British Journal of Nutrition (2005), **93**, Suppl. 1, S73–S90 DOI: 10.1079/BJN20041349

Inulin-type fructans and reduction in colon cancer risk: review of experimental and human data

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Inulin-type fructans (β(2,1)fructans) extracted from chicory roots (*Cichorium intybus*) are prebiotic food ingredients, which in the gut lumen are fermented to lactic acid and SCFA. Research in experimental animal models revealed that inulin-type fructans have anticarcinogenic properties. A number of studies report the effects of inulin-type fructans on chemically induced pre-neoplastic lesions (ACF) or tumours in the colon of rats and mice. In twelve studies, there were twenty-nine individual treatment groups of which twenty-four measured aberrant crypt foci (ACF) and five measured tumours. There was a significant reduction of ACF in twenty-one of the twenty-four treatment groups and of tumour incidence in five of the five treatment groups. Higher beneficial effects were achieved by synbiotics (mixtures of probiotics and prebiotics), long-chain inulin-type fructans compared to short-chain derivatives, and feeding high-fat Western style diets. Inulin-type fructans reduced tumour incidence in APC^{Min} mice in two of four studies and reduced growth and metastasising properties of implanted tumour cells in mice (four studies). The effects have been reported to be associated with gut flora-mediated fermentation and production of butyrate. In human cells, inulin-derived fermentation products inhibited cell growth, modulated differentiation and reduced metastasis activities. In conclusion, evidence has been accumulated that shows that inulin-type fructans and corresponding fermentation products reduced the risks for colon cancer. The involved mechanisms included the reduction of exposure to risk factors and suppression of tumour cell survival. Thus, this specific type of dietary fibre exerted both blocking agent and suppressing agent types of chemopreventive activities.

Inulin-type fructans: Colorectal cancer: Review

Causes of colorectal cancer

Colorectal cancer (CRC) is one of the most frequent causes of death due to cancer in populations of developed countries who consume 'Western style diets' (World Cancer Research Fund, American Institute for Cancer Research, 1997). Studies reported that dietary patterns, lifestyle exposure patterns, physical inactivity and obesity increased CRC risks, especially in genetically predisposed populations (Potter, 1999). CRC is thus causally related to both genes and environment. Environment delivers risk factors that cause mutations and initiate cancer or enhance growth by genetic and epigenetic mechanisms (Ferguson, 1999). Nutrition may supply products which may counteract the causative factors (Johnson *et al.* 1994) and which can be recommended on the basis of a wholesome and complete diet.

Dietary fibre and colorectal cancer risk reduction

One of the protective dietary ingredients may be dietary fibre (non-starch polysaccharides), which, however, has and has not been shown to lower the risk of CRC. No protective effects of fibre were seen in large prospective studies in the USA, Finland and Sweden (Fuchs *et al.* 1999; Pietinen *et al.* 1999; Terry *et al.* 2001). There were no reduced recurrence rates of adenomatous colorectal polyps in large intervention trials with supplements of bran, soluble fibre, or vegetables (Alberts *et al.* 2000; Bonithon-Kopp *et al.* 2000; Schatzkin *et al.* 2000). In contrast, a more recent observational study on dietary fibre in food and protection against CRC in the European Prospective Investigation into Cancer and Nutrition (EPIC) showed an inverse association between dietary fibre intake and incidence of CRC (Bingham *et al.* 2003). The striking overall conclusion of this study was that, in populations with low average intake of dietary fibre, an approximate doubling of total fibre intake from foods could reduce the risk of CRC by 40 % (Bingham *et al.* 2003). Another study, done within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, investigated the relation of fibre intake and frequency of colorectal adenoma using a food frequency questionnaire (Peters *et al.* 2003). The fibre intake of 33 971 participants who were sigmoidoscopy-negative for polyps was compared with 3591 cases with at least one histological verified adenoma in the distal large bowel. The authors reported that high intakes of dietary fibre were associated with a lower risk of colorectal adenoma, after adjustment for potential dietary and non-dietary risk factors. A commentary discussed why these two groups of studies gave such different results from the earlier reports (Ferguson & Harris, 2003). One major difference mentioned was the study size of the different surveys, with the EPIC study clearly covering the largest population studied. The other major difference was seen in the amount of dietary fibre

Abbreviations: AFC, aberrant crypt foci; AI, apoptotic index; AOM, azoxymethane; CRC, colorectal cancer; DMH, 1,2-dimethylhydrazine; EPIC, European Prospective Investigation into Cancer and Nutrition; GST, glutathione *S*-transferase; NSAID, non-steriodal anti-inflammatory drugs.

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consumed. Thus, the EPIC consortium had assessed a range of dietary fibre intakes that was substantially greater than what was determined for the survey in US nurses (Fuchs *et al.* 1999) or what was given in the intervention studies (Alberts *et al.* 2000; Schatzkin *et al.* 2000). It was concluded that dietary fibre intake needs to be increased to about 30 g/d before protection can be demonstrated (Ferguson & Harris, 2003).

Cancer prevention based on type of dietary fibre

Even if the question on whether or not dietary fibre may be protective is developing in favour of the dietary fibre theory, it is still a matter of debate which types of dietary fibres actually may contribute in these cancer-preventive activities. Although polysaccharides are the most abundant components of plant cell walls (Ferguson *et al.* 2001), plant foods contain a wide range of additional components, such as flavonoids, lignans, anthocyanins, etc., that can protect against cancer (Harris & Ferguson, 1993). Hence, such compounds could, at least theoretically, inhibit the development of colon cancer. Therefore, whatever be the reasons for the results reported, eating a diet rich in plant foods, in the form of fruits, vegetables and whole-grain cereals, probably remains the best option for reducing the risk of colon cancer and for the protection of general health (Ferguson & Harris, 2003).

Inulin-type fructans

One dietary fibre that could be of relevance in colon cancer prevention is the group of the inulin-type fructans. A number of foods, such as garlic, onion, artichoke and asparagus, have high levels of inulin-type fructans and their average consumption in the normal human diet has been evaluated to amount to several grams per day (Van Loo, 1995). Inulin is obtained industrially from chicory roots by hot water extraction, followed by refining and spray drying (for more details about the chemistry and nomenclature of inulin-type fructans see Roberfroid, this supplement). The β-2 → 1 glycoside bond inulin-type fructans have been shown to resist hydrolysis by enzymes in the human small intestine. They are fermented extensively by large bowel microflora (Hidaka *et al.* 1986) to lactic acid and to SCFA, which can be absorbed and metabolised by the host (Gibson & Roberfroid, 1995; Campbell *et al.* 1997). Associated with this is the 'bifidogenic' nature or prebiotic effect of inulin-type fructans, which has been shown to result in enhanced concentrations of bifidobacteria in the colon lumen (Roberfroid *et al.* 1998; Bouhnik *et al.* 1999). Accumulating evidence in experimental animals of a preventive effect of inulin against colon cancer has been reviewed in brief (Pool-Zobel *et al.* 2002). The present report will focus on a more detailed review of new findings on the dietary fibre inulin-type fructans and their potential role in cancer prevention. The aim of this review is to reflect the up-to-date data for protective effects during CRC.

Potential mechanisms

Effects of butyrate

The gut flora ferment dietary fibres to yield SCFA together with degraded phytochemicals and plant nutrients. Butyrate, a major SCFA, has considerable physiologic relevance to the integrity and function of the colonic epithelium and may be potentially chemoprotective by a number of different mechanisms. Its effects on the turnover, structure and function of cells have been studied extensively *in vitro*, particularly in colon cancer cell lines, where it has been shown to inhibit cell proliferation, stimulate cell differentiation and induce apoptosis (Kruh, 1982; Augeron & Laboisse, 1984; Hague *et al.* 1993; Johnson, 1995). In normal colonic epithelial cells, it has been difficult to show many of the effects observed in cell lines (Mariadason *et al.* 2001). In fact, butyrate has often been shown to exert seemingly paradoxical effects *in vitro* and *in vivo* (Lupton, 1995, 2004; Hague *et al.* 1997; Gibson *et al.* 1999). *In vivo* butyrate has not been shown to retard tumour growth in animals treated with the colon carcinogen azoxymethane (AOM; Caderni *et al.* 2001). However, dietary fibres which are fermented to yield high amounts of butyrate have been associated with a higher efficacy of protecting from AOM-induced colon tumours in animals (McIntyre *et al.* 1993; Compher *et al.* 1999; McIntosh *et al.* 2001; Perrin *et al.* 2001). In particular the *in vivo* study by Perrin (presented in more detail below) needs to be mentioned in this context, since it included a treatment group with inulin-type fructans. The authors demonstrated that those fibres which promoted a stable butyrate-producing colonic ecosystem decreased the rate of aberrant crypt foci (ACF) in rats, thus adding to the line of evidence that a stable butyrate-producing colonic ecosystem, as related to selected fibres (including inulin-type fructans), reduces risks of developing colon cancer (Perrin *et al.* 2001). Numerous reviews have addressed the reasons for these seemingly opposed effects, and more and more studies are now appearing to help understand the effects of butyrate and other SCFA on prevention of CRC. Some examples are the recent findings on modulated expression of glutathione *S*-transferases (GST) and the complex microarray expression patterns in human colon tumour cell lines by butyrate (Mariadason *et al.* 2000; Ebert *et al.* 2001, 2003). One mechanism by which butyrate may modulate gene transcription is by inhibiting histone deacetylases, which leads to alterations of histone acetylation patterns and is associated with the activation of gene transcription (Kobayashi *et al.* 2003).

Fermentation products of inulin-type fructans

It has now been shown that gut fermentation products of inulin-type fructans beneficially modulated markers of tumour progression in human colon tumour cells as well (Beyer-Sehlmeyer *et al.* 2003; Klinder *et al.* 2004*b*). In one study, inulin was fermented with human faecal slurries *in vitro*, analysed for SCFA, and the corresponding SCFA mixture was prepared. HT29 colon tumour cells were treated for 72 h with individual SCFA or with complex samples containing physiological SCFA concentrations. Growth of cells, GST activities and chemoresistance towards 4-hydroxynonenal were determined. The fermentation sample of inulin contained acetate (87 mM), followed by butyrate (14 mM) and propionate (22 mM), as the major SCFA. It inhibited cell growth more than the corresponding SCFA mixtures based on the SCFA concentrations in the complete fermentation sample. The relative EC_{50} value (that is the concentration resulting in a reduction of cell growth and survival by 50 %) was $5{\cdot}37 \pm 0{\cdot}19$ mM for the inulin fermentation sample and $8{\cdot}78 \pm 0{\cdot}32$ mM for the corresponding SCFA mixture. The SCFA mixture was more active than butyrate of the same concentration available in the mixture, probably due to the presence of

propionate, which also inhibited cell growth. Only butyrate induced the activity of GST, whereas chemoresistance was not caused by the fermentation sample. This could be considered an advantage since, in tumour cells, GST induction could counteract cancer chemoprevention by causing chemoresistance and thus enhancing survival of transformed cells (Ebert *et al.* 2001). Indeed, pre-treatment of HT29 tumour cells with fermentation samples from inulin did not enhance resistance toward genotoxic 4-hydroxynonenal. We concluded that fermented dietary fibres were more potent inhibitors of tumour cell growth than butyrate alone, and also contained ingredients which counteracted the undesired positive selection pressures that higher concentrations of butyrate were shown to induce in tumour cells (Beyer-Sehlmeyer *et al.* 2003).

In addition to this work, it has also been recently shown that gut fermentation products of inulin-derived prebiotics beneficially modulated markers of tumour progression in human colon tumour cells as well (Klinder *et al.* 2004*b*). For this study, samples were prepared in a three-stage fermentation system that simulated the various conditions expected to occur in the three different colon segments (proximal, transverse and distal). Ingredients of the fermentation mixture were oligofructose-enriched inulin (Raftilose® Synergy1), probiotics (*Bifidobacterium lactis* Bb12, *Lactobacillus rhamnosus* GG) and/or faecal inoculates. HT29 or CaCo-2 cells were incubated with supernatants of the fermented samples (2·5–25 % v/v, 24–72 h). Cellular parameters of survival, differentiation, tumour progression and invasive growth were determined. The key results were that fermentation supernatants derived from probiotics and Synergy1 were more effective in inhibiting growth than the corresponding supernatants produced with glucose. Another novel and important finding was that the supernatant derived from the gut model vessel (representing the distal colon) was the most effective of the three vessels for all investigated parameters, possibly on account of the higher butyrate concentrations obtained in vessel three than in vessels two and one (Klinder *et al.* 2004*b*). Thus, these studies show that biological effects of inulin-type fructans on colon cells may have been mediated not only by growth stimulation of the lactic acid-producing bacteria and/or production of butyrate, but also by other bacteria and products of the gut lumen. These properties of the supernatants to inhibit growth and metastases in colon tumour cells were, therefore, considered to be important potential mechanisms of tumour suppression by this type of dietary fibre.

Conclusions: in vitro *studies, butyrate and reductions of colorectal cancer risks*

The present data allow us to conclude that, in colonocytes, butyrate has the potential to inhibit the growth of emerging pre-malignant and malignant cells, which retards tumour progression. Translated to the *in vivo* situation, this could mean that a life-long supply with butyrogenic dietary fibres — including inulin-type fructans — may contribute substantially to dietary colon cancer chemoprevention, a feasible hypothesis, which needs to be substantiated in human clinical trials. The available experimental animal data are largely supportive for these hypotheses and are presented in more detail below.

Animal studies

Inulin-type fructans and their potential impacts on tumour prevention in animal models has been reviewed previously (Pool-Zobel *et al.* 2002). The following adds to this information by documenting animal studies aimed at investigating inulin-type fructans in various stages of the carcinogenesis process.

Effects of inulin on colorectal cancer induced by azoxymethane or 1,2-dimethylhydrazine

The first of the two animal models has been frequently employed to determine the induction of pre-neoplastic lesions in the colon of rats (Bird, 1987) and their prevention (Corpet & Pierre, 2003). The carcinogenic compound commonly used is AOM, an alkylating derivative of 1,2-dimethylhydrazine (DMH) that specifically targets the colon of rats, where it induces DNA damage (Pool-Zobel *et al.* 1996), pre-neoplastic lesions and is detected as aberrant crypts and tumours (Bird, 1987; McLellan & Bird, 1988). AOM-induced tumours share many histopathologic characteristics with human tumours. They, like human tumours, are often mutated on *K-ras* and *β-catenin* genes and show microsatellite instability, but, unlike human tumours, are seldom mutated at the *Apc* gene (15 %), are never mutated at the *p53* gene (DeFilippo *et al.* 1998) and have a low tendency to metastasise (Corpet & Pierre, 2003). The highest tumour incidence was observed in the distal part of the colon. In the usual protocol, rats are injected (subcutaneously) with two AOM doses (2 × 15 mg AOM/kg body weight) at an interval of 1 week. Intermediate endpoints induced by AOM can be detected already after 8 weeks, since it produces large quantities of ACF in the colon. These pre-neoplastic abnormalities are due to the thickening of the wall in the pericarp of the colon crypts that can be stained and counted. Numerous aberrant crypts occur together and are visible as ACF. Most of these lesions, however, are eliminated by repair mechanisms. Only some of them develop into tumours, of which mainly those with high numbers of aberrant crypts per focus (multiplicity) are associated with cancer risks (Magnuson *et al.* 1993). The tumours appear after a period of 45–52 weeks. The application of the model using AOM as the initiator has been developed to study chemoprevention of colon tumours (Wargovich *et al.* 1992; Pereira *et al.* 1994). An overview of the variations of this model that have been used to study inulin and oligofructose for preventive properties is presented in Table 1. A number of studies have shown a reduction of crypt numbers and multiplicity, when adding inulin (10 %) to the diet. In one case, Reddy and co-authors observed that the effects of inulin were numerically more distinct than the effect of oligofructose (Reddy *et al.* 1997). This was attributed to the lower fermentation rate of the inulin in more distal parts of the colon, where the injected carcinogen (AOM) exerts its damaging activity (Reddy *et al.* 1997). In contrast, another group using a similar approach was not able to confirm the ability of oligofructose to reduce ACF incidence (Gallaher & Khil, 1999); albeit, in this study only a low dose of oligofructose (2 % w/w in diet) was used. Oligofructose given at a relatively low level of 6 % in the diet was, however, again reported to be protective in the study by Perrin *et al.* (2001). As indicated earlier in the present review, this study had the specific aim to assess whether long-term stable butyrate production as a consequence of different dietary fibre ingestion would be a prerequisite for reducing incidence of ACF. The authors performed a two-part randomised blinded study in rats, mimicking a prospective study in man, using a low-fibre control diet and three high-fibre diets, one of which was short-chain oligofructose. The rats were fed for 2, 16, 30

Table 1. Summary of studies determining modulation of chemically induced colorectal preneoplasia and tumours after dietary intervention with inulin-type fructans

Intervention	DP	% in diet	Type of diet	Feeding scheme	Animals per group	Type of animal	Sex of animal	Age at start of intervention	Carcinogen dose (mg/kg BW) and age at 1st dose	Age at end of experiment	Biomarker (colon)	Major result	Reference
Oligofructose & bifido-bacteria	NI	5	AIN-76A	I + P	20	CF1 mice	Females	*x* + 0·5 weeks	15 (DMH 6 ×) at 2·5 weeks	16·5, 36·5 & 46·5 weeks	ACF Caecal wt Caecal pH	↓ ↑ ↓	Koo & Rao (1991)
Oligofructose & synbiotic with bifidobacteria	NI NI	2	AIN-76A	P	8–20	Wistar rats	Males	*x* + 2 weeks	15 (DMH 2 ×) at *x* weeks	*x* + 5·5–7 weeks	ACF Cell prolif ACF Cell prolif	↔ ↔ ↓ ↔	Gallaher *et al.* (1996)
Oligofructose Inulin	~4·5 ~25	10 10	AIN-76A	I + P	12	F344 rats	Males	5 weeks	15 (AOM 2 ×) at 7 weeks	15 weeks	ACF	↓	Reddy *et al.* (1997)
Inulin	NI	10	AIN-76A	I + P		F344 rats	Males	7 weeks	15 (AOM 2 ×) at 7 weeks	16 weeks	ACF* β-Glucuronidase Caecal SCFA Caecal butyrate	↓ ↔ ↑ ↑	Rao *et al.* (1998)
Inulin	22–25	5	HF CO25	P	15	SD rats	Males	5–6 weeks	12·5 (AOM 2 ×) at 4–5 weeks	17–18 weeks	ACF Caecal wt Caecal pH Ammonia β-Glucuronidase β-Glucosidase	↓ ↑ ↓ ↓ ↓ ↑	Rowland *et al.* (1998)
& synbiotic with *B. longum*	22–25	5									ACF Caecal wt Caecal pH Ammonia β-Glucuronidase β-Glucosidase	↓ ↑ ↓ ↓ ↓ ↑	
Inulin Inulin	~25 ~25	5 5	SSA CO25	P	6	SD rats	Males	6–7 weeks	12·5 (AOM 2 ×)		ACF	↔ ↓	Bolognani *et al.* (2001)
Oligofructiose	4	6	Purified	I + P	36	BDIX rats	Males & females	8–10 weeks	15 (AOM 2 ×) 14–16 weeks	18–20 weeks	ACF Caecal wt Caecal SCFA Caecal butyrate Cell prolif	↓ ↑ ↔ ↑ ↔	Perrin *et al.* (2001)
Inulin	~25	2·5	AIN93M	I + P	12	F344 rats	Males	12 months	10 (AOM 2 ×) 12·5 months	15 months	ACF Caecal wt Caecal pH	↓ ↑ ↓	Verghese *et al.* (2002*a*)
Inulin	~25	5									ACF Caecal wt Caecal pH	↓ ↑ ↓	
Inulin	~25	10									ACF Caecal wt Caecal pH	↓ ↑ ↓	
Inulin	~25	10	AIN93G	I&P	12	F344 rats	Males	4 weeks	16 (AOM 2 ×) at week 7	16 weeks	ACF Caecal wt	↓ ↑	Verghese *et al.* (2002*b*)
Inulin	~25	10	AIN93G	I†	20			4 weeks		45 weeks	Tumours Caecal wt Caecal pH	↓ ↑ ↔	

Table 1. *Continued*

Intervention	DP	% in diet	Type of diet	Feeding scheme	Animals per group	Type of animal	Sex of animal	Age at start of intervention	Carcinogen dose (mg/kg BW) and age at 1st dose	Age at end of experiment	Biomarker (colon)	Major result	Reference
Inulin	~25	10		P‡				10 weeks			Tumours Caecal wt Caecal pH	↓ ↑ ↓	
Inulin	~25	10		I&P§				4 weeks			Tumours Caecal wt Caecal pH	↓ ↑ ↓	
Oligofructose	2–8	5	Purified	P	20	F344 rats	Males	6 weeks	20 (DMH 4 ×) at 6 weeks	11 & 16 weeks	ACF‖ Caecal wt‖ Caecal pH‖ Caecal SCFA¶,** Caecal butyrate¶,** Cell prolif	↓ ↓ ↑ ↑ ↔ ↑ ↔ ND	Poulson *et al.* (2002)
Oligofructose	2–8	15		P				6 weeks			ACF Caecal wt Caecal pH Caecal SCFA Caecal butyrate Cell prolif††	↔ ↔ ↑ ↑ ↑ ↑ ↑ ↓	
Oligofructose	2–8	15		I&P				3 weeks			ACF Caecal wt Caecal pH Caecal SCFA Caecal butyrate Cell prolif	↓ ↓ ↑ ↑ ↔ ↑ ↑ ND	
Inulin	≥23	5		P				6 weeks			ACF Caecal wt Caecal pH Caecal SCFA Caecal butyrate Cell prolif	↓ ↓ ↑ ↑ ↔ ↑ ↑ ND	
Inulin	≥23	15		P				6 weeks			ACF Caecal wt Caecal pH Caecal SCFA Caecal butyrate Cell prolif	↓ ↓ ↑ ↑ ↑ ↑ ↑ ↓	
Inulin	≥23	15		I&P				3 weeks			ACF Caecal wt Caecal pH Caecal SCFA Caecal butyrate Cell prolif	↓ ↓ ↑ ↑ ↔ ↑ ↑ ND	

Table 1. *Continued*

Intervention	DP	% in diet	Type of diet	Feeding scheme	Animals per group	Type of animal	Sex of animal	Age at start of intervention	Carcinogen dose (mg/kg BW) and age at 1st dose	Age at end of experiment	Biomarker (colon)	Major result	Reference
Oligofructose-enriched inulin	I&O (1:1)	10	HFAIN76	I + P	32	F344 rats	Males	4–5 weeks	15 (AOM 2 ×)	8 months	Tumours	↓	Femia *et al.* (2002)
											Caecal SCFA	↑	
											Caecal butyrate	↑	
											Cell prolif	↓	
											Apoptosis‡‡	↑ ↔ ↔	
											iNos‡‡	↑ ↔ ↓	
											COX-2‡‡	↑ ↔ ↔	
											GSTpi‡‡	↑ ↓ ↓	
Synbiotic with LGG, Bb12	I&O (1:1)	10									Tumours	↓	
											Caecal SCFA	↑	
											Caecal butyrate	↑	
											Cell prolif	↔	
											Apoptosis	↑ ↔ ↔	
											iNos	↑ ↔ ↓	
											COX-2	↑ ↔ ↔	
											GSTpi	↑ ↓ ↓	
Inulin	≥23	10	AIN93G	I + P	12	F344 rats	Males	5 weeks	16 (AOM 2 ×) at week 7	16 weeks	ACF	↓	Verghese *et al.* (2003)
Inulin		10									ACF	↓	
Oligofructose	3–8	10									ACF	↓	
Oligofructose-enriched inulin	I&O (1:1)	10									ACF	↓	
Mixture	I&O (1:2)	10									ACF	↓	

DP, degree of polymerisation; BW, body weight; *B. longum, Bifidobacterium longum;* Oligofructose-enriched inulin, 1:1 mixture of slowly fermentable long-chain fraction (DP ranging from 10 to 65, average 25) and a rapidly fermentable fraction of oligofructose (DP ranging from 3 to 8, average 4); LGG, *Lactobacillus rhamnosus* GG; Bb12, *Bifidobacterium lactis* Bb12; NI, not indicated; I&O, mixtures of inulin and oligofructose at 1:1 or 1:2; I, initiation group; P, promotion group; SD, Sprague–Dawley; *x*, age of rats at beginning of experiment was not indicated; DMH, 1,2-dimethylhydrazine; AOM, azoxymethane; ACF, aberrant crypt foci; wt, weight; prolif, proliferation; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; GSTpi, glutathione *S*-transferase π type.

*Reduction was not seen for total number of ACF but for ACF/cm^2.

†In the initiation (I) group, rats received inulin in the diet 3 weeks before injection 1 until 1 week after injection 2 (5 weeks total). Rats were then switched to the control diet.

‡In the promotion (P) group, the rats received the control diet until 10 weeks of age (2 weeks after the second injection) followed by the inulin for 34 weeks.

§In the I&P group, rats received inulin in the diet throughout the 41-week experiment. The rats were switched to an AIN 93M-based diet at 20 weeks of age.

||First arrow indicates total number of ACF for animals killed at week 11, second arrow is for animals killed at week 16.

¶Data from animals killed at week 11 and 16 were pooled.

**Calculated as weight of caecal content multiplied by the caecal SCFA concentration in μM (total amount).

††Labeling index, effects seen only in proximal colon.

‡‡Parameters were measured in normal and in tumour tissue; first arrow indicates differences between tumour and normal tissues, second and third arrows indicate differences between intervention group *v.* control groups for normal or tumour tissue, respectively.

or 44 d to determine the period of adaptation to the diets, fermentation profiles and effects on the colon, including mucosal proliferation on day 44. Another thirty-six rats fed the same diet for 44 d were injected with AOM and checked for ACF 30 d later. The major findings were that after stabilisation of fermentation for 44 d the inulin-type fructans produced large amounts of butyrate with a trophic effect in the large intestine. Fewer ACF were present albeit proliferation was not affected. In comparison to the other dietary fibre treatment groups, the authors stressed the associations of butyrate production and ACF inhibition (Perrin *et al.* 2001).

Inulin as part of synbiotic treatments (Table 1)

The combination of the prebiotic inulin and the probiotic *Bifidobacterium longum* was shown to inhibit AOM-induced ACF in a synergistic manner (Rowland *et al.* 1998). The effects on the foci with multiplicity of over four crypts, which are thought to be the most relevant markers for tumour formation, were reduced by the combination treatment. The authors also investigated the impacts of the diets on biochemical parameters for which there was experimental evidence that they were associated with a reduced risk for developing colon cancer (Rowland, 1991, 1993). Indeed, in their study on synbiotic intervention (Rowland *et al.* 1998), the authors were able to demonstrate that consumption of diets containing *B. longum*, long-chain inulin or both decreased β-glucuronidase activity and ammonia concentration in the caecum. Also, the inulin-containing diets (with or without *B. longum*) increased caecal weights and β-glucosidase activity, with concomitant decreases of caecal pH. The authors were, therefore, able to conclude that consumption of *B. longum* or inulin was associated with potentially beneficial changes in caecal physiology and bacterial metabolic activity in relation to tumour risk and in the incidence of putative pre-neoplastic lesions in the colon. The effects of *B. longum* were less pronounced for a number of parameters than those observed for inulin, and even more so in comparison to the combination treatment. Since the dietary treatments were started 1 week after the carcinogen dose, the results suggested that *B. longum* and inulin were probably affecting the early promotion phase of the carcinogen. Earlier studies have shown that *B. longum* and other dietary fibres inhibit DNA-damaging activities of genotoxic colon carcinogens (Pool-Zobel *et al.* 1996; Rowland *et al.* 1996; Wollowski *et al.* 1999). The study of Rowland *et al.* on inulin with or without *B. longum* intervention indicates that these dietary measures can influence the carcinogenic process at more than one point, thus markedly increasing their potential effectiveness as cancer-preventing agents. The results also indicated that combined treatment with the two agents was more effective in reducing colonic lesions. This was the first demonstration of an effect now described as 'synbiotic' (Roberfroid, 1998), which has been confirmed by another group (Gallaher & Khil, 1999). This group performed a series of animal studies measuring ACF with the objectives of determining whether the combination of bifidobacteria and oligofructose would have an additive effect (i.e. synbiotic) in reducing colon cancer risk in rats, and whether other oligosaccharides would also be effective as part of a synbiotic combination. The major results were that neither bifidobacteria nor oligofructose (2 %) on its own was able to significantly reduce ACF. Opposed to this, the authors reported that the combination of the same dose of oligofructose with bifidobacteria did reduce aberrant crypt number. Soyabean oligosaccharide and wheat bran oligosaccharide were also fed in combination with bifidobacteria, with mixed results in several experiments. Altogether, the authors concluded that the combination of bifidobacteria and oligofructose was able to reduce colon cancer risk in carcinogen-treated rats, whereas the effect of other oligosaccharides was uncertain. Connected to these data were some findings from a study investigating the effects of lactic acid-producing bacteria or inulin on ACF (Bolognani *et al.* 2001). Strains of *B. longum*, *Lactobacillus casei* and *Lactobacillus acidophilus* were administered to rats fed a purified high-starch diet, under a variety of treatment protocols including daily gavage, via the drinking water and in the diet. The rats were treated with *N*-methylnitrosourea, DMH or AOM to induce ACF. The major results were that, in general, no consistent significant changes in ACF numbers were detected in these experiments. Therefore, in one study, the basal diet of the rats was changed to one containing a higher level of fat (corn oil). Under these conditions, consumption of the high-fat diet alone did not significantly alter the incidence of total ACF, or the incidence of larger foci (mean number of ACF with >4 aberrant crypts/focus was $13{\cdot}5 \pm 12{\cdot}2$ for a semi-synthetic, low-fat diet and $14{\cdot}0 \pm 11{\cdot}1$ for rats fed the high-fat diet). The incorporation of either lactobacilli or inulin into the normal diet was associated with a small, but non-significant decrease in ACF. In contrast, when incorporated into the high-fat diet, both *L. acidophilus* and inulin significantly decreased the number of AOM-induced ACF by 38 % and 48 %, respectively. The authors also observed that the decrease was most apparent in foci with 1–3 aberrant crypts per focus. These findings led the authors to conclude that the type of diet fed can influence the detection of protective effects of lactic acid-producing bacteria and oligosaccharides, and that against the background of a diet with a level of fat typical of a Western diet, evidence for a protective effect of *L. acidophilus* and inulin towards colon cancer was obtained.

Dose effects, age of rats and sensitive stages of tumorigenesis (Table 1)

It has now been shown that the effect of inulin was dose-related (Verghese *et al.* 2002*a*, 2003). In these studies, adult rats were used for the first time for ACF studies. After a 2-week acclimatisation period, 12-month-old Fisher 344 retired male breeders received two subcutaneous injections of AOM dissolved in saline at weekly intervals. In the first AOM dose-finding experiment, six groups received 0, 4, 8, 10, 12 and 16 mg AOM/kg body weight, respectively, at each injection and were fed AIN-93M diet. In the second experiment, four groups of rats were fed 10 mg AOM/kg body weight at each injection based on the results of experiment 1, and were fed 0, 2·5, 5 and 10 g long-chain inulin diets/100 g, respectively. All the rats were killed after 11-week feeding periods. In experiment 1, there was a significant ($P \leq 0{\cdot}05$) AOM dose–response on ACF formation. Rats fed >10 mg of AOM had greater ($P \leq 0{\cdot}05$) mortality. In experiment 2, there was a significant increase in caecal weight and a decrease in caecal pH from 7·17 in the control group to 6·87, 6·61 and 5·76 in the groups fed inulin at 2·5, 5·0 and 10 g/100 g, respectively. Thus, these studies showed that long-chain inulin dose-dependently reduced ACF incidence in the colon ($P \leq 0{\cdot}01$). Compared with rats fed the control diet, the percentage reductions of ACF in rats fed 2·5, 5·0 and 10 g inulin diets/100 g

were 25, 51 and 65, respectively. Thus, the authors concluded that mature rats can be used as models in ACF studies, and dietary long-chain inulin dose-dependently suppressed AOM-induced ACF formation in Fisher 344 mature male rats (Verghese *et al.* 2002*a*). These authors have also recently reported a study designed to determine the effect of 10 % dietary long-chain inulin on the AOM-induced colonic pre-neoplastic ACF and small intestinal and colon tumours at the initiation (I), promotion (P), and both initiation and promotion (I + P) stages (Verghese *et al.* 2002*b*). Fisher 344 male weanling rats were assigned to a control-diet group and to a diet containing 10 % inulin. All the rats received 16 mg AOM/kg body weight dissolved in saline subcutaneously at 7 weeks of age followed by a second injection at 8 weeks of age. An additional group of five rats received only saline and consumed the control diet. The rats received the assigned diets until 16 or 45 weeks of age to determine ACF or tumours, respectively. The major results were that caecal weight was greater in rats fed inulin and caecal pH was lower. The inulin group had fewer aberrant crypts and fewer ACF compared with the control group. Tumour incidences in the small intestine and colon of rats in the control, I, P and I + P groups were 78, 31, 0 and 11 % and 90, 73, 69 and 50 %, respectively. The corresponding values for the distal portion of the colon were 87, 63, 45 and 33 %, respectively. Colon tumours per tumour-bearing rat were 4·2, 3·09, 1·36 and 1·2 for the control, I, P and I + P groups, respectively. All groups differed $P \leq 0{\cdot}05$. The authors concluded that that dietary long-chain inulin suppresses AOM-induced ACF formation, an early pre-neoplastic marker of colon tumorigenesis in rats, and colon tumours, particularly at the promotion stage (Verghese *et al.* 2002*b*).

Inulin composition and chain length (Table 1)

Poulson *et al.* (2002) were able to detect different effects of short- and long-chain inulins on large intestinal physiology and carcinogen-induced ACF in rats. Specifically, the study was designed to investigate the effects of inulin-type fructans on DMH-induced effects in the rat colon. Dietary levels of oligofructose (degree of polymerisation (DP) = 2–8) and long-chain inulin (DP ≥ 23) of 5 and 15 % were included in the diet before (present during initiation and progression phases of carcinogenesis) and after gavage (present only during progression phase of carcinogenesis) of several injections of DMH. Table 1 shows that, in this study, a number of other parameters in addition to ACF were determined at weeks 11 and 16 after the beginning of the experiment. The key results were that feeding with long-chain inulin significantly inhibited the number of small and total ACF after 11 and 16 weeks. The diet with high concentrations of short-chain fructans also inhibited the number of small and total ACF after 11 and 16 weeks, but significantly increased the numbers of medium and large ACF after 10 weeks. This finding was apparent only for the animal group receiving 15 % oligofructose during the promotion phase of the experiment. The authors discussed this finding on the basis of more severe diarrhoea observed simultaneously with the carcinogen exposure in this group of animals, thus making the intestine more sensitive and the fructans less efficient (Poulson *et al.* 2002). Altogether, the authors concluded that the chain length of the inulins influenced the effect on ACF outcome. More recently, another study was also aimed at elucidating the effect of inulin-type fructans of different chain lengths on AOM-induced ACF in Fisher 344 (Verghese *et al.* 2003). After a 1-week period of acclimatisation, ninety male Fisher 344 weanling rats were divided into groups and assigned to eight dietary treatments for 13 weeks during which they received a control diet, or a control diet supplemented with maltodextrin (placebo control) or with inulin made up of oligofructose fractions at various chain lengths. All animals first received a subcutaneous injection of AOM in saline (16 mg/kg body weight) at 7 weeks of age, followed by a second injection at 8 weeks of age. Animals were killed using CO_2 asphyxiation at 16 weeks of age. ACF were scored and the total numbers of ACF, as well as the number of crypts per focus, were recorded. The major results were that numbers of ACF were higher in the distal colon ($P < 0{\cdot}05$) than in the proximal colon. The percentage reductions in ACF in the groups consuming diets containing oligofructose-enriched inulin, inulin and long-chain inulin were 52·2, 29·6 and 46·3 %, respectively. The greatest reduction of ACF incidence was seen with feeding oligofructose-enriched inulin with a reduction of 62·1 and 64·1 %, respectively, in the proximal and distal colon, and an overall reduction of 63·9 %. Oligofructose reduced ACF by 24 %. The authors concluded that the products containing high-molecular-weight inulin fractions had the highest efficacy in reducing the incidence of ACF in the distal colon.

Meanwhile, oligofructose-enriched inulin has now also been investigated for modulation of chemically (AOM) induced colon tumours in rats on a high-fat diet. The aim of this most recent chronic animal study was to assess whether oligofructose-enriched inulin (10 % of the diet), probiotics (*B. lactis* (Bb12) and *L. rhamnosus* (LGG), each at 5×10^8 cfu/g diet) or synbiotics (a combination of the two) protect rats against AOM-induced colon cancer (Femia *et al.* 2002). Male F344 rats were divided into: controls; a 'prebiotic group', which were fed a diet containing oligofructose-enriched inulin; a 'probiotic group', fed a diet containing LGG and Bb12; and a 'synbiotic group', fed a diet containing oligofructose-enriched inulin, LGG and Bb12. Ten days later, the rats were treated with AOM (15 mg/kg subcutaneously, two times); dietary treatments were continued for the entire experiment. Thirty-one weeks after AOM treatment, rats treated with oligofructose-enriched inulin (prebiotic and synbiotic fed groups) had a significantly lower ($P < 0{\cdot}001$) number of tumours (adenomas and cancers) than rats without oligofructose-enriched inulin (mean (SD) colorectal tumours/rat were 1·9 (SD 1·7), 1·1 (SD 1·1), 2·2 (SD 1·4) and 0·9 (SD 1·2) in controls, prebiotic, probiotics and synbiotic groups, respectively). A slight but not significant effect of probiotics in reducing malignant tumours was also observed ($P = 0{\cdot}08$). Caecal SCFA were higher ($P < 0{\cdot}001$) in the groups treated with oligofructose-enriched inulin. Apoptosis was increased in the normal mucosa of the probiotics-fed group, while no variation was observed in the tumours. Colonic proliferation was lower in the prebiotic-fed group as compared with control group. GST π-type expression and inducible NO synthetase were depressed in the tumours from rats in the prebiotic and synbiotic groups. Cyclooxygenase-2 expression was increased in the tumours of control rats but not in those from prebiotic-, probiotic- or synbiotic-fed rats. On the basis of these results, the authors concluded that prebiotic administration in the diet decreased AOM-induced carcinogenesis in rats.

Summary of effects of inulin-type fructans on azoxymethane/1,2-dimethylhydrazine-mediated colorectal cancer (Table 1)

Altogether, twelve different studies have been published which describe experiments to assess the impacts of inulin-type fructans on chemically (AOM, DMH) induced pre-neoplastic lesions

(ACF) or tumours. Eleven studies looked at effects in the colon of rats, and one study in the colon of mice. The twelve studies included twenty-nine individual treatment groups designed to assess the relative effects of different dietary levels of inulin-type fructans, of different chain lengths, applying the fructans at different stages of the carcinogenesis process, including them into high-fat diets and so forth. Of these twenty-nine treatment groups, twenty-four measured ACF and five measured tumours as the major endpoints. There was a significant reduction in the total number of ACF in twenty-one of the twenty-four treatment groups. There was a significant reduction of tumour incidence in five of the five treatment groups. However, there were relatively different degrees of effectiveness depending on the design of the studies. In the three treatment groups that did not report a reduced ACF incidence, the total number of ACF was not significantly different from the control. In one case, this lack of effectiveness could have been due to too low dietary levels (2 %) albeit this level was effective when given in combination with bifidobacteria (Gallaher & Khil, 1999). In a second study, the lack of effectiveness of intervention with inulin could have been due to the low-fat diet, since the same intervention in animals fed a high-fat diet was very effective in reducing ACF incidence (Bolognani *et al.* 2001). Finally, in the third of the three treatment groups not leading to a reduced ACF incidence, the animals had been fed large doses of DMH, which led to diarrhoea that may have prohibited the oligofructose intervention (15 %) from being effective. Moreover, in this treatment group, the number of large ACF was even increased in comparison to the controls that had not received inulin-type fructans in their diet. It is difficult to interpret the relevance of these findings, since such high exposure situations to carcinogens probably do not occur physiologically. However, in the same study, there were reductions of ACF caused by other treatment schedules with inulin-type fructans, in spite of the high carcinogen doses. In summary, the studies on chemically induced pre-neoplasia and tumours in the colon of rats point to a clear-cut non-toxic effect of inulin-type fructans, leading to a marked reduction of colon cancer incidence in animals exposed to the colon carcinogens. The most pronounced effects were reported for inulin-type fructans (designed for favourable fermentation in the colon lumen) and especially longer-chain inulin components (optimal effectiveness at 10 % w/w in diet), animals fed a high-fat Western style diet, intervention together with probiotic bacteria (synbiotic preparations) and intervention throughout the whole carcinogenesis process. The importance of fermentation in ensuing risk-reducing effects in the colon was assessed in seventeen treatment groups by measuring caecal weight, in fifteen groups by measuring caecal pH, and in ten groups by measuring caecal SCFA and caecal butyrate. The results are very much in par with the proposed mechanisms discussed above, since seventeen of the seventeen groups had an increase in caecal weight, all of which were also associated with a decreased incidence of ACF or colon tumours. Eight of fifteen determinations showed a decrease in pH. In five of the same fifteen groups, there was no change of gut luminal pH; however, in four of these five an increase in SCFA and butyrate levels was reported, reflecting mechanisms that lead to pH changes. In contrast, opposed to what would be expected on the basis of putative mechanisms, increases in pH were observed in two treatment groups (15 % dietary levels of inulin and oligofructose, P feeding schedule, see Table 1; Poulson *et al.* 2002). In the oligofructose group, however, intervention led to increases in large ACF. Altogether, nine of ten determinations resulted in increased levels of caecal SCFA; and in nine of ten groups, increased levels of caecal butyrate were observed, mostly in such treatment groups for which a decrease of total ACF or tumours was also reported.

Faecal water genotoxicity post-initiation with azoxymethane (Table 2)

Connected to the above described findings on reduction of tumour incidence with a synbiotic preparation containing oligofructose-enriched inulin, *L. rhamnosus* and *B. lactis* (Femia *et al.* 2002) was the observation of a reduced faecal water genotoxicity in tumour-free, but not in tumour-bearing rats. This analysis was performed under the assumption that measuring faecal water genotoxicity in human colon cells could be a useful biomarker to study effects of diet in the colon (Rafter *et al.* 1987; Schiffman, 1987; Venturi *et al.* 1997; Kok & van Maanen, 2000; Osswald *et al.* 2000). Therefore, the prebiotic, probiotics and the combination of the two (synbiotic) were studied for their potential to modulate genotoxicity of faecal samples from the chronic animal study in order to determine response and possible predictive value of this non-invasive parameter of risk (Klinder *et al.* 2004*a*). The study offered the additional advantage of comparing the obtained biomarker results with the tumour incidence. For this, rat faeces of the tumour study were collected at 0, 10 d and 2, 4, 8 months; caecal contents were collected at 8 months. Aqueous phases of faecal and caecal samples were prepared and tested for genotoxicity in HT29 colon cells using the comet assay as had been described before. The major findings were that the diets with synbiotic supplementation reduced faecal genotoxicity (4 and 8 months). Samples from prebiotic-fed animals were less genotoxic than corresponding control samples. Genotoxicity of the faecal (8 months) and caecal water from the control animals directly correlated with each other. Excretion of genotoxins was lower in tumour-free than in tumour-bearing animals, especially after synbiotic intervention. These effects were not related to the butyrate or SCFA levels in the gut lumen. The study supports the conclusion that inulin-based diets reduced exposure to genotoxins in the faeces, directly reflecting the reported tumour and adenoma incidence in this group of animals. Since genotoxins are expected to be the source of processes leading to cancer cell initiation and are probably also the driving forces for processes of tumour progression, their inhibition can be important for cancer chemoprevention. According to Wattenberg (1992), this type of effect can be classified as 'blocking agent activity'. Altogether, this new study provides evidence that this measurement may be utilised as a biomarker of chemoprevention since (i) faecal water genotoxicity reflects genotoxic exposure in the caecum, (ii) tumour risks and faecal genotoxicity are related, thus allowing the conclusion that (iii) synbiotics reduce tumour risks by reducing exposure to genotoxins in the gut.

Apoptosis and cell proliferation (Tables 1 and 2)

In order to assess suppressing agent activities (which target the transformed cell, instead of targeting the carcinogen), a study was carried out to determine the effects of oligofructose and long-chain inulin on apoptosis and bacterial metabolism associated with carcinogenesis (Hughes & Rowland, 2001). Three groups of six animals were fed one of the three diets: basal, basal with oligofructose (5 % w/w) or basal with long-chain

Table 2. Summary of studies determining modulation of chemically induced colorectal parameters of risk and exposure after dietary intervention with inulin-type fructans

Intervention	DP	% in diet	Type of diet	Feeding scheme	Animals per group	Type of animal	Sex of animal	Age at start of intervention	Carcinogen dose (mg/kg BW) and age at 1st dose	Age at end of experiment	Biomarker (colon)	Major result	Reference
Oligofructose	~4	5	AIN93	I	6	F344 rats	Males	3–4 weeks	20 (DMH 1 ×) at 6–7 weeks	24 h after	Apoptotic cells	↑	Hughes &
										6–7 weeks	Caecal wt*	↔	Rowland (2001)
											Ammonia*	↔	
											β-Glucuronidase	↔	
											β-Glucosidase*	↔	
Inulin	~25	5									Apoptotic cells	↑	
											Caecal wt*	↔	
											Ammonia*	↔	
											β-Glucuronidase	↔	
											β-Glucosidase	↔	
Oligofructose-enriched inulin	I&O (1:1)	10	HF AIN76	I + P	32	F344 rats	Males	4–5 weeks	15 (AOM 2 ×)	2 months	FWgenotox†,‡	↔ ↔ ↔	Klinder *et al.*
										4 months		↔ ↓ ↓	(2004*a*)
										8 months		↑ ↓ ↔	
Synbiotic with LGG, Bb12	I&O (1:1)	10								2 months	FWgenotox†,‡	↑ ↔ ↔	
										4 months		↑ ↓ ↓	
										8 months		↑ ↓ ↓	

DP, degree of polymerisation; BW, body weight; Oligofructose-enriched inulin, 1:1 mixture of slowly fermentable long-chain fraction (DP ranging from 10 to 65, average 25) and a rapidly fermentable fraction of oligofructose (DP ranging from 3 to 8, average 4); LGG, *Lactobacillus rhamnosus* GG; Bb12, *Bifidobacterium lactis* Bb12; I&O, mixture of inulin and oligofructose at 1:1; I, initiation group; I + P, initiation and promotion group; DMH, 1,2-dimethylhydrazine; AOM, azoxymethane; wt, weight.

*Changes followed same trends as in Rowland *et al.* (1998), but did not reach statistical significance.

†Faecal water genotoxicity, determined with the microgelelectrophoresis (comet) assay.

‡First arrow indicates differences between non-tumour and tumour-bearing animals; second and third arrows indicate differences between control group *v.* intervention groups for tumour non-bearing and tumour-bearing animals, respectively.

inulin (5 % w/w) for a 3-week period. All animals were dosed with DMH and killed 24 h later. Numbers of apoptotic cells per crypt were determined by immunohistochemistry, taking positive cells of twenty longitudinal crypt sections as the basis. Results reported were that the mean number of apoptotic cells per crypt was significantly higher in the colon of rats fed oligofructose ($P=0{\cdot}05$) and long-chain inulin ($P=0{\cdot}02$) as compared to those fed the basal diet alone. According to the authors, this suggests that oligofructose as well as the long-chain inulin exerted protection at an early stage in the onset of cancer, as the supplements were effective soon after the carcinogen insult. For all animals, apoptosis was significantly higher in the distal colon as compared to the proximal colon ($P=0{\cdot}002$); however, no significant site-specific effect of diet occurred. The authors concluded that this was the first time that a significant effect of inulin-type fructans on apoptosis had been reported and that the results contribute to the growing evidence that chicory fructans may have cancer-preventing properties.

In the study investigating the effects of oligofructose-enriched inulin, probiotics and the synbiotic combination in rats (described above), Femia *et al.* (2002) also determined *in vivo* apoptosis. The major results were that apoptosis in the normal mucosa was significantly increased in the probiotics-fed animals as compared with the controls, but that no changes were found in the other groups. The analysis of the distribution of apoptosis along the crypt showed that the increase in apoptosis seen in the probiotics-fed animals was due to an increase in the apoptotic index (AI) in the lower third of the crypt (mean (SD) AI 0·33 (SD 0·36), 0·35 (SD 0·34), 0·68 (SD 0·35) and 0·35 (SD 0·37) in the controls, prebiotic, probiotics and synbiotic groups, respectively). The results also showed that the AI in the upper compartment of the crypt tended to be higher in the groups treated with prebiotic, probiotics and synbiotics; but this difference did not show any statistical significance (mean (SD) AI in the upper third of the crypt: 0·22 (SD 0·25), 0·35 (SD 0·27), 0·33 (SD 0·25) and 0·36 (SD 0·25) in the control, prebiotic, probiotic and synbiotic groups, respectively). Apoptosis in the tumours was higher than in the normal mucosa, but the values were not different from each other among the different groups (mean (SD) AI in all the tumours was 3·0 (SD 1·7), *n* 97; Femia *et al.* 2002). One of the reported reasons for the differences to other studies linking apoptosis to cancer risk was that here apoptosis was measured several months after carcinogen application and not shortly after carcinogen treatment; where apoptosis levels may follow different patterns. Femia *et al.* (2002) also determined the proliferative activity in the colonic mucosa, an endpoint which is tightly linked to both apoptosis and carcinogenesis (Terpstra *et al.* 1987; Ames & Gold, 1990; Bartram *et al.* 1993; Levin, 2003). A significantly lower number of labelled cells per crypt was measured in the oligofructose-enriched inulin-fed group as compared with controls; also probiotic-fed animals and synbiotic-fed animals had slightly lower proliferation, although this effect was not statistically significant (Femia *et al.* 2002). The distribution of the proliferative activity along the crypt was similar among the different groups. Since high proliferative activity in the colon mucosa has been associated with an increased risk of colon cancer, the authors concluded that their data suggest that prebiotics, and to a lesser extent probiotics, might act on lowering CRC risk by reducing the rate of cell proliferation (Femia *et al.* 2002).

In one study, the rate of distal colon mucosal cell proliferation was estimated as the labelling index using proliferating cell nuclear antigen, a marker of proliferating cells. Intervention with oligofructose with or without bifidobacteria did not significantly alter this biological parameter (Gallaher & Khil, 1999).

Poulson *et al.* (2002) measured the cell proliferation in distal and proximal regions of the colon using bromodeoxyuridine labelling. The parameter was determined in animals of a DMH control group and in the groups given 15 % oligofructose or 15 % inulin without pre-treatment (P, intervention during the progression phase of carcinogenesis) for 10 weeks. These types of intervention statistically significantly decreased the proximal colon-labelling index of the bottom and middle third of the crypt as well as the entire crypt compared to the control. In contrast, no alterations were detected in the distal colon. In connection with the reported findings of an unchanged number of cells per crypt profile, the authors suggested that the decreased cell proliferation could have reflected a reduced cell turnover and apoptosis. The authors, however, concluded that more studies, including differentiation and apoptosis, would be needed to elucidate the fructan-induced changes in cell dynamics.

Intestinal cancers in transgenic APC^{Min} mouse model (Table 3)

Inulin may also modulate the occurrence of tumours which are not chemically induced. Studies were performed with a genetically predetermined model, the APC^{Min} mouse. This transgenic mouse contains a non-sense mutation in the murine *Apc* gene and it is strongly predisposed to developing intestinal tumours at a relatively young age. It comes close to reflecting the situation of patients with familial adenomatous polyposis, or of individuals carrying the first *Apc* mutation in somatic cells and who are then later predisposed for developing sporadic colon cancer. However, adenocarcinomas are seldom observed in this model, and no typical ACF arise above the intestinal mucosa. Consequently, the ACF to carcinoma progression is not established in this model. Moreover, the *K-ras* mutations observed in many human tumours were not detected in Min mice polyps (Shoemaker *et al.* 1997), and *p53* inactivation, frequent in human cancers, was reported not to raise tumour number in Min mice (Fazeli *et al.* 1997). The major drawback of these mutants as models of human colon cancer is that their tumours occur predominantly in the small intestine and not in the colon. Effects of dietary fibres in the upper intestine may be difficult to interpret, since the fermentation conditions by the gut flora are completely different in the small intestine than in the colon and since the small intestine is not a target tissue of human cancer.

At this time, four individual studies by three different groups of authors have been reported. In the first published study, dietary supplementation with oligofructose (from sucrose) caused a reduction in the incidence of colonic tumours but not of small intestinal tumours (Pierre *et al.* 1997). The number of smaller sized colon tumours was even more significantly inhibited by oligofructose intervention. Moreover, the authors observed that the oligofructose-fed mice had a better-developed gut associated lymphoid tissue than the controls. Using the same mouse model, Mutanen *et al.* (2000) compared the impact of a non-specified fraction of chicory inulin with other prebiotic food ingredients, added at higher dosage levels. Here, inulin was not effective, or according to the authors' conclusions, even 'cancer-enhancing', since higher yields of small-intestinal tumours were scored in the inulin-fed animals than for rye bran-fed animals. The mice fed the rye-bran diet had the lowest number of polyps in the distal small intestine. The inulin group

Table 3. Summary of studies determining modulation of colorectal tumours in APC^{Min} mice after dietary intervention with inulin-type fructans

Intervention	DP	% in diet	Type of diet	Feeding scheme	Animals per group	Sex of animal	Age at start of intervention	Age at end of experiment	Biomarker (colon)	Major result	Reference
Oligofructose	≤4	5·8	CD	P	9–10	Males & females	5–6 weeks	11–12 weeks	Tumours	↓	Pierre *et al.* (1997)
Inulin	~25	2·5	HF	P	7–9	Males	5–7 weeks	10–13 weeks	Tumours β-Catenin* PKC isoforms	↔ ↑ ↔	Mutanen *et al.* (2000)
Inulin	~25	10	HF AIN93G	P	9–11	Males & females	6 weeks	15 weeks	Tumours β-Catenin†, cyt β-Catenin†, nuc β-Catenin†, memb PCNA† p53† β-Catenin‡, cyt β-Catenin‡, nuc β-Catenin‡, memb PCNA‡ p53‡	↑ ↑ ↑ ↑ ↔ ↑ ↔ ↑ ↔ ↑ ↓ ↔ ↓ NI ↔ ↓ ↔ ↓ ↑ ↓	Pajari *et al.* (2003)
Oligofructose-enriched inulin	I&O (1:1)	10	NWD	P	10	Males	5–6 weeks	15 weeks	Tumours	↓	Lipkin (in preparation)

DP, degree of polymerisation; Oligofructose-enriched inulin, 1:1 mixture of slowly fermentable long-chain fraction (DP ranging from 10 to 65, average 25) and a rapidly fermentable fraction of oligofructose (DP ranging from 3 to 8, average 4); I&O, mixture of inulin and oligofructose at 1:1; CD, low-fibre diet with 2% cellulose; NWD, HF, high fat; North Western style diet; P,promotion group; PKC, protein kinase C; cyt, cytosolic fraction; nuc, nuclear fraction; memb, membrane fraction; PCNA, proliferating cell nuclear antigen; NI, not indicated.

*Measured in cytosolic and particulate fractions of distal small intestinal mucosa.

†Measured in adenoma; first arrow indicates (trend) change from week 9 to week 15, second arrow indicates change caused by inulin.

‡Measured in normal appearing mucosa samples; first arrow indicates (trend) change from week 9 to week 15, second arrow indicates change caused by inulin.

on the other had differed significantly from the rye-bran group in the distal small intestine. The number of animals bearing tumours in the colon and caecum was only 33 % in the rye-bran group when compared with 100 % in the inulin group. However, as already discussed in detail (Pool-Zobel & Cherbut, 2003), these conclusions may be misleading, since there was no significant difference between inulin-fed animals and those from the non-fibre-fed control group. In a follow-up study by the same group, inulin (10 % w/w) was fed in a high-fat diet for up to 15 weeks. Inulin increased the number and size of adenoma in the small intestine. In both studies, β-catenin levels were additionally investigated in the same group of animals to assess potential mechanisms, in particular to explore the possibility that intracellular accumulation of β-catenin may arise as a consequence of loss of function of either the *Apc* gene or the APC–β-catenin-GSK–3β complex (Pajari *et al.* 2003). Although β-catenin levels did not accumulate significantly in the first of the two studies, the authors reported, in the second study, the interesting findings that tumorigenesis was accompanied by an accumulation of cytosolic β-catenin in the adenoma tissue at week 15, whereas membrane β-catenin was reduced in the inulin-fed mice.

Finally, in the most recent study, the feeding of inulin-type fructans again significantly reduced the incidence of tumours in APC^{Min} mice (Lipkin, in preparation). Thus, discrepancies are apparent when analysing the results in the transgenic mouse system. Opposite results were obtained in the same animal model by the group of Mutanen on one hand (Mutanen *et al.* 2000; Pajari *et al.* 2003) and by the two independent groups, Pierre *et al.* (1997) and Lipkin (in preparation), on the other. The reasons for these differences among the studies are not immediately explainable, but may be discussed based on differences in the animal strains, composition of the basal diets, inulin-type of fructans used for the intervention, dose and duration of intervention, and so forth. All studies were performed with C57BL/6J Min mice, although from different sources. The diets seemed to differ more than the animals, even though they did not reveal any pattern indicating that a certain diet would or would not favour protective or non-protective effects by inulin-type fructans. The most apparent difference was the type of intervention in the fourth study of Lipkin, showing protective effects, in comparison to the others. In Lipkin's study, the animals received oligofructose-enriched inulin (Raftilose® Synergy1), instead of inulin, which could have been more favourably fermented in the colon of the mice than inulin. Although dietary fermentation patterns have hardly been studied in mice, the study by Femia *et al.* (2002) reported SCFA production in rats after intervention with Synergy1®. Major results were that SCFA in the caecum of the groups treated with the prebiotic oligofructose-enriched inulin (prebiotic- and synbiotic-fed animals) were significantly higher than that in the samples from the groups which had not received the prebiotics ($P<0{\cdot}001$). However, it is not known whether pH, SCFA pool and intestinal bacteria in the APC^{Min} mice are similar to those from the rats of the AOM models and from man. Thus, it remains speculative to explain different intervention effects on this basis, and studies are needed to answer the questions.

The positive tumour-enhancing properties reported by the Finnish group need to be carefully regarded. Similar puzzling and opposing results with non-steroidal anti-inflammatory drugs (NSAID) have been reported as well. Reports have claimed that NSAID (Piroxicam® and Sulindac®) strikingly increased tumour yields in mutant mice susceptible to spontaneous colon tumours (Jacoby *et al.* 2001; Yang *et al.* 1999, 2001), although NSAID are widely accepted as chemopreventive agents for such cancers in humans (Thun & Henley, 2002). These results raised questions about either the animal model or the NSAID protection, discussed in detail in a recent review comparing dietary chemoprevention studies for the two animal models of CRC (Corpet & Pierre, 2003). The authors compared the efficacy of agents in the Min mouse model and the AOM rat model, and found that they correlated ($r = 0{\cdot}66$; $P<0{\cdot}001$), although some agents afforded strong protection in the AOM rat model but still increased the tumour yield in the large bowel of the mutant mice. The reason for this discrepancy could not be explained as a whole, but it was suggested that the process of fermentation could explain some of the discrepant findings.

Effects on growth of transplantable mouse tumours (Table 4)

Another group of studies has been performed to assess the influence of inulin-type fructans on the later stages of tumorigenesis and on their impacts during cancer treatment strategies. In the first study, 15 % inulin or oligofructose was incorporated into the basal diet to assess effects on the growth of transplantable mouse tumours (ascitic and solid tumours; Taper *et al.* 1998). The dietary treatment was initiated 7 d prior to tumour transplantation and then continued until death of the animals. The major results were that the mortality due to growth of both forms of transplantable mouse tumours was significantly inhibited by the supplementation of the diet with inulin or oligofructose. As an extension of this study, the authors investigated the influence of inulin and oligofructose on breast cancer and tumour growth (Taper & Roberfroid, 1999). In female Sprague–Dawley rats with mammary carcinogenesis induced by treatment with *N*-methylnitrosourea, the intervention with 15 % oligofructose, added to the basal diet, inhibited carcinogenesis. A lower number of tumour-bearing rats and a lower total number of mammary tumours were observed for the oligofructose-fed rats in comparison to the group fed the basal diet alone. The effect of dietary non-digestible carbohydrates (15 % oligofructose, inulin or pectin incorporated into the basal diet) was then investigated for their potential to modulate the growth of intramuscularly transplanted mouse tumours, namely a transplantable liver tumour and a mammary tumour cell line (TLT and EMT6). The major reported results were that supplementing the diet with non-digestible carbohydrates significantly inhibited the growth of both tumour lines. This allowed the authors to conclude that these non-toxic dietary treatments could be a feasible adjuvant factor in the classical protocols of human cancer therapy. Inulin and oligofructose also effectively inhibited the development of cancer metastases in an animal model (Taper & Roberfroid, 2000*a*). In this study, the development of lung metastases of a transplantable tumour to young male C3H mice was measured after dietary intervention with 15 % inulin or oligofructose. The major finding was that, in the inulin- and oligofructose-fed groups, significantly fewer animals developed lung metastases than in the control group. This is an important finding that could be used for the benefit of human health, and therefore necessitates more in-depth investigations on involved mechanisms and further evaluation of potential use in human tumour therapy. In an extension of these findings, the authors investigated how dietary intervention could modulate protocols of cancer chemotherapy using their established animal models (Taper & Roberfroid, 2000*b*, 2002). For this, inulin- or

Table 4. Summary of studies determining modulation of autochtonous and of transplanted breast and other tumours after dietary intervention with inulin-type fructans

Intervention	DP	% in diet	Type of diet	Feeding scheme	Animals per group	Type of animal	Sex of animal	Age at start of intervention	Tumour type route	Survival time or age at killing*	Biomarker	Major result	Reference
Oligofructose Inulin	~4 ~25	15	AO4 UAR		10–12 (*n* 4)	Mice		*x*	Ascites i.p.on day *x* +7 days	25 days	Mortality Mortality	↓ ↓	Taper *et al.* (1998)
Oligofructose Inulin	~4 ~25				10–12 (*n* 3)			*x*	Solid i.m. on day *x* +7 days		Mortality Mortality	↓ ↓	
Oligofructose	~4	5–15†	AO4 UAR		9	SD rats	Female	6 weeks	MNU s.c. 50 mg/kg 5 weeks	27 weeks	Size	↓	Taper & Roberfroid (1999)
Oligofructose Inulin	~4 ~25	15	AO4 UAR		9–11	BALB/c mice	Female	*x*	EMT6 i.m. on day *x* +7 days	26–46 days	Size Size	↓ ↓	
Oligofructose Inulin	~4 ~25				10–12	NMRI mice	Male	*x*	TLT i.m. on day *x* +7 days	24 days	Size Size	↓ ↓	
Oligofructose Inulin	~4 ~25				6 18	CH3 mice	Male	*x*	TLT i.m. on day *x* +7 days	4–5 months	Metastases Metastases in lung	↓ ↓	Taper & Roberfroid (2000*a*)
Oligofructose Inulin	~4 ~25	15	AO4 UAR		10–12 (*n* 2–3)	NMRI mice	Male	*x*	Ascites i.p. and cytotoxic drugs	25 days	ILS ILS	↑ ↑	Taper & Roberfroid (2000*a*, 2002)

DP, degree of polymerisation; SD, Sprague–Dawley; *x*, age of mice at beginning of experiment was not indicated; i.p., intraperitoneally; i.m., intramuscularly; MNU, *N*-methylnitosourea-induced mammary tumours (adenocarcinoma); s.c., subcutaneously; EMT6, mammary carcinoma cell line; TLT, transplantable liver tumour cell line; ILS, increased life span.

*Mean survival time indicates mortality rates (days) after tumour transplantation, or time of killing of animals at end of study.

†One week after carcinogen injection dose of 5%, next week dose of 10% for 1 week, followed by dose of 15% until end of experiment.

oligofructose-fed mice (which had been injected with an ascitic form of a TLT) were intraperitoneally injected with a single and sub-therapeutic dose of cancer chemotherapy drugs; the increase in life span was calculated. The marked result was that both inulin and oligofructose were significantly effective in increasing life span, and in over half of the studies there was a synergistic beneficial effect observed with the combination treatment (anticancer drug + inulin-type fructans) in comparison to either component on its own. The authors concluded that albeit further investigations are necessary to understand mechanisms and effects in other treatment protocols, this approach holds great promises for human application and should be considered in future trials (Taper & Roberfroid, 2002).

Human data

Faecal water genotoxicity as a biomarker of risk reduction

On the basis of the promising findings showing associations of faecal water genotoxicity and tumour occurrence in rats treated with a synbiotic (Klinder *et al.* 2004*a*), we were interested in further developing this biomarker to assess how faecal water would indicate the carcinogen burden in the gut. There is at least one recent human study which has now provided evidence of a reduced faecal water genotoxicity as a consequence of ingesting probiotic yoghurts containing 1 % oligofructose (Oberreuther-Moschner *et al.* 2004). Rationale of the study was that probiotics reduce colon cancer risks by inhibiting carcinogen-induced DNA damage in animals (Wollowski *et al.* 1999) but analogous data in man was lacking. Therefore, to enhance the knowledge of the effects in man, the genotoxicity of faecal water was investigated after dietary intervention with standard yoghurt or milk fermented with the probiotics *L. acidophilus* 145 and *B. longum* 913. For this study, faeces were collected from nine healthy volunteers after intervention with milk fermented with probiotics and after intervention with yoghurt. Faecal water was isolated and incubated with human colon tumour cells HT29clone19A. DNA-strand breaks, oxidised DNA bases and damage after challenge with H_2O_2 were determined by microgel electrophoresis. The major results were that, in comparison to the solvent control (physiological NaCl), faecal water was genotoxic but protected against H_2O_2-induced DNA-strand breaks. The intervention with fermented milk significantly lowered faecal water genotoxicity compared to yoghurt. However, probiotic intervention also increased oxidative damage, which either reflected pro-oxidative activity or perhaps stimulation of endogenous defence systems.

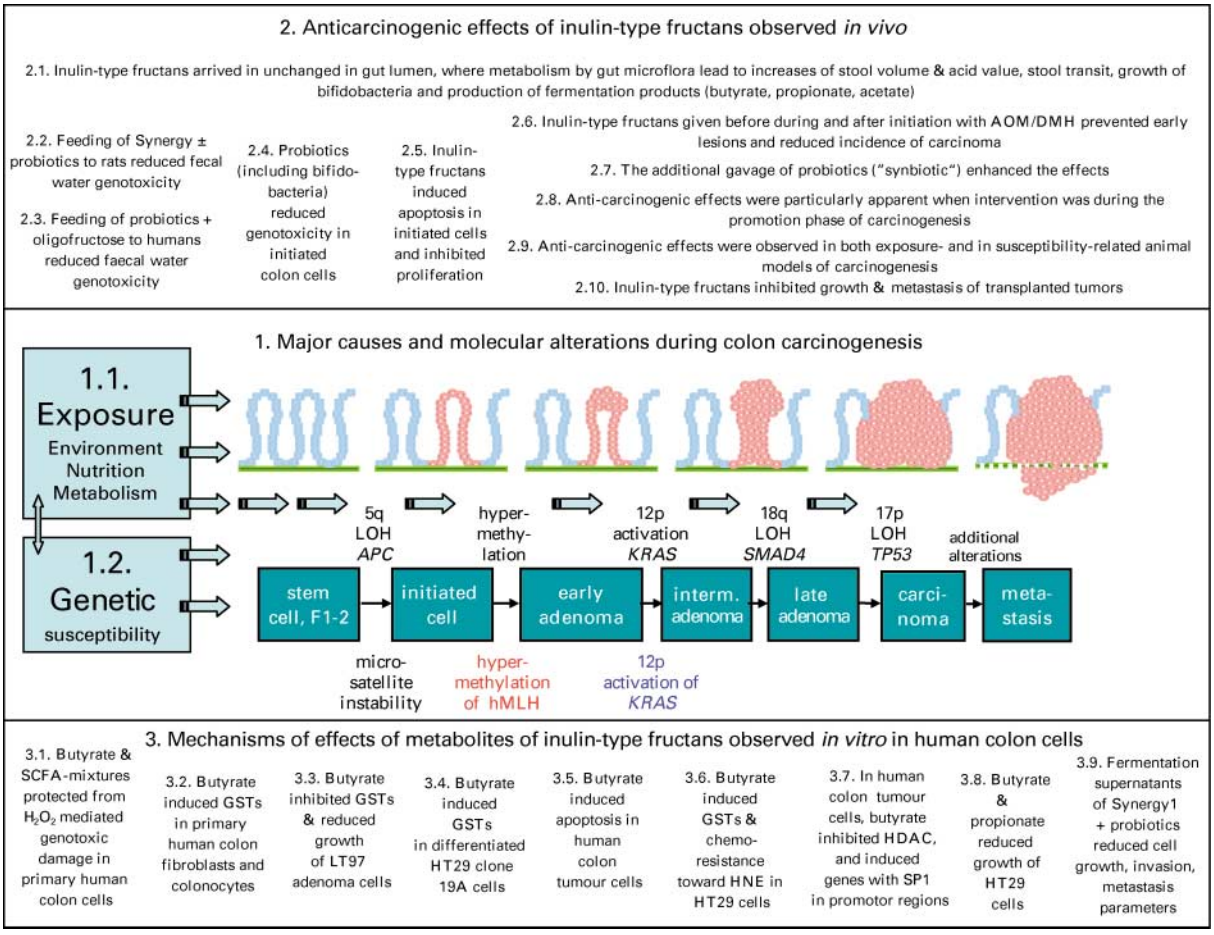

Fig. 1. Schematic presentation of the findings on (1) major causes and molecular alterations during colon carcinogenesis, (2) anticarcinogenic effects of inulin-type fructans observed *in vivo*, and (3) possible mechanisms by which metabolites of inulin-type fructans act chemoprotectively *in vitro* in human colon cells. The mechanisms of CRC are based on Fearon & Vogelstein (1990) and Fodde *et al.* (2001). Literature and explanations related to mechanisms of chemoprevention by the inulin-type fructans are detailed in the text and summarised in the conclusions.

Altogether, the balance of effects favoured protection, since faecal water from the probiotic group reduced overall genetic damage, and the consequences of the pro-oxidative effects are subject of current investigations.

Human study with synbiotic intervention 'SYNCAN'

The core of an EU-funded project SYNCAN (European Communities, specific RTD programme 'Quality of Life and Management of Living Resources', Key Action 1 'Food, Nutrition and Health') was to assess whether a synbiotic could reduce the risk of CRC (for more details about that study see Van Loo & Collins, this supplement). Volunteer patients (reselected RC or polyps) at high risk for CRC consumed a synbiotic, added to the diet, for 3 months (12 weeks). The synbiotic was composed of a prebiotic (oligofructose-enriched inulin) and two probiotics (*Lactobacillus* GG and *Bifidobacterium* Bb12), previously already assessed for anticancer properties on the AOM animal model (see above). Intervention was for 12 weeks with either the synbiotic or the placebo (maltodextrose). At several timepoints in the study, biopsies and samples of blood, urine and faecal matter were collected from each volunteer. Each sample was analysed for a range of activities by a 'Biomarker network' of collaborating scientists (http://www.syncan.be). Presently the biomarker analysis has been completed, the code has been revealed and analysis of the extensive data has been performed. Some of the interesting results pertaining to the content of this present review were that the synbiotic treatment in polyp patients reduced DNA damage, cell proliferation in colonocytes and faecal water genotoxicity. These findings are strikingly in par with the data from *in vitro* cell culture experiments and *in vivo* animal studies (Table 1), thus indicating a potential risk preventing property of this intervention in man.

Conclusions: summary of effects and potential mechanism

In conclusion, new rat and human studies have shown that inulin-type fructans containing diets reduced colon cancer risks by reducing exposure to genotoxic carcinogens in the gut (Fig. 1, sections 2·2 and 2·3) or by reducing their genotoxic impacts (Fig. 1, sections 3·1 and 3·6). In addition, novel studies in human cell systems have shown that other mechanisms of CRC risk reduction by inulin-based products are the inhibition of growth (Fig. 1, sections 3·3 and 3·8), modulation of gene expression (Fig. 1, sections 3·2, 3·4 and 3·7) and reduction of metastasis activities of colon tumour cells (Fig. 1, section 3·9). The available animal studies largely support the assumption that inulin-type fructans may reduce CRC incidence when given during the initial stages of cancer development (Fig. 1, sections 2·6 and 2·7). The reasons for reported non-beneficial effects of inulin in singular studies will need careful attention in the future, and it remains to be determined whether this was due to a lack of 'beneficial' gut fermentation. Nonetheless, marked effects have been observed showing that inulin-type fructans inhibited progression of already formed pre-neoplastic and neoplastic lesions (Fig. 1, section 2·8). These effects may have been the results of the mechanisms previously shown to occur in human colon cells *in vitro*, but now have also been reported for the *in vivo* situation in animals (Fig. 1, section 2·5). Moreover, newer findings in animals and also in human subjects have served as the basis for developing a wide range of novel functional biomarkers that are based on the described mechanisms. These hold promise to assess in more detail how inulin-type fructans may contribute to CRC risk reductions in human populations.

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