

## Oligosaccharides: state of the art

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Oligosaccharides, consisting of a mixture of hexose oligomers with a variable extent of polymerisation, are food products with interesting nutritional properties. They may be naturally present in food, mostly in fruits, vegetables or grains, or produced by biosynthesis from natural sugars or polysaccharides and added to food products because of their nutritional properties or organoleptic characteristics. The dietary intake of oligosaccharides is difficult to estimate, but it may reach 3–13 g/d per person (for fructo-oligosaccharides), depending on the population. The extent of resistance to enzymic reactions occurring in the upper part of the gastrointestinal tract allows oligosaccharides to become ‘colonic nutrients’, as some intestinal bacterial species express specific hydrolases and are able to convert oligosaccharides into short-chain fatty acids (acetate, lactate, propionate, butyrate) and/or gases by fermentation. Oligosaccharides that selectively promote some interesting bacterial species (e.g. lactobacilli, bifidobacteria), and thus equilibrate intestinal microflora, are now termed prebiotics. The pattern of short-chain fatty acid production in the caeco-colon, as well as the prebiotic effect, if demonstrated, are dynamic processes that vary with the type of oligosaccharide (e.g. extent of polymerisation, nature of hexose moieties), the duration of the treatment, the initial composition of flora or the diet in which they are incorporated. Experimental data obtained *in vitro* and *in vivo* in animals, and also recent data obtained in human subjects, support the involvement of dietary oligosaccharides in physiological processes in the different intestinal cell types (e.g. mucins production, cell division, immune cells function, ionic transport) and also outside the gastrointestinal tract (e.g. hormone production, lipid and carbohydrates metabolism). The present paper gives an overview of the future development of oligosaccharides, newly recognised as dietary fibre.

### Oligosaccharides: Fermentation: Colon: Dietary fibre

#### From chemical structure to the prebiotic effect

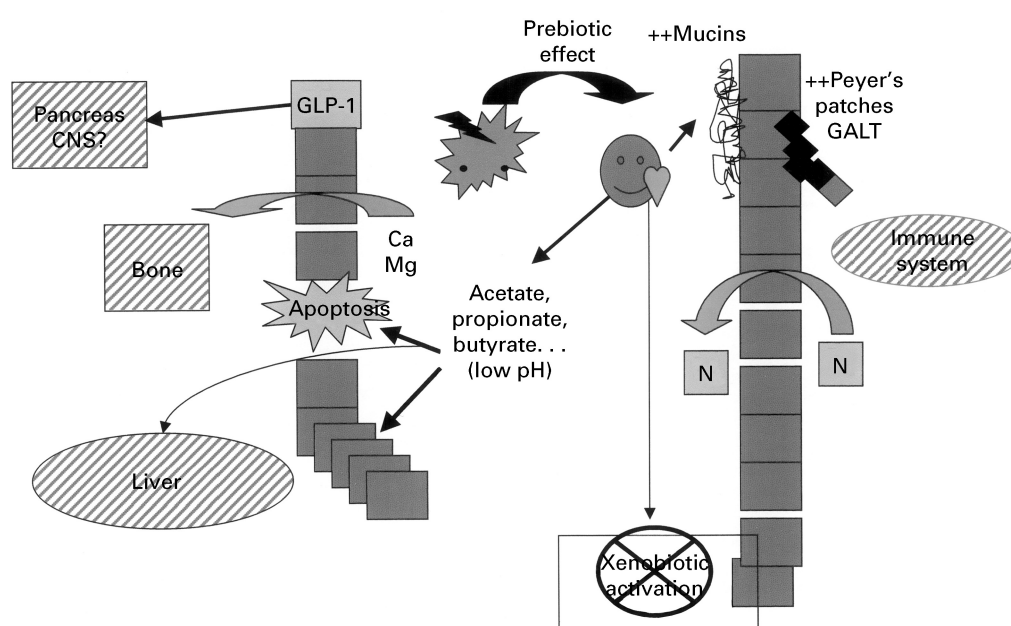
Oligosaccharides present in the diet differ from one another in their chemical structure: the number (from 2 to 20) or the type of hexose moieties (e.g. glucosyl-, fructosyl-, galactosyl-, xylosyl-); the position and conformation ( $\beta$ - v.  $\alpha$ -) of links between the hexose moieties. All these characteristics not only have consequences on the physical properties of oligosaccharides, and then on their putative usefulness as food ingredients, but also on their fate in the gastrointestinal tract. As illustrated in Table 1, natural sources of oligosaccharides exist (e.g. galacto-oligosaccharides in breast milk, fructans in onion (*Allium cepa*), leeks (*Allium porrum*) and garlic (*Allium sativum*), stachyose in soyabean) but, because of their nutritional interest, biotechnology (enzymic or thermal processes) has been applied to obtain new types of oligosaccharides, by

either enzymic synthesis from simple sugars or enzymic hydrolysis from more complex carbohydrates (Murphy, 2001). Short-chain fructo-oligosaccharides, for example, may be thus obtained by synthesis from saccharose, or through controlled and partial hydrolysis from chicory (*Cichorium intybus*) roots, i.e. inulin (for a review, see Roberfroid & Slavin, 2000). Oligosaccharides are readily water-soluble and exhibit some sweetness, which decreases with increasing chain length. Water-binding and gelling properties, and so the putative use as a fat substitute, increases with the number of hexose molecules and reticulation. These properties, together with some other interesting physiological effects (e.g. low energy value (about 6.3 kJ/g oligosaccharides), low carcinogenicity, prebiotic effect, improvement of mineral absorption; Fig. 1), support the addition of some oligosaccharides to foodstuffs that normally contain low or negligible amounts of such

**Table 1.** Dietary oligosaccharides available in food products on the market and the type of source available (adapted from Murphy, 2001)

Type of oligosaccharides	Natural occurrence	Industrial production process*
Fructo-oligosaccharides	Fruits and vegetables onions, banana, garlic, etc.	Synthesis from saccharose Hydrolysis from chicory-root inulin
Galacto-oligosaccharides	Human milk	Enzymic synthesis from lactose
Lactulose		Synthesis from lactose
Lactosucrose, glycosylsucrose		Synthesis from saccharose and/or lactose
(Iso)malto-oligosaccharides		Hydrolysis or glycosyl transfer from starch
Xylo-oligosaccharides		Hydrolysis from polyxylans
Stachyose, raffinose	Soyabean	
Palatinose-oligosaccharides		
Gentio-oligosaccharides		
Cyclodextrin		Synthesis from starch

\*Most synthetic products are obtained through enzymic reactions.



**Fig. 1.** Presentation of most of the physiological effects occurring in the caeco-colon following the ingestion of fructo-oligosaccharides, and their putative consequences outside the gastrointestinal tract. These effects are described in detail in the text, but may be summarised as follows: feeding with oligosaccharides may lead to a prebiotic effect, allowing a (re)equilibrium of the colonic microbiota. The production of short-chain fatty acids allows oligosaccharides (1) to play a role in cell proliferation in normal or altered colonic cells (butyrate); (2) to lower pH, with consequences on cation absorption; (3) to reach the liver, to play a role in lipid and glucose homeostasis. Bacterial colonic changes may also contribute to the lowering of carcinogen activation in the colon, modulate mucin production and stimulate the immune system, with consequences on host resistance to infection and other immune system-dependent processes (perhaps cancer). Other interesting effects occur such as an increase in faecal N excretion, and a promotion of the production of intestinal hormones (for example, glucagon-like peptide-1; GLP-1). CNS, central nervous system; GALT, gut-associated lymphoid tissue.

nutrients. Thus, quantification of the dietary intake of oligosaccharides in adult human subjects is difficult. In one study, based on the intake of fructo-oligosaccharides in fruits and vegetables, the estimated daily intake of inulin-type fructans for a 75 kg person was 3.2–11.3 g in Western Europe, and 1–4 g in the USA (Van Loo *et al.* 1995).

#### Oligosaccharides in the gastrointestinal tract: cooperation between bacterial and mammalian cells

One important characteristic of oligosaccharides, once ingested, is their relative resistance to digestion by hydrolytic

enzymes secreted into, or active in, the intestine (e.g.  $\alpha$ -glucosidase, maltase and isomaltase), which is dependent on the extent of polymerisation. Isomalto-oligosaccharides have a good resistance with an extent of polymerization  $>3$ . The links between the hexose moieties of  $\beta$ 1–2 fructans are completely resistant, whereas  $\beta$ 2–6-linked fructo-oligosaccharides may be partially hydrolysed at low pH. Oligosaccharides, which mostly escape digestion in the upper gastrointestinal tract, are important sources of energy for bacteria in the caeco-colon that express enzymes such as  $\beta$ -fructosidase,  $\beta$ -galactosidase, xylanase or any other hydrolases (Bernalier *et al.* 1999). The ingestion of some

oligosaccharides may lead to the proliferation of certain types of bacteria that are generally considered to be beneficial (e.g. bifidobacteria and lactobacilli), to the detriment of more harmful bacteria. The (re)equilibration of the colonic biotope, first defined as the 'prebiotic effect' by Gibson & Roberfroid (1995), has been demonstrated for dietary fructans in many studies in animals and in man; promising results have also been obtained with other oligosaccharides (Delzenne & Williams, 2002). Recent data obtained with fructo-oligosaccharides demonstrate that the dose and the duration of oligosaccharide intake, the place where fermentation mainly occurs (proximal or distal colon), as well as the initial composition of faecal flora, are important factors influencing the extent of the prebiotic effect, i.e. the increase in bifidobacteria (Rao, 2001; Tuohy *et al.* 2001a,b).

The production of short-chain fatty acids through fermentation of oligosaccharides by colonic flora is an important outcome. The pattern of fermentation, i.e. the proportion of the different short-chain acids acetate, propionate, butyrate and lactate, produced in the caecum varies with the nature of the oligosaccharides, at least in animals. Acetate:propionate is six-fold higher in the caecum of rats fed a diet containing galacto-oligosaccharides than in that of animals fed a diet containing similar levels of fructo-oligosaccharides (Sakaguchi *et al.* 1998). The proportion also changes with the duration of the treatment, which was well illustrated by the study of Le Blay *et al.* (1999), who reported a transient increase in caecal lactate and a persistent increase in caecal butyrate in rats fed a diet enriched in fructo-oligosaccharides for 27 weeks.

The data obtained in human subjects are more difficult to interpret, since they often take into account the short-chain fatty acids excreted in the faeces, which might be unrepresentative of the actual production in the colon itself.

The short-chain fatty acids have important effects in the intestinal tract. It is largely accepted that butyrate has an essential role in maintaining the metabolism, proliferation and differentiation of the different epithelial cell types (Blottière *et al.* 1999). Interestingly, short-chain fructo-oligosaccharides, known to produce substantial amounts of butyrate in the colon, increase mucosal crypt height and epithelial cell density in neo-natal pigs. 'Protective' sulfomucins in the caeco-colonic mucosa are higher in rats given a human faecal flora and fed a diet enriched in fructo-oligosaccharides (for a review, see Cherbut, 2002). All these effects could have contributed to the amelioration of symptoms associated with colitis in several experimental models (Butel *et al.* 2002; Cherbut, 2002).

Intestinal immunity is also enhanced by dietary oligosaccharides (Fig. 1). Synthetic fructans at a dose of 50–80 g/kg diet increase Peyer's patches in mice, and at a dose of 100 g/kg promote caecal and colonic macrophages in rats (Schley & Field, 2002). The mechanism of such an effect is unknown, but could be attributed to the prebiotic effect of oligosaccharides; bifidobacteria are able to stimulate production of cytokine (tumour necrosis factor  $\alpha$ , interleukin 6) and reactive molecules (NO,  $H_2O_2$ ) by macrophages *in vitro*. The accessibility of bacteria to Peyer's patches remains, however, an argument against a direct effect of intact lactic-acid bacteria as stimulators of immunity via gut-associated lymphoid tissue; bacterial

cell by-products (cell-wall peptido-glycans, cytoplasmic antigens) are more credible candidates (Schley & Field, 2002). Whatever the mechanism, it is quite interesting that an overall improvement in immunity (fewer physician visits, fewer fever episodes, higher immunological response) has also been reported in human subjects (infants and adults) ingesting a diet enriched in short-chain fructo-oligosaccharides (Saavedra & Tschernia, 2002).

Several results suggest that fermentation of oligosaccharides in the caeco-colon could contribute to the protection against colon cancer: (1) an enhanced immunity and modulation of mucin production, as described earlier; (2) the production of butyrate, which could exert a role in the regulation of altered cell proliferation, i.e. through its pro-apoptotic potency; (3) the prebiotic effect, since bifidobacteria and lactobacilli, whose growth is promoted by several oligosaccharides, exhibit low  $\beta$ -glucuronidase and nitroreductase activity; this phenomenon could contribute to lower activation of carcinogenic molecules in the colon (Fig. 1). An increase in apoptotic index and a decrease in aberrant crypt foci have been observed in the colon of rats receiving 25–100 g inulin-type fructo-oligosaccharides/kg diet, mainly after carcinogenic treatment (in the promotion phase). Synthetic oligosaccharides decrease colonic tumours in transgenic mice prone to develop aberrant crypt foci, a phenomenon associated with an increased activity of gut-associated lymphoid tissue (for a review, see Pool-Zobel *et al.* 2002). The most protective effect was observed when fructo-oligosaccharides, together with lactic acid-producing bacteria, were added to a diet with a level of fat typical of a Western diet (Bolognani *et al.* 2001). Human studies are essential to evaluate the relevance of such effects to human colon cancer risk. A dietary intervention study (European syncan project; [www.synscan.be](http://www.synscan.be)) is now in progress to evaluate the potency of a mixture of short- and long-chain fructo-oligosaccharides (10 g/d) given together with probiotics (*Lactobacillus rhamnosus* GC and *Bifidobacterium bifidum* Bb;  $12^{10}$ – $10^{10}$  colony-forming units/d) to modulate several criteria associated with high risk of developing colon cancer.

#### Oligosaccharides and mineral homeostasis and nitrogen-containing substrates

The distal part of the intestine plays an important, and often underestimated, role in mineral absorption, and is also important in the excretion of N.

The fermentation of the oligosaccharides (fructo-oligosaccharides, galacto-oligosaccharides) reaching the caeco-colon contributes to increased cation solubility, i.e. through the decrease in pH. This effect may facilitate the dissociation of bivalent cations–phytate complexes, thus counteracting the 'anti-nutrient' effect of dietary components (Greger, 1999; Lopez *et al.* 2000). Caecal hypertrophy and increased blood flow associated with oligosaccharide fermentation could also promote cation absorption. There could also be some form of collaborative relationship between fermentable carbohydrates; a combination of inulin-type fructo-oligosaccharides and resistant starch not only promotes an increase in the concentrations of caecal

soluble Ca and Mg, but also increases the apparent intestinal absorption of both minerals in rats (Younes *et al.* 2001). Several factors influence the potential of oligosaccharides to promote ion absorption: (1) the effect is only associated with certain cations (mainly Ca and Mg, and to a much lower and less-well established extent, Zn, Cu and Mn); (2) the structure and extent of polymerisation, together with the pH-modulating effect of oligosaccharides; (3) the duration of oligosaccharide treatment (in rats supplemented with arabinoxylans there is an increase in the soluble caecal Ca pool up to day 20 of treatment); (4) the presence of other nutrients (Ca and Mg absorption is increased by fructo-oligosaccharides in the presence of resistant starch; fructo-oligosaccharides decrease the anti-nutrient effect of phytate; Lopez *et al.* 2000; Younes *et al.* 2001; Scholz-Ahrens & Schrezenmeir, 2002).

It has been shown recently in human subjects that the increase in Mg and Ca absorption is associated with an improvement in the bioavailability of these nutrients, at least in some cases. In a recent double-blind crossover study in post-menopausal women it was shown that short-chain fructo-oligosaccharides given at the dose of 10 g/d for 5 weeks increased  $^{125}\text{Mg}$  absorption (+12.5 %) and the plasma Mg level (Tahiri *et al.* 2001). An increase in  $^{46}\text{Ca}$  absorption was observed in girls at menarche who had received 5 g fructo-oligosaccharides/d (Griffin *et al.* 2002). An increase in Ca absorption in adults (by >60 %) has also been demonstrated in some studies (for a review, see Scholz-Ahrens & Schrezenmeir, 2002). The improvement in Ca availability associated with some oligosaccharides (i.e. inulin-type oligosaccharides) is now considered an interesting 'functional' effect of these nutrients.

Oligosaccharide feeding may also influence polyamine metabolism. The fermentation of fructo-oligosaccharides in rats is associated with an increase in putrescine, and a decrease in spermidine in the caecal contents, a phenomenon that probably reflects modulation of the bacterial ecosystem (Delzenne *et al.* 2000). However, the increase in total polyamine concentration in the caeco-colonic tissue itself reflects an increase in ornithine decarboxylase activity (related to cell proliferation) rather than absorption of bacterial-derived polyamines.

In addition to the effects on polyamines, the displacement of N excretion to the colon and then faeces by oligosaccharide feeding is of great interest. Feeding rats a diet supplemented with inulin or oligofructose (a short-chain inulin-derived fructo-oligosaccharide) at a dose of 100 g/kg for a few weeks decreases uraemia in both normal and nephrectomized rats. Dietary fructo-oligosaccharides effectively enhance both faecal and renal N excretion in rats (Roberfroid & Delzenne, 1998; Fig. 1). It has been proposed that their osmotic effect in the small intestine allows the transfer of urea into the distal ileum and the large intestine, where a highly ureolytic microflora proliferates. When fermentable oligosaccharide intake is high, the amount of  $\text{NH}_4$  required to sustain maximal bacterial growth may become insufficient, and blood urea is then required as a ready source of N for protein synthesis by caecal bacteria (Scheppach *et al.* 2001). The relationship between fermentable fructo-oligosaccharides and hyperammonaemia, which appears to be a fermentation-dependent process, has been demonstrated for lactulose, which is used

as a pharmacological agent in the treatment of hepatic encephalopathy.

### Systemic effect of non-digestible oligosaccharides

The ingestion of non-digestible oligosaccharides may also have pleiotropic effects outside the gastrointestinal tract, involving various mechanisms. Most of the earlier published data are related to experimental studies performed in animals, but some promising effects have also been observed in man (i.e. on the lipids involved in glucose homeostasis).

In animals the putative protection against tumour cell development provided by oligosaccharides is not only restricted to colonic tumours, but has also been shown in several models, including tumour cell proliferation after transplantation in femoral muscle or intraperitoneally (Taper & Roberfroid, 2002). Fructo-oligosaccharides also decrease the mortality due to systemic infection with *Listeria monocytogenes* or *Salmonella typhimurium* (Buddington *et al.* 2002). The mechanism of such protection against pathology developing outside the gastrointestinal tract has not been elucidated, but is consistent with enhanced immune function in response to changes in the colonic microbiota.

Oligosaccharides have been shown to modulate hepatic lipid metabolism in rats and hamsters, with consequences on triacylglycerol accumulation in the liver and/or serum lipids (Fig. 1). In non-obese rats and hamsters fed a high-carbohydrate diet a decrease in hepatic and serum triacylglycerols is observed when inulin-type fructans are added to the diet at concentrations of 25–100 g/kg for several weeks (from 2 to 12 weeks; for a review, see Delzenne & Williams, 2002). In animals reduced triacylglycerolaemia is often linked to a decrease in *de novo* lipogenesis in hepatic cells, but not in adipose tissue cells. A decrease in the expression of key hepatic lipogenic enzymes, reflected by a decrease in fatty acid synthase mRNA, seems to be involved in the lower lipogenic capacity observed after supplementation with inulin-type fructo-oligosaccharide (also found with resistant starch; Delzenne *et al.* 2002). In rats fed a lipid-rich diet containing 100 g fructo-oligosaccharides/kg, a decrease in triacylglycerolaemia also occurs without any protective effect on hepatic triacylglycerol accumulation and lipogenesis, suggesting a possible peripheral mode of action (Kok *et al.* 1998b). By contrast, in obese Zucker rats dietary supplementation with fructans reduces hepatic steatosis, with no effect on postprandial triacylglycerolaemia (Daubioul *et al.* 2000). This effect probably results mainly from reduced availability of non-esterified fatty acids from adipose tissue, since fat mass and body weight are decreased by the treatment. The protection against steatosis is strongly dependent on fermentation pattern (Daubioul *et al.* 2002). The high proportion of propionate produced in the caecum (which reaches the liver through the portal vein) is, at least in animals, a key factor in the reduction of hepatic triacylglycerol synthesis observed when oligosaccharides are fed to normal and obese Zucker rats. Short-chain fatty acids are known to modulate hepatocyte metabolism; acetate may be considered to be both a lipogenic and cholesterologenic substrate, whereas propionate acts as an inhibitor

of hepatic lipid synthesis (Demigné *et al.* 1999). Propionate decreases lipogenic gene expression in cultured rat hepatocytes, and competitively inhibits monocarboxylate transporters specific to acetate transfer to hepatocytes, thus acetate to a large extent is not incorporated into liver cholesterol and fatty acids (Delzenne *et al.* 2002; M Lasa, unpublished results). The relevance of such a mechanism in man remains controversial, and will require further studies with appropriate techniques (e.g. the use of stable isotope-containing substrate).

Reported effects of oligosaccharides on circulating blood lipids in human subjects are variable. Both positive and negative outcomes have been obtained in a small number of well-designed human studies that investigated the effect of dietary supplementation with fructo-oligosaccharides (doses from 8 to 20 g/d) exhibiting prebiotic properties (Delzenne & Williams, 2002). Three of eleven studies reported significant reductions in serum triacylglycerols, whilst five studies reported modest reductions in total cholesterol and LDL-cholesterol. Studies have been conducted in both normo- and moderately-hyperlipidaemic subjects. There appear to be no obvious differences in the type of oligosaccharide, the dosages employed and duration of treatment between studies reporting negative and positive outcomes. Subjects with serum cholesterol >2500 mg/l tended to have the greatest reduction in cholesterol after supplementation with inulin-type oligosaccharide. Research into the putative effect of fructo-oligosaccharide supplementation on hepatic steatosis and lipogenesis in obese subjects and in non-alcoholic steatohepatic patients is now in progress in the author's laboratory.

Some effects of oligosaccharides on glucose metabolism and hormone metabolism have been described. Feeding fructo-oligosaccharides at 100 g/kg diet to male Wistar rats for 30 d reduces postprandial glycaemia without affecting the glycaemic response during a glucose-tolerance test after overnight fasting (Kok *et al.* 1998a; Daubioul *et al.* 2000). Feeding streptozotocin-treated (diabetic) rats a diet containing 100 g oligofructose/kg for 3 weeks decreases postprandial glycaemia, and partially restores insulin secretion. This effect seems to be linked to the higher secretion of glucagon-like peptide 1 in the portal vein, as a result of an increase in the colonic production of this peptide (Kok *et al.* 1998a; C Daubioul, P Cani, C Remacle, B Reusens and N Delzenne, unpublished results). The fact that non-digestible or fermentable carbohydrates may influence intestinal hormone production is interesting, and it could be suggested that these hormones may be a link between the outcome of fermentation in the lower part of the gut and systemic consequences of 'colonic food', such as oligosaccharide intake.

### Conclusion

Oligosaccharides have recently been recognised as components of dietary fibre because of their interesting physiological effects, which are similar to those of well-known 'soluble' fibres (Flamm *et al.* 2001). American Association of Analytical Chemists methods have been developed for some fructo-oligosaccharides in order to allow their quantification in food products. These methods

will facilitate studies into the relationship between human health status and oligosaccharide feeding. The availability of food products containing non-digestible oligosaccharides continues to increase. They may now be recognised as being carbohydrates with interesting functional effects, in some cases similar to those described for other dietary fibres (e.g. effect on lipids and on intestinal function; Scheppach *et al.* 2001), and in other cases more specific (such as the prebiotic effect). Together with resistant starch, they should be now considered as 'colonic nutrients' that may help elucidate the key role of nutrients in the lower part of intestine that have consequences on whole-body function.

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