BRITISH Nutrition FOUNDATION

Prebiotic inulin-type fructans: nutritional benefits beyond dietary fibre source

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Summary

For several years, there has been an increasing awareness of the fundamental role that the complex bacterial ecosystem plays in our health. Inulin and oligofructose are prebiotic dietary fibres naturally present in the chicory root. They escape metabolism in the small intestine, and their selective fermentation by the human colonic microflora leads to a shift in the composition of the indigenous bacterial ecosystem, in favour of health-promoting bifidobacteria. In addition to their dietary fibre effects on improved bowel regularity, several physiological advantages are linked to their specific pattern of fermentation in the colon, including improved mineral absorption, enhanced natural host defences and colonic protection, improved gut health, and beneficial influence on appetite regulation. The aim of the present review was to highlight the nutritional benefits of inulin-type fructans, focusing on key physiological functions.

Keywords: calcium absorption, colonic protection, inulin, oligofructose, prebiotic, satiety

Introduction

Inulin and oligofructose are food components that are naturally present in many edible fruits and vegetables such as onions, garlic, leeks, asparagus, wheat, bananas, artichokes and chicory (Van Loo *et al*. 1995). Native chicory inulin is extracted industrially from the root of the chicory plant (*Cichorium intybus*), by diffusion in hot water. Inulin is a mixture of linear oligomers and polymers of fructose with a glucose unit generally at the terminal position. Inulin is symbolised as G-Fn (G, glycosyl moiety; F, fructosyl moiety; n, number of fructose units). The degree of polymerisation (DP) or number of fructose units of native chicory inulin varies between 3 and 60, with an average DP of 10. Native inulin can be partially hydrolysed through the use of an endo-inulinase, to produce oligofructose in which the DP varies from 2 to 8 (average DP = 4). Oligofructose is comprised of the same fructose monomers as inulin, and is a mixture of both G-Fn (the inulin fraction containing the sucrose part) and Fm molecules (the fragmented tail of the inulin). High-performance inulin (HP-inulin), consisting of the long-chain inulin fractions with a chain length ranging from 12 to 65 (average DP = 25), is obtained by physical separation technology. Synergy1, a second-generation prebiotic, is an oligofructose-enriched inulin, combining a specific ratio of oligofructose and HP-inulin.

Inulin-type fructans consist almost exclusively of fructose units, linked together by β(2-1) bonds, which are resistant to hydrolysis by the human small intestinal digestive enzymes. Therefore, inulin and oligofructose are neither hydrolysed nor absorbed in the human upper gastrointestinal tract. Inulin and oligofructose are

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therefore classified as 'non-digestible carbohydrates', and are recognised as dietary fibres in most countries worldwide. Data supporting the non-digestibility of inulin-type fructans have been collected in ileostomised volunteers. These are patients whose large bowel has been removed, and hence the food bolus present at the end of the ileum can be collected as representative material that would normally enter the colon. It was demonstrated that 90% of the amount of ingested inulin or oligofructose is recovered intact at the end of the ileum (Ellegård *et al*. 1997).

Fermentation properties

Inulin and oligofructose reach the colon intact, where they are completely fermented by bacteria colonising the large bowel. Feeding studies in humans showed that inulin and oligofructose are not recovered in the stools (Lewis 1912; Alles *et al*. 1996; Castiglia-Delavaud *et al*. 1998). Through fermentation by lactic acid-producing bacteria, they are completely converted into bacterial biomass, short-chain fatty acids (SCFAs), lactic acid and gases (CO_2 and H_2). The major part of these SCFAs (acetate, propionate and butyrate) is rapidly used by the colonic flora or absorbed through the mucosa; only a very small fraction is excreted in the stools. Butyrate is used by the mucosal cells and contributes to the maturation and maintenance of the colonic epithelium. It also stimulates apoptosis of cancerous cells and is considered as a putative preventive agent in carcinogenesis and ulcerative colitis (Kleessen *et al*. 2001). Certain SCFAs (mainly propionate and lactate) can also reach the general circulation, and be further used as energy substrates by the liver and the muscles. The caloric value of inulin-type fructans is estimated to be about 4.2 kJ (1 kcal)/gram and 6.3 kJ (1.5 kcal)/gram for inulin and oligofructose, respectively (Roberfroid *et al*. 1993).

Dietary fibre effects and bowel habits

Inulin and oligofructose comply with most definitions of dietary fibres, and they are labelled as such in almost all countries worldwide. Through their fermentation and stimulation of bacterial growth, inulin and oligofructose improve bowel habits. The mechanism of such effect implies an increased bacterial biomass in the colon that results in an increased stool weight, as bacteria contribute substantially to the faecal mass and water content of the stools. This effect stimulates bowel peristalsis, facilitates excretion, and increases stool output and stool frequency (Gibson *et al*. 1995; Castiglia-Delavaud *et al*. 1998; Scholtens *et al*. 2006). Consumption of inulin-type fructans results in a regularisation of bowel habits and a relief of constipation (Kleessen *et al*. 1997; Den Hond *et al*. 2000).

Prebiotic properties

The original definition of 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' (Gibson & Roberfroid 1995). Prebiotics distinguish themselves from other dietary fibres through their selective fermentation pattern. The main prebiotic demonstration that has been widely studied is a selective stimulation of the growth of colonic bifidobacteria, considered as indicator organisms of a well-balanced flora, in the complex microbial community that colonises the colon. Bifidobacteria exert important anti-pathogenic activities, thus preventing or alleviating several gastrointestinal disorders. Bifidobacteria are also able to modulate the immune system, influence allergic inflammation and play a key role in the establishment of a healthy balanced infant microbiota (Salminen *et al*. 2005).

In vitro studies have demonstrated the preferential fermentation of inulin and oligofructose by bifidobacteria in anaerobic batch culture fermenters inoculated with either pure culture species or mixed human faecal flora. In all cases, the selective growth of several bifidobacteria species was observed when oligofructose or inulin was added in the medium as the sole source of carbon and energy. Other tested carbohydrate sources (polydextrose, pectin and starch) had a non-selective effect by stimulating several bacterial genera (Wang & Gibson 1993; Gibson & Wang 1994a, 1994b; Hopkins *et al*. 1998). The prebiotic properties of inulin-type fructans originate from the capacity of bifidobacteria to break down and utilise them specifically. Bifidobacteria display an inducible β-fructo-furanosidase activity, allowing them to efficiently use inulin and oligofructose as growth substrates in a competitive environment such as the colon.

In humans, this selective ability to stimulate bifidobacteria has been repeatedly proven in well-controlled intervention studies, all pointing out a selective and significant increase in bifidobacteria after the intake of inulin-type fructans (Gibson *et al*. 1995; Kleessen *et al*. 1997; Menne *et al*. 2000; Rao 2001; Tuohy *et al*. 2001; Harmsen *et al*. 2002; Bouhnik *et al*. 2007). Gibson *et al*. (1995) first demonstrated that the inclusion of 15 g/day of either inulin or oligofructose in the diet of healthy volunteers selectively stimulated the growth of bifido-

bacteria, which was accompanied by a reduction in the counts of other bacteria such as bacteroides, clostridia and fusobacteria. With a dosage as low as 5 g/day, oligofructose ingestion was reported to stimulate bifidobacteria by almost 1 logarithmic unit after 11 days of intake (Rao 2001). Similar results were reported after the intake of 5 g/day of inulin (Bouhnik *et al*. 2007). Molecular-based approaches, such as fluorescence *in situ* hybridisation, also confirmed the specific quantitative increase in bifidobacteria counts as a consequence of inulin-type fructans ingestion (Tuohy *et al*. 2001; Harmsen *et al*. 2002). By contrast with other prebiotic studies evaluating the modifications of bacterial counts in the colonic lumen (planktonic flora), Langlands *et al*. (2004) investigated the impact of a combination of 7.5 g/day of oligofructose and 7.5 g/day of long-chain inulin, given for 2 weeks to volunteers prior to colonoscopy, on the mucosa-associated intestinal flora. Biopsy samples were collected from the colonic mucosa, allowing the quantification of the impact of the prebiotic on the mucosa-associated flora. Prebiotic intake significantly ($P \leq 0.05$) increased mucosal bifidobacteria and lactobacilli in both the proximal and distal parts of the colon. The bacterial flora colonising the epithelial surface of the intestine is likely playing a specific role in modulating the immune system reactivity.

By compiling currently available data in humans, Roberfroid (2005) concluded that the daily dose of a prebiotic does not correlate with the magnitude of the bifidogenic response. At the population level, the prebiotic efficacy rather correlates with the basal composition of the faecal flora and is inversely proportional to the number of bifidobacteria initially present in the colon. Volunteers with the lower initial population levels will be subject to a higher bifidobacteria increase after inulin-type fructans consumption.

Thus, inulin and oligofructose both exert important bifidogenic properties, and based on available data, the quantitative increase in bifidobacteria counts seems to be rather independent of the fructan chain length. However, a difference in profile and rate of fermentation appears among components with different chain length distribution. By comparing the degradation rate of inulin-type fructans during *in vitro* fermentations, Roberfroid *et al*. (1998) showed that the rate of fermentation of fructans with longer chains (DP > 10) was about twice as slow as that of molecules with a DP < 10. This means that oligofructose is rapidly fermented, and hence this metabolic process is thought to take place in the proximal part of the colon. Longer-chain molecules (as in HP-inulin), on the other hand, show a slower fermentation rate and reach more distal parts of the colon, which are primarily susceptible to colonic chronic disease. Oligofructose-enriched inulin (Synergy1) combines both short-chain oligofructose and long-chain inulin, and results in a wider spread and more sustained fermentation pattern that will benefit a more extended length of the colon.

Nutritional benefits of prebiotics

The health benefits of inulin-type fructans originate not only from their impact on the composition of the intestinal microflora, but also on the metabolic activity of the micro-organisms they stimulate, that is, the production of SCFAs (acetate, propionate and butyrate), as well as numerous yet unidentified compounds, and their influence on miscellaneous bacterial enzyme activities or on other end-products of fermentation.

Enhanced host resistance and colonic protection

Scientific evidence supports the role that the commensal gut microbiota plays in our resistance to invasion by pathogens. By increasing the numbers of bifidobacteria, prebiotics impair the proliferation of potentially harmful bacteria such as *Escherichia coli*, *Campylobacter jejuni*, *Salmonella enteritidis* or *Clostridium perfringens* (Wang & Gibson 1993; Fooks & Gibson 2002). SCFAs also lower the colonic pH that contributes to preventing the overgrowth of pH-sensitive pathogenic bacteria. Results from human intervention studies suggest that inulin and oligofructose positively influence the gut-associated lymphoid tissue. Together, these effects contribute to strengthening our resistance towards colonisation and translocation of pathogens, accelerate the recovery of the gastrointestinal tract after disturbances, and ameliorate disease symptoms. Most human intervention trials with prebiotics have studied modulations of the microbiota in healthy individuals, which in turn may contribute to maintaining health and wellbeing, notably through an increased barrier function and colonisation resistance in the gut. An important area of investigation into the benefits of prebiotics includes populations that are already subjected to gastrointestinal disease and susceptible to infections. In patients hospitalised for *Clostridium difficile*-associated diarrhoea, for instance, Lewis *et al*. (2005) have shown that oligofructose increased bifidobacterial counts and decreased relapses of diarrhoea. For an extensive review of these effects, the reader is referred to Bosscher *et al*. (2006) and Seifert and Watzl (2007).

By modifying the composition of the microflora, inulin-type fructans can influence the risk of chronic

colonic disease and have been shown to reduce inflammation in ulcerative colitis (Furrie *et al*. 2005) or disease activity in Crohn's disease (Lindsay *et al*. 2006), in addition to their clear bifidogenic properties. Promising results have also been obtained in patients with increased colon cancer risk in a double-blind, placebo-controlled trial where Synergy1, in combination with probiotics, once again proved its prebiotic effect and positively altered several risk markers for colon cancer (Rafter *et al*. 2007). Work on these latter areas of research is at an early stage, but these preliminary findings highlight the interest of prebiotics in pathological conditions affecting primarily the lower gastrointestinal tract.

The fermentation process of inulin-type fructans does not only benefit the gastrointestinal tract, but it also creates favourable metabolic conditions that positively modulate mineral absorption (mainly calcium), thus improving host health 'beyond the gut'. Inulin and oligofructose are moreover able to influence entero-endocrine functions and to modulate hormones involved in the regulation of appetite and satiety. In the context of this paper, we will focus more closely on these effects.

Improved mineral bioavailability

Several studies using experimental animal models have demonstrated that inulin-type fructans have the ability to increase calcium and magnesium absorption. This effect was shown in young growing rats (Delzenne *et al*. 1995), as well as in ovariectomised female rats (Scholz-Ahrens *et al*. 2002), an experimental model for post-menopausal osteoporosis. In this latter model, bone mineralisation in femur and lumbar vertebrae was also enhanced and prevented ovariectomy-induced loss of trabecular bone (tibia) structure. These findings suggest that inulin-type fructans have a potential beneficial effect towards bone loss caused by oestrogen deficiency. A positive effect on bone mineral density was further confirmed in growing rats (Roberfroid *et al*. 2002). One study compared several inulin-type fructans (oligofructose, HP-inulin and Synergy1) and showed that all three components increased calcium absorption, but that the highest increase was observed with an oligofructose-enriched inulin (Synergy1) combining both short and longer fructan chains (Coudray *et al*. 2003).

In humans, several intervention studies have confirmed the observations made in rats. Van den Heuvel *et al*. (1999) found a significant increase in true calcium absorption ($P < 0.05$) in adolescent boys who were given 15 g/day of chicory oligofructose for 1 week. The fractional calcium absorption was increased by 26% compared with the placebo group (sucrose). In 29 adolescent girls (11–14 years) supplemented with calcium (daily intake of 1500 mg/day), Griffin *et al*. (2002) saw a significant effect on calcium absorption with a low dose of Synergy1 (8 g/day), but not with oligofructose given at the same dose for 3 weeks. Synergy1 intake resulted in 38.2% true calcium absorption compared with 32.2% in the placebo group ($P = 0.01$). Griffin *et al*. (2003) extended the results of this study by recruiting an additional pool of 25 girls in a study with a protocol identical to the previous one, so that finally, 54 adolescents were involved. The results confirmed the beneficial impact of Synergy1 on true fractional calcium absorption compared with the placebo (36.1% vs. 33.1%; $P < 0.05$).

Holloway *et al*. (2007) supplemented 15 post-menopausal women with 6 g of either Synergy1 or a placebo twice a day for 6 weeks in a randomised cross-over design. A significant increase ($P < 0.05$) for both calcium and magnesium absorption was observed after the ingestion of Synergy1 compared with the placebo. The treatment was also found to affect markers of bone turnover. Urinary deoxypyridinoline cross-links (a marker of bone resorption) were transiently decreased after 3 weeks ($P = 0.08$), but the levels were higher than baseline at 6 weeks of intervention ($P < 0.05$). Conversely, the levels of osteocalcin (indicative of bone formation activity) showed a clear upward trend at 3 weeks, and were significantly increased at 6 weeks of prebiotic intake ($P < 0.05$). The efficacy of the intervention seemed to be even more important in women with lower initial bone density of the lumbar spine.

The most important findings come from a study demonstrating that the enhancement of calcium absorption consecutive to Synergy1 intake leads to an increased bone mineralisation. A total of 100 adolescents in early puberty (aged 9–13 years) were involved in a 1-year randomised, double-blinded intervention study performed by Abrams *et al*. (2005). During the whole intervention period, the subjects were supplemented with 8 g/day of Synergy1 or placebo (maltodextrin). True calcium absorption was significantly enhanced after 8 weeks in the Synergy1 group (38.5%) compared with the placebo group (30%), and this beneficial effect was maintained during the whole year (37.7% for Synergy1 vs. 31.7% for controls) ($P < 0.05$). At the end of the experimental period, the supplementation with Synergy1 resulted in a greater increment in whole-body bone mineral content (change: 245 g/year vs. 210 g/year) and whole-body bone mineral density (change: 47 mg/cm^2/year vs. 32 mg/cm^2/year) ($P = 0.01$). The net

benefit of supplementation in calcium accretion to the skeleton was an average of approximately 30 mg/day.

Abrams *et al*. (2007a) have recently confirmed that increased calcium absorption in response to inulin-type fructans intake (Synergy1) primarily originates in the colon. An isotope-based kinetic technique (with ^{42}Ca given orally and ^{46}Ca measured intravenously) has been used to follow the relative time course for the increase in calcium absorption after the prebiotic intake. The colonic phase of calcium absorption was estimated to occur at least 7 hours after the isotope administration (before 7 hours, calcium was calculated to be absorbed in the small intestine). Healthy subjects were supplemented with 8 g/day of Synergy1 for 8 weeks. The calculation of the total area under the curve (AUC) was based on oral tracer enrichment in the blood during 26 hours after dosing. Synergy1 increased calcium absorption. The relative amount of the total AUC achieved at 7 hours did not differ between baseline and after Synergy1 intake. The relative difference in increased calcium absorption in the Synergy1 group thus occurred after the 7-hour measurement following the prebiotic intake, which demonstrates that the benefit derived from Synergy1 is mainly associated with the colonic phase of calcium absorption.

All together, these data highlight the potential for inulin-type fructans, and more specifically for Synergy1, in increasing calcium absorption, which appears to enhance bone mineralisation leading to a greater bone mass during adolescence.

Impact on entero-endocrine activities and regulation of food intake

Experimental data have recently accumulated demonstrating that inulin-type fructans, and in particular oligofructose and Synergy1, are able to modulate the expression of gut hormones (and their subsequent release into the blood) that are involved in appetite regulation. Among these hormones, glucagon-like peptide-1 (GLP-1) and grehlin have been investigated in animal studies. GLP-1 is a peptide released from the entero-endocrine L-cells present in the ileum and the colon, in response to nutrient ingestion. GLP-1 promotes insulin secretion and satiety. Grehlin is a potent orexigenic peptide.

It was shown that the addition of oligofructose or Synergy1 (10%) to the diet of animals significantly lowered energy (and food) intake, which was accompanied by lower body fat mass development in growing rats, and lower fat deposition coming from high-fat diets. These effects occurred together with an increased expression of GLP-1 in the colon and/or portal blood (Daubioul *et al*. 2002; Cani *et al*. 2004, 2005). One study has shown that the plasma levels of grehlin remained significantly lower in oligofructose- and Synergy1-fed rats, compared with the control group. Proglucagon (mRNA), the precursor of GLP-1, was significantly increased in the caecum and colonic tissue as well as in the portal blood of rats receiving oligofructose (Cani *et al*. 2004, 2005). The production of GLP-1 might obviously constitute a link between the outcome of fermentation in the colon and the modulation of food intake. Cani *et al*. (2006a) confirmed the role of GLP-1 for the systemic effects observed with oligofructose. High-fat diet-fed mice supplemented with oligofructose exhibited lower food intake and body weight gain as well as anti-diabetic effects. Treatment of the mice with the GLP-1 receptor agonist (Ex 9-39), on the contrary, totally prevented the beneficial effects seen with oligofructose. The importance of the GLP-1 receptor in mediating the effects of oligofructose was also confirmed through the use of GLP-1 receptor knock-out mice (GLP-1R -/-), in which the mice were totally insensitive to the effects of oligofructose on body weight, food intake and parameters of glucose metabolism.

The effects of inulin-type fructans on appetite and energy intake were ultimately investigated in humans, in a placebo-controlled intervention study including 10 healthy volunteers (8 g of oligofructose twice daily). Oligofructose intake resulted in increased satiety after breakfast and dinner ($P < 0.05$) as well as reduced hunger and prospective food consumption at dinner ($P \leq 0.05$). This led to a lower total energy intake during the day ($P = 0.05$) as compared with the control group (Cani *et al*. 2006b). Data of a 1-year intervention trial in adolescents ($n = 100$) further showed that the administration of Synergy1 (8 g/day) resulted in a significantly lower body mass index, lower body weight and lower body fat mass ($P < 0.05$) compared with the placebo group, thus supporting adequate weight management during early adolescence (Abrams *et al*. 2007b).These data highlight the potential importance of food ingredients that act on the gut–brain axis in modulating appetite and hence weight gain.

Technological advantages

The use of inulin and oligofructose as fibre ingredients is easy and often leads to better organoleptic properties. Inulin has a bland neutral taste and it does not negatively modify the sensory properties of foods in which it is incorporated. Inulins, and in particular long-chain inulins, are well-known fat replacers owing to their

ability to stabilise water into a particle gel network with a fat-like, creamy texture. They provide the same mouth feel as fat. Inulin is also used to improve the stability of foams and emulsions. Oligofructose, on the other hand, is highly soluble and has a moderately sweet taste (about 35% compared with sucrose), which allows for natural sugar replacement. Inulin-type fructans are already successfully applied in several well-known dairy products, baked goods and cereal products, desserts, processed meat products, baby food, drinks, and meal replacers, thus improving the nutritional profile of processed foods.

Conclusions

Inulin and oligofructose are normal constituents of our diet, and these are also available as food ingredients, contributing in a significant way to the fibre content of our diet. Besides their fibre properties, inulin and oligofructose have been widely demonstrated to beneficially affect the composition of the gut microflora, by selectively stimulating the growth and the activity of beneficial bifidobacteria, both in the gut lumen and at the colonic mucosal surface. These modifications play a major role in gastrointestinal physiology, and evidence supporting the nutritional advantages of inulin-type fructans is rapidly accumulating. Inulin and oligofructose have the potential to maintain health and wellbeing in healthy individuals, and offer a promising approach in disease conditions primarily affecting the gut, in which changes in microbiota composition might affect the outcome of the disease. Moreover, several studies have demonstrated that inulin-type fructans offer a clear benefit in calcium absorption and bone health, hence offering interesting strategies for osteoporosis prevention. Lastly, recent data have unveiled new perspectives for the use of inulin and oligofructose to increase satiety and lower food (energy) intake. This last topic of research requires confirmation in further human studies.

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