals with lower Ca absorption during the placebo period showed the greatest benefit. Therefore the study concluded that inulin has the ability to enhance Ca absorption mainly in girls with lower Ca absorption levels.

All of the above-mentioned studies were of relatively short duration (9 days to 5 weeks), so an important consideration in terms of the effects of inulin on calcium bioavailability is whether the benefits of these ingredients to calcium absorption persist in the longer term (Cashman, 2006). Abrams *et al* (2005) initiated a study to determine the effects on Ca absorption and bone mineral accretion after 8 weeks and one year of supplementation with an inulin type fructan. Pubertal adolescents (50 boys and 50 girls) were randomly assigned to receive 8g/d of inulin or a maltodextrin placebo. Bone mineral content and bone mineral density were measured before randomization and after one year. Ca absorption was measured using the stable isotope method at baseline, 8 weeks and one year. The results indicated that the Ca absorption was significantly higher in the fructan group than the control at both 8 weeks (difference 8.5±1.6%) and one year (difference 5.9±2.8%). After one year the fructan group had greater increment in whole body bone mineral content (difference 35±16g) and whole body bone mineral density (difference 0.015±0.0004 g/cm^2) than did the control group. The authors concluded that daily consumption of inulin type fructans significantly increases Ca absorption and enhances bone mineralization during pubertal growth both in the short and more importantly in the long term.

In another recent study headed by the same chief author (Abrams *et al.*, 2007), the mechanism of action in increasing Calcium absorption by inulin-type fructans was evaluated using a kinetic technique which employed oral ^{42}Ca and i.v. ^{46}Ca doses. Those who responded to an 8 week supplementation with 8g of a mixed short and long degree of polymerisation inulin-type fructans by increasing their calcium absorption had kinetic measurements analysed to evaluate the time course of absorption. The area under the curve of the oral tracer in the blood during the 26 hours post-ingestion of the dose was calculated and the time dependence of the increased absorption was actuated. The findings suggested that the effects of inulin-type fructans on Calcium absorption occur mainly in the colon. The study therefore further strengthens similar findings reported in previously published work (Roberfroid, 2005; Nyman, 2002; Cummings and MacFarlane, 2002; Cashamn, 2006).

Coxam (2007) in her review summarises that in adult humans, an improvement in intestinal Calcium absorption can be observed following the consumption of inulin-type fructans if the right methodology is used. The author also postulates that the absorption occurs mainly through the colon based on

evidence from trials involving subjects with an ileostomy. However she also cautions that this lack of effect may be due to the increased transit time offering less opportunity for the inulin-type fructans to influence the fermentation process. Reviewing research regarding inulin-type fructans in late postmenopausal women, the author states that although these compounds do not appear to affect absorption of Calcium during the first 5 years after the onset of menopause, they appear to be effective later on, probably because hormonal changes occurring during menopause become less important, leaving room for other mechanisms of regulation. However she cautions that only long term studies will confirm if the extra calcium absorbed is deposited in bones.

Another aspect that must be considered is that caecal fermentation and absorption may be more important in rats than humans (van den Heuval,1998). Their study reports no effect of ingestion of oligofructose and inulin on Ca and Fe absorption in human adults, but did observe an increased absorption of Ca in adolescents upon oligofructose ingestion.

CONCLUSIONS AND FUTURE DIRECTION

Ca is a key nutrient that should be consumed in adequate quantities as it is required to maintain a strong skeletal system; inadequate intakes could lead to many irreversible conditions such as osteoporosis, osteoarthritis, bone deformations and fractures especially in the latter stages of life (Cashman, 2002). Studies have also shown that the occurrence of bone conditions in the latter stages of life is directly correlated with the Ca intake in the first fifteen years after birth (Cashman, 2001). In order to improve Ca intake, we need to increase the quantity consumed as well as maximize its absorption from food. Inulin is currently used widely in functional foods throughout the world for its favourable properties in relation to health and well being and its role as a prebiotic is well accepted.

As discussed in this review, there is much evidence that inulin type fructans enhance mineral absorption in some situations although its specific mechanisms are yet to be determined. It is clear in animal studies that Ca absorption is significantly increased when fed diets containing inulin. But the effect inulin has on humans require further research before conclusions may be reached. Research up to now demonstrates that inulin may have a significant effect on mineral absorption in adolescents, but hardly any work has been carried out to determine its effects on adults. Therefore two main areas can be proposed for further research. The first are further studies to understand the underlying mechanisms of how inulin actually increases Ca absorption. Animal studies may be employed towards this end, which can also be applied to study inulin's effects on bone architecture. The second is to extend some of the recent findings in animal models to man. Particular attention should be given to determining whether inulin can reduce bone resorption in post-menopausal women as demonstrated in numerous studies involving ovariectomized rats. Long term studies on humans should be further initiated to determine the effect of inulin type fructans on Ca absorption and bone density.

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