

Prebiotics and synbiotics: concepts and nutritional properties

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The main role of diet is to provide enough nutrients to meet the requirements of a balanced diet, while giving the consumer a feeling of satisfaction and well-being. The most recent knowledge in bioscience supports the hypothesis that diet also controls and modulates various functions in the body, and, in doing so, contributes to the state of good health necessary to reduce the risk of some diseases. It is such an hypothesis which is at the origin both of the concept of 'functional food' and the development of a new scientific discipline of 'functional food science'. In the context of this paper the potential 'functional foods' to be discussed are the prebiotics and the synbiotics. The prebiotics developed so far are the non-digestible oligosaccharides and especially the non-digestible fructans among which chicory fructans play a major role. The chicory fructans are β (2-1) fructo-oligosaccharides classified as natural food ingredients. They positively affect various physiological functions in such a way that they are already or may, in the future, be classified as functional food ingredients for which claims of functional effects or of disease risk reduction might become authorized. They are classified as prebiotic and have been shown to induce an increase in the number of bifidobacteria in human faecal flora. As part of a synbiotic-type product, they are already bifidogenic at a dose of 2.75 g/d and the effect lasts for at least 7 weeks. The other potential functional effects are on the bioavailability of minerals, but also, and more systemically, on the metabolism of lipids. Potential health benefits may concern reduction of the risk of intestinal infectious diseases, cardiovascular disease, non-insulin-dependent diabetes, obesity, osteoporosis and cancer. However, except for the prebiotic effect, and tentatively the improvement of calcium bioavailability, the evidence to support such effects is still missing in humans though hypotheses already exist to justify nutrition studies.

Functional foods: Prebiotics: Synbiotics: Gut flora: Bifidobacteria: Chicory fructans: Fructooligosaccharides

Functional foods: concept and strategy

The main role of diet is to provide enough nutrients to meet the requirements of a balanced diet, while giving the consumer a feeling of satisfaction and well-being. The most recent knowledge in bioscience supports the hypothesis that diet also controls and modulates various functions in the body, so as to contribute to the maintenance of a state of good health and reduce the risk of some diseases. It is such an hypothesis which is at the origin both of the concept of 'functional food' and the development of a new scientific discipline of the 'functional food science'.

A 'functional food' is a 'food which contains (in adequate concentration) one or a combination of components which affects functions in the body so as to have positive cellular or physiological effects' (Roberfroid, 1996). Research and development of 'functional food' is part of the science of nutrition and the benefit associated with its intake has to be evaluated in the context of the diet. The strategy for research and development of a 'functional food' requires:

- (1) the demonstration of an interaction with (a) function(s) in the body;

- (2) some understanding of the mechanism thereof;
- (3) the establishment of the effect in relevant biological systems;
- (4) the formulation of sound hypotheses;
- (5) the testing of these hypotheses in human nutrition studies.

The concepts of prebiotics and synbiotics

In the context of this paper the potential 'functional foods' to be discussed are the prebiotics and the synbiotics (Gibson & Roberfroid, 1995). A prebiotic is defined as 'a nondigestible 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' (Gibson & Roberfroid, 1995). It is a substance which modifies the composition of the colonic microflora in such a way that a few of the potentially health-promoting bacteria (especially lactobacilli and bifidobacteria) become predominant in numbers. A synbiotic is the product in which both a probiotic and a prebiotic are combined in a single product. It is defined as 'a mixture of a probiotic and a prebiotic that beneficially

affects the host by improving the survival and the implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria'. The prebiotics developed so far are the non-digestible oligosaccharides (Delzenne & Roberfroid, 1994) and especially the non-digestible fructans among which chicory fructans play a major role.

The chicory fructans

The chicory fructans (inulin and its enzymatic hydrolysate oligofructose) are an important and emerging group of dietary carbohydrate (Cummings & Roberfroid, 1997; Roberfroid & Delzenne, 1998). They are β (2-1) fructo-oligosaccharides classified as natural food ingredients (Van Loo *et al.* 1995). In chicory inulin, both $G_{py}F_n$ (α -D-glucopyranosyl- $[\beta$ -D-fructofuranosyl] $_{n-1}$ -D-fructofuranoside) and $F_{py}F_n$ (β -D-fructopyranosyl- $[\beta$ -D-fructofuranosyl] $_{n-1}$ -D-fructofuranoside) compounds are considered to be included under the same nomenclature and n , the number of fructose units, varies from two to more than sixty (De Leenheer & Hoebregs, 1994; De Leenheer, 1996). Inulin-type fructans are present in significant amounts in miscellaneous edible fruits and vegetables. Average daily consumption has been estimated to be 1–4 g in the USA and 3–11 g in Europe, the most common sources being wheat, onion, banana, garlic and leek (Van Loo *et al.* 1995). Chicory inulin and oligofructose are officially recognized as food ingredients in most European countries in which, for food labelling purposes, they have also been given a dietary fibre status and have a self-affirmed GRAS (generally regarded as safe) status in the USA. Chicory fructans positively affect various physiological functions in such a way that they are already, or may in the future be, classified as functional food ingredients for which claims of functional effects or of disease risk reduction might become authorised (Roberfroid, 1995, 1996). The potential functional effects are on the gastrointestinal physiology, the bioavailability of minerals, but also, and more systemically, on the metabolism of lipids. Potential health benefits may include reduction of the risk of intestinal infectious diseases, cardiovascular diseases, non-insulin-dependent diabetes, obesity, osteoporosis and cancer.

Chicory fructans as prebiotics and functional food ingredients

The prebiotic effect

Chicory fructans resist digestion in the upper part of the gastrointestinal tract (Ellegård *et al.* 1996); moreover, there is evidence that they are not absorbed to any significant extent and are classified as 'colonic food', i.e. 'a food entering the colon and serving as substrate for the endogenous bacteria, thus indirectly providing the host with energy, metabolic substrates' (Gibson & Roberfroid, 1995). The prebiotic effect of chicory fructans is extensively discussed in the paper by Gibson but, as a summary, it can be reported that:

- (1) chicory fructans are fermented by bacteria colonizing the large bowel as shown by a large number of *in vitro* (both analytical and microbiological) and *in vivo* studies which, in addition, confirm the production of lactic and short-chain carboxylic acids as endproducts of the fermentation (Roberfroid *et al.* 1997);
- (3) in human *in vivo* studies, this fermentation leads to the selective stimulation of growth of the bifidobacteria population, making chicory fructans the prototype prebiotic (Gibson *et al.* 1995; Gibson & Roberfroid, 1995; Roberfroid *et al.* 1995, 1997; Kleessen *et al.* 1997).

Functional effects

Besides being prebiotic and bifidogenic, chicory fructans have other nutritional properties which make them good candidates for classification as functional food ingredients. The most extensively studied effects are on calcium bioavailability, on lipid metabolism and on the risk of developing precancerous colonic lesions (i.e. aberrant crypt foci).

Improvement of calcium bioavailability. In growing rats, it has been shown that chicory fructans enhance calcium and magnesium absorption as well as iron and zinc balance without having significant effect on copper bioavailability (Delzenne *et al.* 1995). These conclusions are based on mineral balance measurements. Doses of chicory fructans in the rat diet varied from 5 to 20%. The hypotheses most frequently proposed to explain this enhancing effect on mineral absorption are: (a) the osmotic effect; (b) acidification of the colonic content due to fermentation and production of short-chain carboxylic acids; (c) formation of soluble calcium and magnesium salts of these acids; and (d) hypertrophy of the colon wall. In addition and more recently, Ohta *et al.* (1997) reported that feeding rats fructo-oligosaccharides reduced the concentration of calbindin D9K in the small intestine while enhancing it in the colon. Similar positive effects on calcium absorption have been reported for other non-digestible carbohydrates, in particular resistant starch (Younes *et al.* 1996).

More recently, and based on consistently repeated observations in rats, *in vivo* human studies have been performed which confirm the positive effect of chicory fructans on the absorption and balance of dietary calcium but not of iron, magnesium or zinc. In the first published report, nine men (21.5 ± 2.5 years) taking in ± 850 mg calcium/d and receiving a dietary supplement of 40 g/d of chicory inulin had a significant increase in the apparent absorption (33.7 v. 21.3 %) and balance ($+100$ mg/d) of calcium without any change in urinary excretion (Coudray *et al.* 1997). In the second study, twelve 15–18-year-old boys consumed 15 g chicory fructans/d and their calcium balance, measured by the double stable isotope technique, showed a significant increase (60.1 v. 49.1 %; $P < 0.05$) with no effect on urinary excretion (van den Heuvel & Schaafsma, 1997). In conclusion chicory fructans are likely to positively affect calcium absorption and calcium balance, including in humans, without modifying urinary excretion. Within the strategy for functional food development presented above, the evidence for an effect of chicory fructans on calcium bioavailability

has been assessed as 'promising' (Roberfroid & Delzenne 1998).

Effects on lipid metabolism. The effects of chicory fructans on triglyceridaemia have been studied mainly in rats, but preliminary human studies have already been reported (see Table 1 for summary). In rats, a decrease in serum triglyceridaemia (both in fed and fasted state) has consistently been reported in several studies. In healthy normolipidaemic humans, only fasting lipid parameters have been measured and the results are contradictory (Canzi *et al.* 1995; Pedersen *et al.* 1997). Data concerning the effects of chicory fructans on cholesterolaemia are scarce, but one study performed on slightly hypercholesterolaemic volunteers has reported a significant reduction both in total LDL cholesterol (Davidson *et al.* 1998). In rats, feeding a diet supplemented with chicory fructans (10% in the diet) significantly lowers serum triglycerides and phospholipid concentrations (Delzenne *et al.* 1993), but does not modify free fatty acid concentration in the serum. The hypotriglyceridaemia is mostly due to a decrease in the concentration of plasma VLDL (Fiordaliso *et al.* 1995). This effect is likely to be due to a decrease in the hepatic synthesis of triglycerides rather than to a higher catabolism of triglyceride-rich lipoproteins (Kok *et al.* 1996a). Hepatocytes isolated from these rats have a 40% decreased capacity to synthesize triglycerides from ^{14}C -acetate (Fiordaliso *et al.* 1995; Kok *et al.* 1996a,b). These data support the hypothesis of a decreased *de novo* lipogenesis in the liver, through a coordinated reduction in the activity of all lipogenic enzymes as the key event in the reduction of VLDL-triglyceride secretion in the chicory fructans fed rats. Because hepatic *de novo* lipogenesis is likely to be the target of the hypotriglyceridaemic effect of fructans in the rats, the apparent lack of effect observed in healthy humans, who eat much less carbohydrates but more lipids than rodents, is not surprising and does not demonstrate an absence of effect. Some experiments should be performed either in obese patients or in insulin-resistant individuals, or in subjects eating high carbohydrate-high caloric diets (Aarstrand *et al.* 1996). When assessing these data with regard to functional effects of chicory

fructans, it may be concluded that preliminary evidence exists for an hypotriglyceridaemic effect but that, at the present stage of knowledge, it is impossible to conclude a hypocholesterolaemic effect. Because a mechanism of the hypotriglyceridaemic effect has tentatively been identified, an hypothesis can be formulated which can be tested in relevant human studies.

Reduction of risk of developing precancerous lesions in the colon. Experimental data have been recently published which demonstrate that feeding rats with chicory fructans significantly reduces the incidence of the so-called aberrant crypt foci induced by colon carcinogens like azoxymethane or dimethylhydrazine (Reddy *et al.* 1997; Rowland *et al.* 1998). Furthermore, Taper *et al.* (1997) have reported that supplementing the diet of mice with these food ingredients slows down the growth rate of two different types of implanted tumours as compared to control-fed mice. Fontaine *et al.* (1996) have reported that, in heteroxenic rats harbouring a human colonic flora, inulin stimulates the production of sulphomucin and a reduction in sialomucin, two effects known to be associated with a reduced risk of colon cancer (Cassidy *et al.* 1990; Satchithanandam *et al.* 1990). In the strategy for functional food development described above, these cancer-inhibitory effects in experimental animals correspond to the first step (i.e. 'identification' of effects) which, because of their potential implications in human health, need careful evaluation including in relevant human studies.

Chicory fructans in synbiotic preparations

Whereas the concept of prebiotic has become very popular since its introduction in 1995 (Gibson & Roberfroid, 1995), the concept of synbiotic has, up to now, not really been applied to the development of new foods especially functional foods. Furthermore, even when new products indeed contain a mixture of pro- and prebiotics they are seldom presented or defined as synbiotics. This concept thus remains open for validation and further research is needed. For chicory fructans, which, as reviewed above, are prebiotics, two examples already exist of

Table 1. Summary of the effect of chicory fructans on serum lipids in rats and humans

	Dose	Duration (weeks)	Effects	References
Rats	10% in diet	5	Hypo TG/VLDL Hypo chol	Fiordaliso <i>et al.</i> (1995)
		4	Hypo TG	Delzenne & Roberfroid (1994)
		4	Hypo TG	Kok <i>et al.</i> (1996a)
	20% in diet	5	Hypo TG Hypo chol (LDL)	Delzenne <i>et al.</i> (1993)
Humans	14 g/d	4	No change TG/chol	Pedersen <i>et al.</i> (1997)
	9 g/d	4	Hypo TG Hypo chol	Canzi <i>et al.</i> (1995)
	hypercholesterolaemic*	18 g/d	3	Hypo chol (LDL)

Hypo = statistically significant reduction in TG (triglycerides) or chol (cholesterol). LDL indicates that, in addition to an effect on total cholesterol, there was also an effect on LDL cholesterol.

* These particular volunteers were slightly hypercholesterolaemic.

synbiotic-type products which may have additional interesting properties.

Synbiotics and the composition of the faecal flora

When combining both a probiotic and a prebiotic in a single food product, the expected benefits are, as stated in the synbiotic definition, an improved survival during the passage of the probiotic bacteria through the upper intestinal tract and a more efficient implantation in the colonic microbiota together with a stimulating effect of the prebiotic on the growth and/or the activities of both the exogenous (probiotic) and endogenous bacteria (e.g. bifidobacteria).

Without specifically referring to it as a 'synbiotic approach', Bouhnik *et al.* (1996) have assessed, in healthy humans, the effects of prolonged ingestion of *Bifidobacterium* spp. fermented milk with or without inulin (equivalent to 18 g/d) on faecal bifidobacteria. They concluded that the *Bifidobacterium* spp. fermented milk substantially increased the number of bifidobacteria colony-forming units (cfus) in the faecal flora, but that the concurrent administration of inulin did not enhance the effect. This observation is not surprising in view of the fact that the number of bifidobacteria cfus in the faeces of probiotic-feed volunteers was already so much increased (from $10^{7.7}$ to 10^9) that they could hardly be additionally increased by the prebiotic. Indeed it has recently been argued that the stimulation of growth of bifidobacteria by prebiotics very much depends on their initial level (Roberfroid *et al.* 1997). Moreover, an

in-depth analysis of the data reported by these authors reveals that 2 weeks after stopping the consumption of the dairy products, the volunteers ($n = 6$) who received the synbiotic-type product still had a significantly ($P < 0.01$) higher number of bifidobacteria cfus than those ($n = 6$) receiving the probiotic alone. This could indicate either a better implantation of the probiotic bifidobacteria in the colonic microbiota or a prebiotic-type effect on endogenous bifidobacteria.

Another trial has been performed at the Institut Paul Lambin Brussels, Belgium (Menne *et al.* personal communication) who have fed a group of human volunteers ($n = 11$; aged 20–50 years; BMI 18–25) a daily supplement of a synbiotic composed of 125 ml *Lactobacillus*-fermented milk containing 2.75 g oligofructose continuously for 7 weeks. Every week, freshly collected faecal samples were analysed for total anaerobes, bifidobacteria, lactobacilli and coliforms using selective media as described by Gibson *et al.* (1995). A MANOVA-type statistical analysis of the results (Fig. 1) revealed that, globally, the treatment had a statistically significant effect ($P = 0.02$) on the composition of faecal flora and that this effect was most exclusively due to an increase ($P = 0.025$) in the number of bifidobacteria cfus leading to an equivalent and parallel increase in total anaerobes cfus ($P = 0.02$). The coliform cfus were not modified ($P > 0.05$) but, surprisingly, the number of lactobacilli cfus was decreased ($P = 0.02$). Even though this study does not really demonstrate the efficacy of the synbiotic approach, the results are interesting

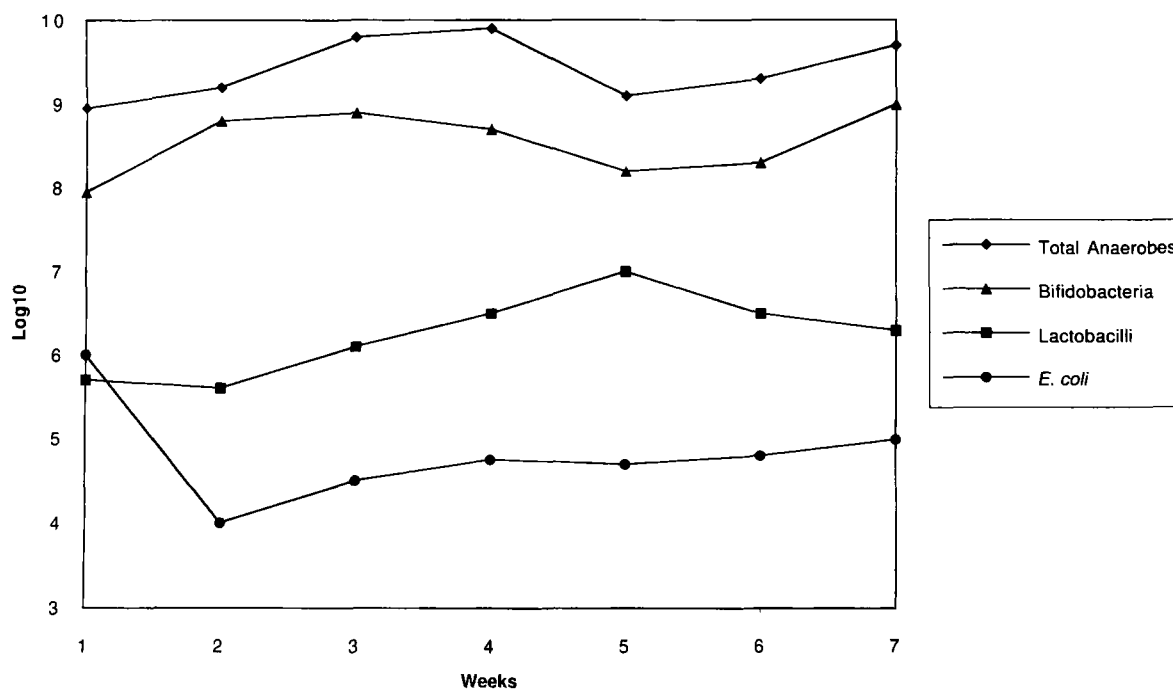


Fig. 1. Evolution, as a function of time (week 1–7), of various bacterial populations in fresh faecal samples of healthy human volunteers consuming daily 2.75 g of oligofructose in 125 ml of a lactobacilli-fermented milk. Data are presented as means of eleven individual values expressed as \log_{10} of the number/g of faeces of the colony forming units (cfus) in selective culture media. A MANOVA statistical analysis revealed that, globally, the treatment had a statistically significant effect ($P = 0.02$) on the composition of the faecal microflora mainly due to an increase in the number of bifidobacteria cfus ($P = 0.025$) which induced a parallel increase in total anaerobes ($P = 0.02$). In addition there was a decrease in lactobacilli cfus ($P = 0.02$) but no change in coliform cfus.

because they demonstrate the bifidogenic/prebiotic effect of quite a low daily dose (2.75 g) of a chicory fructan (oligofructose) continuously fed over a period of 7 weeks. It may be hypothesized that such an effect could result from the synbiotic-type product, but this needs further investigation.

Synbiotics and the reduction of risk of precancerous lesions

In their study reporting an inhibitory effect of chicory fructans on the development of aberrant crypt foci in the colon of rats treated with azoxymethane, Reddy *et al.* (1997) have used a prebiotic-type approach. More recently Rowland *et al.* (1998) have revisited the same hypothesis but using a synbiotic-type treatment. Indeed, they have supplemented the rat diets with either 5 % chicory inulin, *Bifidobacterium longum* 25 ($7 \cdot 10^8$ cfus/g) or both. In rats previously treated with azoxymethane (2×12.5 mg/kg s.c.) and fed these diets for 12 weeks, both inulin and *B. longum* showed a statistically significant reduction in the total number of aberrant crypt foci (−29 % and −21 %, respectively) as well as in the number of foci with one to three aberrant crypts (−41 % and −26 %, respectively) but not in the number of larger foci (\geq four aberrant crypts). The most interesting observation is that the synbiotic product was the most efficient treatment for reducing the total number of aberrant crypt foci, the number of foci with one to three aberrant crypts/foci and the number of large foci (\geq four aberrant crypts/foci) by 74 %, 80 % and 59 %, respectively. These data support the hypothesis that, at least for some of the health-promoting effects, a synbiotic could be more efficient than either a probiotic or a prebiotic alone. This needs further research to be fully validated.

Conclusion

The non-digestible oligosaccharides (Delzenne & Roberfroid, 1994), especially the chicory fructans, have nutritional properties which make them potential candidate functional food ingredients. They are classified as prebiotics and have been shown to induce an increase in the number of bifidobacteria in the faecal flora. This bifidogenic activity may already be statistically significant for a daily intake as low as 2.75 g for up to 7 weeks. Associated with *Bifidobacterium longum* in a fermented milk product, they prolong, at least partly, the bifidogenic effect for at least 2 weeks. Chicory fructans are thus good candidates for incorporation into synbiotic products, even if this concept still needs to be further validated.

Besides modifying the composition of the faecal flora, the daily consumption of chicory fructans may increase the bioavailability of calcium ions in humans. Moreover, experimental data and preliminary evidence in humans support the hypothesis that these natural food ingredients could help to balance the metabolism of lipids, especially triglycerides. In a strategy for functional food development, the time has come for hypothesis-driven human studies. Finally, the inhibitory effect on aberrant crypt foci which are recognized as 'markers' for colon cancer risk, an effect which is moreover supported by similar data on other non-digestible oligosaccharides such as the synthetic

fructo-oligosaccharides (Koo & Rao, 1991; Pierre *et al.* 1997), open interesting perspectives which need further investigations.

The colonic foods, i.e. the probiotics, the prebiotics and the synbiotics are promising tools for the development of functional foods. Indeed, miscellaneous interactions with body functions have been identified and demonstrated in relevant models, some mechanisms of these effects are already understood and human data are accumulating.

References

- Aarsland A, Chinkes D & Wolfe RR (1996) Contributions of *de novo* synthesis of fatty acids to total VLDL-triglyceride secretion during prolonged hyperglycemia/hyperinsulinemia in normal man. *Journal of Clinical Investigation* **98**, 2008–2017.
- Bouhnik Y, Flourie B, Riottot M, Bisetti N, Gailing M, Guibert A, Bornet F & Rambaud J (1996) Effects of fructo-oligosaccharides ingestion on fecal bifidobacteria and selected metabolic indexes of colon carcinogenesis in healthy humans. *Nutrition and Cancer* **26**, 21–29.
- Canzi E, Brighenti F, Casiraghi MC, Del Puppo E & Ferrari A (1995) Prolonged consumption of inulin in ready-to-eat breakfast cereals: effects on intestinal ecosystem, bowel habits and lipid metabolism. *Cost 92, Workshop on Dietary Fiber and Fermentation in the Colon*. Helsinki, 15–17/04.
- Cassidy MM, Satchithanandam S, Calvert RJ, Vahouny GV & Leeds AR (1990) Quantitative and qualitative adaptations in gastrointestinal mucin with dietary fiber feeding. In *Dietary Fiber: Chemistry, Physiology, and Health Effects*, pp. 67–88 [Kritchevsky D, Binfield C, Anderson JW, editors]. Plenum Press, London.
- Coudray C, Bellanger J, Castiglia-Delavaud C, Rémésy C, Vermorel M & Demigné C (1997) Effect of soluble and partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. *European Journal of Clinical Nutrition* **51**, 375–380.
- Cummings JH & Roberfroid MB (1997) A new look at dietary carbohydrate: chemistry, physiology and health. *European Journal of Clinical Nutrition* **51**, 417–423.
- Davidson MH, Synecki C, Maki KC & Drenman KB (1998) Effects of dietary inulin in serum lipids in men and women with hypercholesterolemia. *Nutrition Research* **3**, 503–517.
- De Leenheer L (1996) Production and use of inulin: industrial reality with a promising future. In: *Carbohydrates as Organic Raw Materials*, vol. III, pp. 67–92 [Van Bekkum H, Röper H & Voragen F, editors]. VCH Publications, New York and Verlagsgesellschaft, Weinheim, Germany.
- De Leenheer L & Hoebregs H (1994) Progress in the elucidation of the composition of chicory inulin. *Starch* **46**, 193–196.
- Delzenne N, Kok N, Fiordaliso M, Buoyser D, Goethals F & Roberfroid M (1993) Dietary fructooligosaccharides and modify lipid metabolism in rats. *American Journal of Clinical Nutrition* **57**, 820S.
- Delzenne N & Roberfroid MB (1994) Physiological effects of nondigestible oligosaccharides. *Lebensmittel Wissenschaft und Technologie* **27**, 1–6.
- Delzenne N, Aertssens J, Verplaetse H, Roccaro M & Roberfroid M (1995) Effect of fermentable fructo-oligosaccharides on mineral, nitrogen and energy digestive balance in the rats. *Life Sciences* **57**, 1579–1587.
- Ellegård L, Andersson H & Bosaeus I (1996) Inulin and oligofructose do not influence the absorption of cholesterol,

- or the excretion of cholesterol, Ca, Mg, Zn, Fe, or bile acids but increases energy excretion in ileostomy subjects. *European Journal of Clinical Nutrition* **51**, 1–5.
- Fiordaliso MF, Kok N, Desager JP, Goethals F, Deboyser D, Roberfroid M & Delzenne N (1995) Dietary oligofructose lowers triglycerides, phospholipids and cholesterol in serum and very low density lipoproteins of rats. *Lipids* **30**, 163–167.
- Fontaine N, Meslin JC, Lory S & Andrieux C (1996) Intestinal mucin distribution in the germ-free rat and in the heteroxenic rat harbouring a human bacterial flora: effect of inulin in the diet. *British Journal of Nutrition* **75**, 881–892.
- Gibson GR, Beatty ER, Wang X & Cummings JH (1995) Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* **108**, 975–892.
- Gibson GR & Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition* **125**, 1401–1412.
- Gibson GR & Wang X (1994) Enrichment of bifidobacteria from human gut contents by oligofructose using continuous culture. *FEMS Microbiology Letters* **118**, 121–128.
- Kleessen B, Sykura B, Zunft HJ & Blaut M (1997) Effects of inulin and lactose on faecal microflora, microbial activity, and bowel habit in elderly constipated persons. *American Journal of Clinical Nutrition* **65**, 1397–1402.
- Kok N, Roberfroid M & Delzenne N (1996a) Involvement of lipogenesis in the lower VLDL secretion induced by oligofructose in rats. *British Journal of Nutrition* **76**, 881–890.
- Kok N, Roberfroid M & Delzenne N (1996b) Dietary oligofructose modifies the impact of fructose on hepatic triacylglycerol metabolism. *Metabolism* **45**, 1547–1550.
- Koo M & Rao V (1991) Long term effect of bifidobacteria and neosugar on precursor lesions of colonic cancer in mice. *Nutrition and Cancer* **16**, 249–257.
- Ohta A, Motohashi Y & Sakuma K (1997) Dietary fructooligosaccharides change the intestinal distribution of calbindin 9kD in rats. In *Non-digestible Oligosaccharides: Healthy Food for the Colon? Proceedings of the International Symposium held at Wageningen, 1997*, p. 153 [Hartemink R, editor]. Heteren, The Netherlands, Vermeulen Publications.
- Pedersen A, Sandström B & VanAmelsvoort JMM (1997) The effect of ingestion of inulin on blood lipids and gastrointestinal symptoms in healthy females. *British Journal of Nutrition* **78**, 215–222.
- Pierre F, Perrin P, Champ M, Bornet F, Meflah K & Menanteau J (1997) Short-chain fructo-oligosaccharides reduce the occurrence of colon tumors and develop gut-associated lymphoid tissue in *Min* mice. *Cancer Research* **57**, 225–228.
- Reddy BS, Hamid R & Rao CV (1997) Effect of dietary oligofructose and inulin on colonic preneoplastic aberrant crypt foci inhibition. *Carcinogenesis* **18**, 1371–1374.
- Roberfroid MB (1995) A functional food: chicory fructooligosaccharides, a colonic food with prebiotic activity. *World of Ingredients* March–April, 42–44.
- Roberfroid MB, Bornet F, Bouley C & Cummings JH (1995) Colonic microflora: nutrition and health. *Nutrition Review* **53**, 127–130.
- Roberfroid MB (1996) Functional effects of food components and the gastrointestinal system: chicory fructooligosaccharides. *Nutrition Review* **54**, S38–S42.
- Roberfroid MB, Van Loo JAE & Gibson GR (1997) A review of the bifidogenic nature of chicory inulin and its hydrolysis products. *Journal of Nutrition* **128**, 11–19.
- Roberfroid MB & Delzenne N (1998) Dietary fructans. *Annual Review of Nutrition* **18**, 117–143.
- Rowlands IR, Rumney CJ, Coutts JT & Lievens LC (1998) Effect of *Bifidobacterium longum* and inulin on gut bacterial metabolism and carcinogen-induced crypt foci in rats. *Carcinogenesis* **19**, 281–285.
- Satchithanandam S, Vargofcak-Apker M, Calvert RJ, Leeds AR & Cassidy MM (1990) Alteration of gastrointestinal mucin by fiber feeding in the rat. *Journal of Nutrition* **120**, 1179–1184.
- Taper H, Delzenne N & Roberfroid MB (1997) Growth inhibition of transplantable mouse tumors by non digestible carbohydrates. *International Journal of Cancer* **71**, 1109–1112.
- van den Heuvel EGHM & Schaafsma G (1997) A double blind cross-over trial on the effect of fructo-oligosaccharides on calcium absorption in 15–18 year old males. *American Journal of Clinical Nutrition* (submitted).
- Van Loo J, Coussement P, De Leenheer L, Hoebregs H & Smits G (1995) On the presence of inulin and oligofructose as natural ingredients in the western diet. *CRC Critical Review in Food Sciences Nutrition* **35**, 525–552.
- Wang X & Gibson GR (1993) Effects of the *in vitro* fermentation of oligofructose and inulin by bacteria growing in the human large intestine. *Journal of Applied Bacteriology* **75**, 373–380.
- Younes H, Demigné C & Rémésy C (1996) Acidic fermentation in the caecum increases absorption of calcium and magnesium in the large intestine of the rat. *British Journal of Nutrition* **75**, 301–314.