Brouns 2002 TIFS. butyrate and resistant starch review

DATASET · OCTOBER 2012

READS
224

10 AUTHORS, INCLUDING:

Fred Brouns
Maastricht University
199 PUBLICATIONS **5,453** CITATIONS
SEE PROFILE

Michiel A van Nieuwenhoven
Örebro University Hospital
34 PUBLICATIONS **422** CITATIONS
SEE PROFILE

Renato Amadò
ETH Zurich
173 PUBLICATIONS **2,836** CITATIONS
SEE PROFILE

ELSEVIER

Trends in Food Science & Technology 13 (2002) 251–261

TRENDS IN FOOD SCIENCE &TECHNOLOGY

Resistant starch and "the butyrate revolution"

Fred Brouns*,†,‡, Bernd Kettlitz‡ and Eva Arrigoni§

†Nutrition and Toxicology Research Institute Maastricht, Maastricht University, Maastricht, The Netherlands

‡Cerestar Vilvoorde R&D Centre, Havenstraat 84, B-1800 Vilvoorde, Belgium (fax: +32-2-2570740; e-mail: fbrouns@be.cerestar.com)

§ETH, Inst Food Technology and Food Science, Zurich, Switzerland

Early epidemiological studies indicated that populations that consume a high proportion of non-starch polysaccharide (NSP) dietary fibre (DF) in their daily diet suffer less from gastrointestinal diseases, in particular colorectal cancers, than populations that consume diets that are high in fat and protein but low in NSP fibre. In this respect, diet, by increasing the amount of vegetables and NSP DF's, has been suggested to contribute as much as 25–35% to risk reduction for colorectal cancer. A reduction of fat intake may further reduce the risk by 15–25%. Based on these observations, DF's and substances that are part of the fibre complex such as antioxidants, flavonoids, sulphur containing compounds and folate have been proposed as potentially protective agents against colon cancer. However, results from controlled prospective studies in which beta-carotene and vitamin E or isolated dietary fibres were given to high risk groups showed disappointing results. There are recent indications that the regular consumption of certain subclasses of highly fermentable dietary fibre sources result in gut associated immune and flora modulation as well as a significant production of short chain fatty acids. In vitro studies as well as animal studies indicate that in particular propionate and butyrate have the potential to support the maintenance of a healthy gut and to reduce risk factors that are involved in the development of gut inflammation as well as colorectal cancer. A suggestion put forward is that beneficial effects may be obtained in particular by the consumption of resistant starch (RS) because of the high yield of butyrate and propionate when fermented. These SCFA are the prime substrates for the energy metabolism in the colonocyte and they act as growth factors to the healthy epithelium. In normal cells butyrate has been shown to induce proliferation at the crypt base, enhancing a healthy tissue turnover and maintenance. In inflamed mucosa butyrate stimulates the regeneration of the diseased lining of the gut. In neoplastic cells butyrate inhibits proliferation at the crypt surface, the site of potential tumour development. Moreover, models of experimental carcinogenesis in animals have shown the potential to modify a number of metabolic actions and steps in the cell cycle in a way that early events in the cascade of cancer development may be counteracted while stages of progression may be slowed down. The present review highlights a number of these aspects and describes the metabolic and functional properties of RS and butyrate.

Introduction

In a world of rapidly changing food habits and stressful life styles it is more and more recognised that a healthy digestive system is essential for overall quality of life. This is not surprising since the recognition that the intestinal tract is the organ with the largest surface and metabolic capacity of our body. It is the organ, which absorbs the nutrients that are required for growth, development and health and excretes undesired and waste substances. Impairment of the health status of the intestinal tract may lead to events such as diarrhoea, constipation, inflammation and the passage of undesired substances and bacteria from the intestinal lumen into the body. This recognition has lead to the development of foods that are designed to contribute to a healthy digestive system and indirectly to the maintenance of general well being. Fibre rich food products and probiotics, defined as strains of living bacteria that survive the passage of the stomach and are able to

* Corresponding author at second affiliation..

0924–2244/02/$ - see front matter
PII: S0924-2244(02)00131-0

adhere to the intestinal wall of the small intestine and to colonise the large intestine, are examples of such foods.

Prebiotics are another example. Prebiotics are defined as "non-digestible food substances that beneficially affect the host by selectively stimulating the growth and (or) activity of one or a limited number of bacterial species already resident in the colon and thus attempt to improve the host health (Gibson & Roberfroid, 1995). Non digestible carbohydrates/dietary fibres, such as specific oligosaccharides, are examples of prebiotics.

One of the factors that is being recognised to be of major importance for the maintenance of a healthy digestive system is the colonic flora, especially its bacterial composition and the "nutrients" that it metabolises.

This micro-flora is important for the production and absorption of a number of essential nutrients and for the competition with harmful bacteria, to reduce their negative impact on health. Through the consumption of fermentable carbohydrates the flora may also create a metabolic environment that supports the elimination of toxic and carcinogenic substances. Indeed, a substantial number of studies have pointed to the fact that a desired modification of the intestinal flora and physiology is possible (Gibson & Roberfroid, 1995). Although hard clinical endpoints on beneficial long-term effects are difficult to measure, it is generally believed that pro- and prebiotics may contribute to overall health maintenance (Conway, 2001).

The dietary fibre challenge

A few decades ago it was observed in epidemiological studies that populations that consume a high proportion of non-starch polysaccharide (NSP) dietary fibre (DF) in their daily diet suffer less from gastrointestinal diseases, in particular colorectal cancers, than populations that consume diets that are high in fat and protein but low in NSP fibre. This has lead to thousands of publications on the significance of an increased DF consumption for intestinal health and the prevention of colon cancer.

Although DF was classically defined as non-starch polysaccharides that are not digested in the small intestine and enter the colon to be fermented, it is now recognised that many other sources of non-digestible carbohydrates fall under this definition as well. Examples are different types of non-digestible oligosaccharides and resistant starch (Gibson, Willems, Reading, & Collins, 1996; Champ *et al.*, 2002), even though these are not measured by the current AOAC method for the determination of DF (Champ, Langkilde, Brouns, Kettlitz, & Le Bail-Collet, 2002).

Epidemiological studies have shown that environmental factors are the main reasons for geographical differences in colorectal cancer incidence. It was observed that populations that consume diets high in meat and fat have a higher cancer incidence than populations that consume diets high in starch and low in fat and meat (Bingham, 1996). It was also shown that the low incidence populations had mainly cancers in the upper part of the colon whereas high incidence populations had mainly colorectal cancers in the lower part of the large bowel. This leads to the suggestion that the majority of large bowel cancers can potentially be prevented by modification of environmental factors. In this respect, diet has been suggested to contribute as much as 25–35% to risk reduction for colorectal cancer by increasing the amount of vegetables and NSP DF's. A reduction of fat intake may further reduce the risk by 15–25% (Bingham, 1996). Based on these observations, DF's and substances that are part of the fibre complex such as antioxidants, flavonoids, sulphur containing compounds and folate have been proposed as potentially protective agents against colon cancer.

However, results from controlled prospective studies, in which beta-carotene and vitamin E or isolated dietary fibres were given to familial multiple polyposis cohorts or adenomatous polyps patients, showed disappointing results (Bingham, 1996; Fuchs *et al.*, 1999; Trock *et al.*, 1990). Accordingly, more recent prevention programmes have been focussed primarily on aspects of a healthy general lifestyle (Wasan & Goodlad, 1996).

The disappointing results mentioned above may be subject to several explanations such as:

- the different types of DF used,
- the absence of other dietary components that normally are part of the DF complex but get lost with the isolation of DF,
- the "basal diet",
- the chosen biomarkers and
- the stage of colon adenoma development/carcinogenesis.

However, there are indications that certain subclasses of highly fermentable dietary fibre, such as specific oligosaccharides and resistant starch, that result in significant flora modulation and production of short chain fatty acids, in particular propionate and butyrate, may have the potential to reduce risks of developing colorectal cancer.

In this respect, it is interesting to note that Cassidy, Bingham, and Cummings (1994) found a significant negative correlation between the consumption of starch and with it (assuming an RS fraction of 5% within total starch consumed) resistant starch on colorectal cancer incidence. In her study she assessed the starch consumption of populations in 12 countries as measured in individual surveys. A positive correlation with fat and protein consumption was observed but only a weak and non-significant correlation with NSP intake. There was, however, a strong inverse correlation between colorectal cancer incidence and starch intake ($r = -0.70$) and this association was maintained after controlling for partial correlation due to meat and fat consumption.

A suggestion put forward is that this beneficial effect may be related to the fact that the RS consumed is subject to complete fermentation, particularly leading to high levels of butyrate (molar quantity in SCFA produced, 20–28%) compared to slow and less fermentable fibers such as NSP (10–15%) in the large bowel.

Health aspects of resistant starch consumption

This has lead to an immediate growing interest in the field of gut health related to the consumption of Resistant Starch (RS) (Asp, van Amelsvoort, & Hautvast, 1996). More recently it was observed in African populations that consume relatively low fibre but high starch diets, that the occurrence of intestinal cancer was substantially lower compared to low starch consuming populations (O'Keeffe 1999).

Not only the amount of RS reaching the colon seems to be important but especially the molecular composition and physical structure seem to affect the prebiotic and butyrogenic properties of RS. This correlates well with the scientific consensus that the products of bacterial fermentation, especially propionate and butyrate, rather than the presence of dietary fibre itself determine the physiology in the large intestine and the resulting health effects (Archer, Meng, Shei, & Hodin, 1998; Gamet, Daviaud, Denis-Pouxviel, Remesy, & Murat, 1992; Goodlad, Ratcliffe, & Fordham, 1989; Scheppach, 1994, 1995; Smith, Yokoyama, & German, 1998; Thornton, Dryden, Kelleher, & Losowsky, 1987).

Scientific studies support the view that retrograded resistant starch (RS) is the most powerful butyrate producing substrate (Bird, Brown, & Topping, 2000; Topping & Bird, 1999). Recently a number of publications have highlighted the physiological properties as well as the effects of resistant starches on intestinal digestion, physiology, flora and fermentation (Baghurst, Baghurst, & Record, 1996; Bird, Brown, & Topping, 2000; Conway, 2001; Cummings et al., 1996; Hylla, Gastner, Dusel *et al.*, 1998; Silvester, Bingham, Pollock *et al.*, 1995; Topping & Bird, 1999). For a summary of the potential actions of RS as derived from *in vitro*, animal and human studies described in the reviews that are listed above, see Table 1.

Table 1. Functional potential of resistant starches

Effects on intestinal flora and metabolism
- Completely fermented by intestinal flora
- Low levels of gas formation when being fermented
- Elevates colonic butyrate levels more than NSP's when being fermented
- Reduces intestinal pH in dose dependent manners
- Selectively utilised by lactobacilli and bifidobacteria.
- Promotes colonisation of lactobacilli and bifidobacteria
- Reduces intestinal pathogen levels
- Reduces secondary bile acids
- Reduces faecal water toxicity

Effects on health, gut function and physiology
- Reduces symptoms of diarrhoea (duration and fluid loss)
- Increases stool weight
- Mild laxative effect at higher intakes
- Reduces energy intake when substituted for normal starch in foods
- Reduces insulin response compared to normal starch/carbohydrates
- Increase satiety response in the late post absorptive phase
- Increases Ca and Mg absorption
- Stimulates immune system
- Reduces risk factors related to large bowel cancer

Resistant starch consumption

Originally* resistant starch is classified as 3 types (Euresta):

1. RS Type I: starch that is physically inaccessible for digestive enzymes because it is "packed" in fiber material, e.g. grains and seeds.
2. RS Type II: raw starch granules as present in raw potato and green banana.
3. RS Type III: starch mainly represented as recrystallized (retrograded) amylose. This type is mainly present in cooked and subsequently cooled potato, in bread and in corn flakes.

The resistance of these 3 RS types is attributed to particular physical structures, whilst molecular parameters of the starch (D-glucose units connected by α-1,4/α-1,6 glucosidic bonds) remain substantially unchanged. *Recently also some papers mention a category IV covering some chemically modified starches. In this case the resistance to digestion is due to chemical changes in the starch back bone.

There are some indications that RS consumption has historically declined. A French study showed a reduction from initially 7–9 to 3–7 g/day over a period of 40 years, most probably because of changed food habits related to a rapidly industrialised world and a reduced bread consumption (Fig. 1, Brousseau, Dufour, & Volatier, 1998). In comparison, mean current intakes in Western countries (Europe as well as Australia) range from about 4 to 10 g/day, which is comparable to intakes in Australia (Brousseau *et al.*, 1998; Brighent, Casiraghi, & Baggio, 1998, Baghurst *et al.*, 1996). However, some individuals, who consume high amounts of starch containing foods, may have a daily intake as high as 30—40 g/day. Whilst no specific recommendations exist for RS intake, some experimental studies suggest that intakes in the order of $\geq$20 g/day RS may be needed to obtain some of the bowel related benefits (Baghurst *et al.*, 1996).

Biomarkers related to the study of gut health

Currently there is extensive debate on how to study the effects of selected nutrients on body functions and metabolism. The fact that the populations in Western

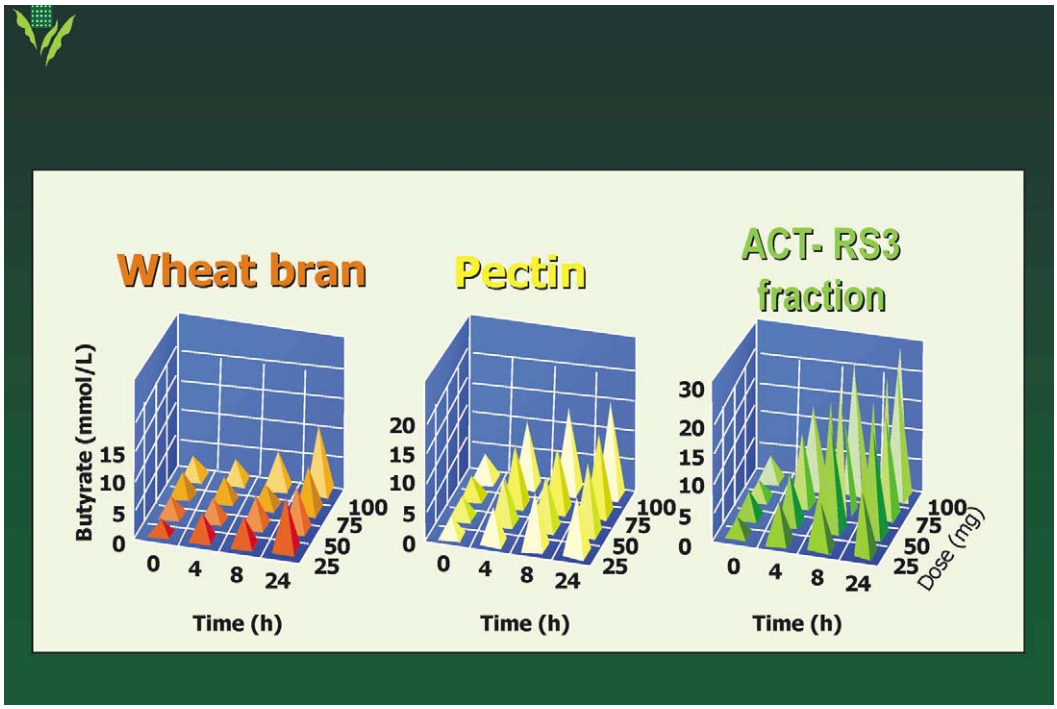

Fig. 1. Starch consumption in France declined significantly during the last 5 decades, most importantly by a reduced consumption of bread and potatoes. Accordingly also the calculated consumption of RS declined from 7–9 g/day initially to 3–7 g in recent years.

countries grow significantly older than a few decades ago has aroused governments as well as health professionals to identify environmental factors that influence health. Reducing risk factors that are associated to disease has become an international target. Accordingly, producers of health foods and food supplements aim at placing disease risk reduction claims on the product packaging.

In this respect there is one generally recognizsed problem. The ingestion of the daily food will lead to the intake of a large number of compounds that may have some kind of bio-active effect.

The latter may be an inhibition as well as an activation of a metabolic event. As such the effect of any dietary intervention is always the effect of a concerted action of all food components ingested. This makes the study of the effect of a single food component in terms of disease prevention a very difficult one. For example, if the study population would be composed of healthy individuals it would require maybe a few thousand of subjects and a study duration of 10–20 years or more to be able to detect a meaningful effect. For economical reasons this is mostly impossible. Related to this issue is the question whether you can make a healthy individual healthier anyway? To avoid such problems the focus generally is on the study of high risk populations, such as adenoma patients, in whom the recurrence of adenoma is studied as a marker of efficacy of nutritional compounds to reduce colorectal carcinogenesis. Also in this case it would require a high number of subjects and a long duration. Moreover, the question could also be raised as to whether such a patients group is representative enough to obtain results that are relevant to the general non-high risk population. This question has not been answered and the studies that have been done with adenoma patients generally have produced disappointing results. Other possibilities concern the study of animals that are exposed to selected carcinogens or inflammatory substances. Such studies can be done in short term and will help to unravel mechanistic aspects of the biochemical actions of the food compound studied. Using experimental gut carcinogenesis and gut inflammation models much data has been obtained on the possible role that products of fermentation may have on either reduction of cancer development and gut inflammation in these animals or recovery from it.

Again, also in this respect, the question is justified as to whether the observations done in animals are meaningful to the human situation. A last alternative is the use of human cell lines that are studied in vitro. On the one hand this comes much closer to influence on human tissues and organs, on the other hand many other influences that normally are present in the human body are eliminated in this situation. For example the concerted action of hormonal and neural influences and the bioactive effects of a many compounds that normally circulate with body fluids but are absent in vitro. There is one other aspect that should not be overlooked. Since the human gut, especially the colon, is very difficult to

access for long-term study, science has to rely largely on animal models and in vitro work. It is in this light of these limitations that we have to deal with the current knowledge on gut health as specified in the sections that follow below.

Butyrate actions and/or immunity effects?

Important is also the recognition that the large bowel plays a significant role in our immune defence against disease. Research has shown that impairments in butyrate supply to colon cells induce gut atrophy and functional impairments, including reduced immune responses. In contrast, enhanced butyrate supply to the colon cells induces growth of the gut epithelium, gut cell differentiation and improvement of immune-surveillance. (Cummings, & Englyst, 1991; Roediger, 1990; Scheppach, 1994).

During the last decade it has been recognised that especially resistant starch induces a high butyrate and propionate production (Bird *et al.,* 2000). Essential in this respect is that this production is also present in the lower part of the large bowel. It is this part (descending colon and rectum) where ''faecal concentration'' takes place and where the risks of developing colon cancer are a 1000 fold higher than in other parts of the intestine (Goodlad & Wright, 1995).

Butyrate is known to have beneficial effects on the reduction of risk factors involved in the etiology of colon cancer and adenoma development (Smith *et al.*, 1998). Butyrate oxidation has been shown to make up for more than 70% of the oxygen consumption by the human colonic tissue (Roediger, 1980), indicating that butyrate is the prime energy substrate of the colonocyte.

Butyrate is an anti-neoplastic agent in vitro, and has been implicated in the protective effect of fibre in rodents (McIntyre, Gibson, & Young, 1993; Scheppach *et al.*, 1995). Sodium butyrate (NaB) exerts an anti-proliferative activity on many cell types. It induces differentiation of colon carcinoma cell lines. It also has been observed to induce gene expression, to influence the rate of gene expression through its effects on post translational modifications, to induce apoptosis (naturally programmed cell death) and to reverse the resistance of colonic cancer (uncontrolled growth) cells to apoptosis (Archer *et al.*, 1998; Caderni, Luceri, Lancioni, Tessitore, & Dolara, 1998; Smith, Yokoyama, & German, 1998; Velàzquez, Howard, & Rombeau, 1996). Various lines of evidence suggest that the pathways of programmed cell death provide a means of protection against carcinogenesis by removing genetically damaged cells before they can give rise to pre-cancerous lesions. This has been reviewed in detail by Johnson (2001). *In vitro* experimental work further showed a decreased hydrogen peroxide induced DNA damage in human colon cancer cells following incubation with butyrate Rosignoli *et al.* (2001). Additionally it was shown colon in cancer cells (CACO2) that butyrate improves intestinal barrier function by reducing its paracellular permeability after an epithelial damaging event Mariadason, Barkla, and Gibson (1997). SCFAs inhibited colon cancer cell (SW116) invasion. Effects were more potent for butyrate (Emenaker & Basson, 1998).

There is data suggesting that the ingestion of wheat bran promotes histone acetylation (most probably by the action of butyrate), a process involved in apoptosis induction in rat epithelial cells *in vivo* (Boffa, Lupton, Mariani *et al.*, 1992).

Butyrate stimulates the immunogenicity of the cancer cells. The phenotype of the weakly immunogenic rat colon cancer PROb cells was modified with sodium butyrate. After a 4-day *in vitro* sodium butyrate treatment, the lymphokine-activated killer cell sensitivity, the expression of Major Histocompatibility Complex class I, and the intercellular adhesion molecule 1 of PROb cells, were increased in a dose-dependent manner (Perrin *et al.*, 1994).

Perrin *et al.* (1994) also tested the efficacy of the immune factor interleukin 2 (IL-2) and sodium butyrate (NaB), alone or in combination, against experimental carcinomatosis, induced in rats by intraperitoneal injection of 2×10^6 PROb colon carcinoma cells.

IL-2/butyrate combination resulted in cases of complete cure of carcinomatosis with specific protection against PROb cells.

This complete regression of tumour masses may be attributed, to butyrate-induced effects on apoptosis (reintroduction) and an increase of the immunogenicity of the cancer cells.

Based on these experimental observations it is hypothesised that the ingestion of indigestible carbohydrates as an indirect source of butyrate to the large bowel may be beneficial in terms of reducing risk factors for colorectal cancer.

Highly interesting for health maintenance is also the specific action of butyrate to promote the growth and development of normal intestinal cells while inhibiting processes that lead to diseased and abnormal cells (Velazquez *et al.*, 1996). When produced in higher amounts in the colon, butyrate may also have small systemic effects after absorption and circulation through the body. Accordingly it has been suggested in scientific literature that butyrate may not only protect against the initiation and development of large bowel cancer (Avivi-Green, Polak-Charcon, Madar, & Schwartz, 2000) but perhaps also breast (Heerdt, Houston, Anthony, & Augenlicht, 1999) and prostate cancer (Ellerhorst *et al.*, 1999).

Studies on the effect of butyrate, propionate and acetate on gut metabolism of patients and experimental animal models suffering from gut inflammation have shown that a sufficient and sustained level of SCFA may be essential for the maintenance of a healthy gut.

Patients with inflammatory bowel disease but also patients with colon cancer typically have low levels of butyrate in the gut (Chapman *et al*., 1994) and have a low rate of butyrate oxidation by the mucosa.

This may be related to a poor supply of fermentable substrate and a non-balanced intestinal flora, in which sulfate reducing bacteria are present in too large quantities. These bacteria produce increased luminal concentrations of mercaptans, sulfides and sulfites.

Mercaptans are known to inhibit the uptake of butyrate by the colon cells (Stein, Schröder, Milovic, & Caspary, 1995). Thus, in a situation of poor supply of fermentable substrate and accordingly low levels of SCFA production, an appropriate supply of butyrate to the colonocytes is further diminished by the action of mercaptans.

This may lead to a shortage of energy for optimal functioning and to a breakdown of adenine nucleotides along with the formation of free radicals through the xanthine oxidase reaction. Both a shortage of energy and the presence of free radicals will promote the inflammation and ultimately the necrosis process in the intestine (Jacobash, Schmiedl, Kruschewski, & Schmehl, 1999)

The fact that butyrate indeed plays an important role is confirmed by studies in which patients with inflammatory bowel disease received sodium butyrate enema. The supply of butyrate reduced inflammation related symptoms significantly in a number of trials (Breuer *et al*., 1997; Cummings, 1997; Scheppach *et al*., 1992). The extent of, as well as the recovery from inflammatory bowel disease in a situation of impaired butyrate availability vs enhanced butyrate availability after the ingestion of RS3 was most pronounced in controlled rat experiments (Butzner, Parmar, Bell, & Dalal, 1996; D'argenio *et al*., 1996). Recently Jacobasch *et al*. (1999) reviewed a number of studies while concluding that the synthesis of new laminin, the protein that makes up most of the basal membrane in the colon mucosa, is closely related to the onset of cell proliferation.

The latter is stimulated by butyrate obtained from RS3 consumption. Apoptosis (controlled cell death) was introduced more rapidly when butyrate was available. Accordingly colonic epithelial cells do have better regenerative properties when supplied with RS derived butyrate. A detailed review on mechanisms of diet related apoptosis is given by Johnson (2001). SCFA also are known to stimulate bowel motility, blood flow (Kamath, Philips, & Zinsmeister, 1988) and reduce enteral feeding associated diarrhoea by enhancing colonic water uptake (Bowling, Raimundo, Grimble, & Silk, 1993).

Of significant interest is also the very recent observation that there is a close relationship between the production of butyrate and enterolactone in the colon, as observed in an ileal cannulated and catheterised pig model ($n=4$) (Bach Knudsen, Serena, Glitsø, & Adlercreutz, 2001). Enterolactone is a mammalian lignan that is produced by the intestinal flora, in the upper part of the colon, from lignans (a class of phytoestrogens) that are present in certain plant foods like whole grain, especially rye (Glitsø *et al*., 2000). Lignans have been described to protect against a number of Western diseases through improving the host's health (Adlercreuz, 1990). This new observation suggests that modulations of either intestinal bacterial metabolism and/or intestinal flora after the consumption of well fermentable dietary fibers most probably leads to an induction of enterolactone production. When confirmed in other studies, the potential inclusion of resistant starch as ingredient in whole grain products may become very attractive.

Observations as outlined above have prompted the food industry to give more focus on dietary fibres that lead to a high butyrate production in a large segment of the colon. In this respect, Baghurst *et al*. (1996) indicated that "for the food industry, one potential advantage of resistant starch, over non-starch polysaccharides as agent for "fibre like" activity, is the much greater potential to increase intake in the community, through the development of new high resistant starch products". Baghurst *et al*. (1996) further commented, that "for some core starch foods such as breads, it has already been possible to increase resistant starch consumption by some 8–10 fold. A similar increase in non-starch polysaccharides (NSP) content is very difficult to achieve in whole foods. If resistant starch proves to be as physiologically effective on a weight for weight basis as NSP, then the health potential of this "new" food component will be high".

A new type of natural RS3 with potential gut health properties

From the above mentioned observations it appears that the amount of butyrate produced in a relatively large segment of the colon is of significant importance. The average ratio of NSP derived acetate / propionate/ and butyrate is 60:25:10 respectively (Cummings, 1981). A substantial number of studies have shown that the short chain fatty acid profile resulting from RS fermentation differs from that of NSP fermentation. RS fermentation generally results in a relatively large butyrate production in the order of 20–28 mol% compared to about 10–15 mol% for NSP. This has been shown both *in vitro* and *in vivo* thus, not the NSP fraction itself but the quantity of fermentable starch, reaching the colon, is a strong determinant for the quantity of butyrate being produced (Perrin, 2001). Recently a new type of natural highly crystalline RS3 (Actistar®,(Act*-RS3)) has been developed from maltodextrins as starting material. (US Patent 6,043,229 (2000). Act*-RS3 has a relatively low average molecular weight and a much

homogeneous molecular weight distribution than RS products based on high amylose starch. Due to raw material choice and production process. It has a very neutral taste. Regular shape and low particle size are the reason for a pleasant mouth feel without any sandiness. It can be easily dispersed in cold and hot fluids without lumping and gelling tendency respectively. The physiological properties seem to be a new challenge for the food industry in their attempts to target the Gut health market. A number of laboratory trials have given insight in the technical as well as functional properties of Act*-RS3® and its metabolism.

SCFA production

The molar proportion of SCFA produced during fermentation depends on the quality of the RS. That is, the extent to which the RS is accessible to the microflora.

RS3 is a very good fermentable substrate. The supplementation of RS3 to food may more than double in the luminal concentrations of butyrate and propionate (Kleesen *et al*., 1997), which may be essential in terms of sustained effects on colonic metabolism.

An *in vitro* fermentation assay of ACT*-RS3® has been performed in a batch system with human faeces according the methodology described by Barry *et al*. (1995). Its fermentation pattern was shown to be complete and rapid, resulting in a dose dependent increase of the total SCFA's (Fig. 2) and a concomitant drop in pH. Butyrate represents 21–28 mol% of total SCFAs, (for comparison, most NSP's result in 10–15% butyrate)

Arrigoni, Rochat, and Amado (2001) used an *in vitro* batch system with pre-digested substrates, as described by Lebet, Arrigoni, and Amado (1998). They compared various oligosaccharides and RS.

Compared to indigestible short-chain oligosaccharides (fructo-oligosaccharides, xylo-oligosaccharides) the fermentation of RS is relatively slow. The production of hydrogen after RS intake is lower and the production of butyrate was significantly higher. According to the authors this may be due to the specific presence of partially crystalline retrograded amylose in the product (RS3). When compared to retrograded debranched high amylose corn starch (Cristalean, Opta Foods) and RS2 (thermally modified granular high amylose corn starch, Novelose 240, National Starch, USA), ACT*-RS3® resulted in a significantly faster substrate disappearance, higher total SCFA and butyrate production at 8 hrs of incubation. (Figs. 3 and 4 and Table 3) (Arrigoni *et al*., 1999, 2001). This indicates that the product is easier fermentable by the colonic microflora. Interestingly, a study by Martin, Dumon, Lecannu, and Champ (2000) in pigs* suggested that butyrate produced from RS3 is more distally fermented in the colon and could be more beneficial for a healthy colon than raw potato starch

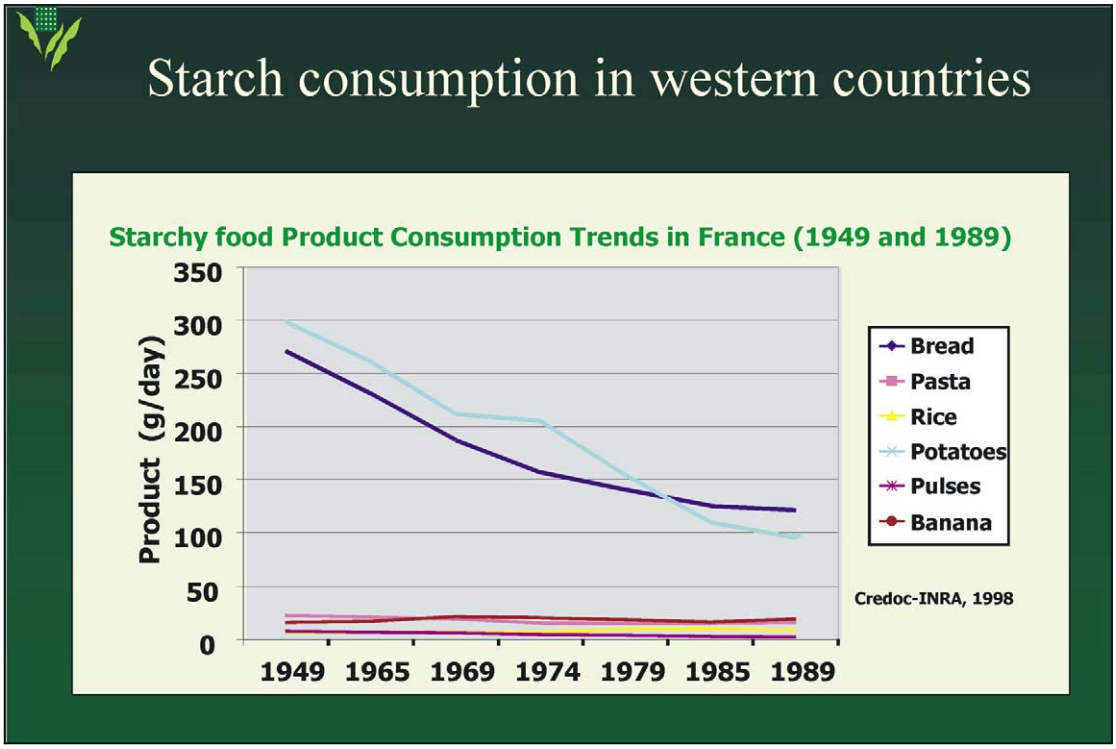

Fig. 2. Production of butyrate from wheat bran, pectin and Act*-RS3. The SCFA production was measured in an *in vitro* batch system with human faeces.

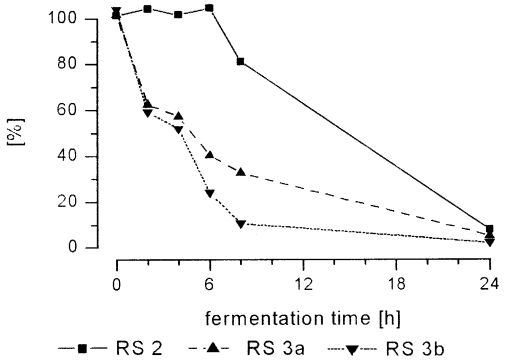

Fig. 3. Substrate disappearance during *in vitro* fermentation of pre-digested resistant starch, using human faeces. RS2 = thermally modified granular high amylose cornstarch, RS3a = retrograded debranched high amylose corn starch, RS3b = retrograded starch produced from potato maltodextrin (Act*-RS3).

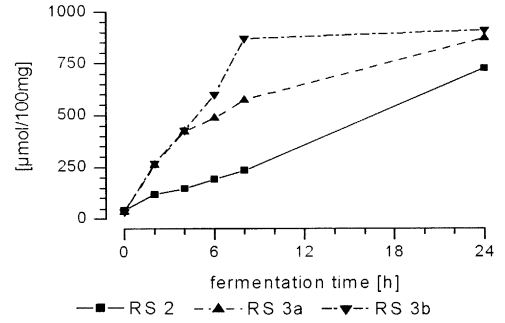

Fig. 4. Short chain fatty acid production during in vitro fermentation of pre-digested resistant starch, using human faeces. RS2 = thermally modified granular high amylose cornstarch, RS3a = retrograded debranched high amylose corn starch, RS3b = retrograded starch produced from potato maltodextrin (Act*-RS3). The fact that RS3b did not increase anymore after 8 hrs is explained by the complete fermentation at that moment. It is expected that reintroduction of substrate will elevate molar ratio of butyrate at 24 h significantly.

(RS2) (* pigs are very close to humans in terms of the digestive system).

Immunogenicity

Experimental studies using animal models that are assumed to be most representative to study the effects of nutrients on the initiation and progression of colon cancer have shown that Act*-RS3® consumption induces the rejection of pre-cancerous cells (aberrant crypt foci) via direct actions, most probably through its butyrate delivery. It has been suggested that this effect is further indirectly supported by an improved immunogenicity of the colon cells as well as a modulation of blood immune parameters (Menantau *et al.*, 1998; Perrin *et al.*, 2001; Pierre *et al.*, 1997).

Digestibility

In vivo human studies using ileostomy patients as model have shown that the product contains 54% (DS 60%) of type 3 RS that reaches the colon for fermentation. The ileostomy model was earlier shown to give reliable results for *in vivo* measurement of RS (Langkilde, Philipsson, Andersson, & Brouns, 2001). The other fraction of 46% is digestible and absorbable carbohydrate that will contribute to daily carbohydrate intake (Langkilde 2001). This observation implicates that consumption will reduce caloric intake as well as insulinemia when compared to the consumption of normal starch. Accordingly, the inclusion of RS3 in foods may be suitable for a wide variety of food products designed for overweight individuals and type II diabetics.

Digestive tolerance

In order to promote an increased regular consumption of type 3 RS consumers desire that a reasonable daily consumption of 20–30 g will not induce digestive side effects that may disturb the quality of life. Inherent to increased fermentation of most carbohydrates is the increased flatus production. With rapidly fermentable carbohydrates this may also result in less desired bloating of the gastrointestinal tract. In this respect a recent study on digestive tolerance of RS3 was conducted in healthy volunteers ($n = 41$) at the Nutritional Bio-sciences Department of the Salford University (UK) (Lee, Storey, Bornet, & Brouns, in press). This study evaluated the effects of an acute daily dose of ACT*-RS3® (0, 20, 40, 60, 80, 100 and 120 g presented in random order).The second objective was to study the effect of gradually, increasing the dosages over a period of 21 days with the goal to finally reach a threshold dose equal to the dose that was maximally tolerated *without disturbing* digestive problems in the acute intake condition.

Symptoms were considered to be: nausea, bloating, borborygmi, colic, flatus, diarrhoea in such a way that the subject judged these to be disturbing. With the acute dose, three subjects had a threshold tolerance of 80 g, 8 subjects of 100 g and 30 subjects of 120 g resp. Regression analysis showed that there was no significant increase in mean symptom scores for any symptom. Mean numbers of toilet visits to pass faeces of any consistency: normal, watery or hard faeces, following consumption of increasing doses of RS3 did not change either.

From the chronic consumption data, it could be concluded that repeated ingestion of RS3 did not cause any increase in the number of subjects experiencing severe

symptoms, compared to the acute condition. Accordingly, the authors concluded that up to 60 g RS per day (equivalent to 120 g ACT*RS3) was well tolerated by subjects but a mild laxative effect at 60 g was suggested (Lee *et al*., in press).

Food application characteristics

Low hygroscopicity, low water binding capacity and very low and stable suspension viscosity enable an easy incorporation in numerous food recipes. Under most normal processing conditions the resistant retrograded structure is stable. The use of ACT*-RS3® in bread, biscuits, or pasteurised milk products resulted in a 95–100% recovery of RS structures. This means that substantial amounts of crystalline structures (RS) are recovered after heat treatment of food preparations. Even under very harsh conditions such as UHT (137°C for 5 s) about 2/3 of the retrograded structure is retained. Possible inclusion levels of RS3 in foods and drinks are presented in Table 2.

Table 2. RS III-enriched products: Inclusion levels tested without taste effects compared with non-enriched standard products (*note: *in vivo* resistant starch content of ACTISTAR® amounts 54% (60%DS))

	Proposed % ACT-RS3® inclusion*
Cereal-based products	
Bread	8
Rusks	3
Breakfast cereals	14
Muesli	20
Pasta	5
Dessert and biscuits	
Savory biscuits and cookies	8
Vegetables	
Soup	2.5
Dairy products	
Milk	2
Yoghurt	5

Table 3. Molar proportion of the three main short chain fatty acids produced from pre-digested resistant starch preparations after 24 h of *in vitro* fermentation (mol%)

	Acetate	Propionate	Butyrate
RS 2	53	16	30
RS 3a	66	12	20
RS 3b	62	12	24

Summary/conclusions

Food consumption studies show that RS consumption has declined over the last decades. There are reasons to suggest that increasing the RS content in the daily diet will be of benefit for the maintenance of gut health and the reduction of risk factors associated with the development of intestinal inflammation and colorectal cancer. A significant resistant starch consumption and its related fermentation pattern may act on the colon in several ways:

1. stimulation of the local immune system (GALT)
2. modulation of blood immune parameters
3. modulation of DNA synthesis and repair by butyrate
4. stimulation of normal cell growth by butyrate
5. inhibition of abnormal cell growth and development by butyrate
6. promotion of recovery from epithelial inflammation.

A new type RS3 has been developed with excellent technical and functional properties for application in food:

- contains 54% resistant starch (60%DS)
- high butyrate as well as substantial propionate production in colon.
- low glycemic and insulinemic response.
- remarkably good digestive tolerance
- non-starchy taste, no sandy mouth feel.

Future research with foods that have significant inclusion levels of RS3 should focus on validating the promising results from experimental animal studies in a human situation. Seen the fact that different types of RS may have a different fermentation profile and butyrate production, it is important that results are obtained especially on RS3, the resistant starch type mostly consumed by humans and most distally fermented in the colon in animal experiments (Martin *et al*., 2000)

References

Adlercreutz, H. (1990). Western diet and Western diseases: some hormonal and biochemical mechanisms and associations. *Scandinavian Journal of Clinical and Laboratory Investigation*, *50*(Suppl. 201), 3–32.

Archer, S., Meng, S., Shei, A., & Hodin, R. A. (1998). P21WAFI is required for butyrate-mediated growth inhibition of human colon cancer cells. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 6791–6796.

Arrigoni, E., Rochat, F., & Amado, R. (2001). Short-chain fatty acid production during in vitro fermentation. Abstract Proceedings Vienna Int. Conference Nutrition

Arrigoni, E., Jann, A., Rochat, F. & Amado, R. (1999). In vitro fermentability of indigestible oligons and polysaccharides. In: Latz-

tity (Ed.), *Proc. of Euro Food Chem* (pp. 481–488) Budapest: Publishing Company of TUB.

Asp, N. C., van Amelsvoort, J. M. M., & Hautvast, J. G. A. J. (1996). Nutritional implications of resistant starch. *Nutritional Research Review*, *9*, 1–31.

Avivi-Green, C., Polak-Charcon, S., Madar, Z., & Schwartz, B. (2000). Apoptosis cascade proteins are regulated in vivo by high intracolonic butyrate concentration: correlation with colon cancer inhibition. *Oncology Research*, *12*, 83–95.

Bach Knudsen, K. E., Serena, A., Glitsø, V., & Adlercreutz, H. (2001). Colonic formation and absorption of mammalian lignans and butyrate. In K. Liukkonen, A. Kuokka, & K. Poutanen (Eds.), *Proceedings conference whole grain and human health* (pp. S30–S31). Finland: Haikko Manor.

Baghurst, P. A., Baghurst, K. I. & Record, S. J. (March, 1996). Dietary fibre, non-starch polysaccharides and resistant starch—a review. *Suppl. To Food Australia 48* (3).

Barry, J. L., Hoebler, C., MacFarlane, G. T., MacFarlane, S., Mathers, J., Reed, K. A., Mortensen, P. B., Norgaard, I., Rowland, I. R., & Rumney, C. J. (1995). Estimation of the fermentability of dietary fibre in vitro: a European interlaboratory study. *British Journal of Nutrition*, *74*, 303–322.

Bingham, S. A. (1996). Epidemiology and mechanisms relating diet to risk of colorectal cancer. *Nutrition Research Reviews*, 197–239.

Bird, A. R., Brown, I. L., & Topping, D. L. (2000). Starches, resistant starches, the gut microflora and human health. *Curr. Issues Intest. Microbiol.*, *1*, 25–37.

Boffa, L. C., Lupton, J. R., Mariani, M. R., *et al*. (1992). Modulation of colonic cell proliferation, histone acetylation and luminal short chain fatty acids by variation of dietary fiber (what bran) in rats. *Cancer Research*, *52*, 5906–5912.

Bowling, T. E., Raimundo, A. H., Grimble, G. K., & Silk, D. B. (1993). Reversal by short-chain fatty acids of colonic fluid secretion induced by enteral feeding. *Lancet*, *342*, 1266–1268.

Breuer, R. I., Soergal, K. H., Lashner, B. A., Christ, M. L., Hanauer, S. B., Vanagunas, A., *et al*. (1997). Short-chain fatty acid rectal irrigation for left-sides ulcerative colitis : a randomised, placebo-controlled trial. *Gut*, *40*, 485–491.

Brighenti, F., Casiraghi, M. C., & Baggio, C. (1998). Resitant starch in the Italian diet. *British Journal of Nutrition*, *80*, 333–341.

Brousseau, A. D., Dufour, A., Volatier, J. L. (1998). Assessment of resistant starch consumption in France and the nutritional impact of its use in an increasing number of foodstuffs. Internal Report of CREDOC, Département Prospective de la Consommation, Paris, France

Butzner, J. D., Parmar, R., Bell, C. J., & Dalal, V. (1996). Butyrate enema therapy stimulates mucosal repair in experimental colitis in the rat. *Gut*, *38*, 568–573.

Caderni, G., Luceri, C., Lancioni, L., Tessitore, L., & Dolara, P. (1998). Slow-release pellets of sodium butyrate increase apoptosis in the colon of rats treated with azoxymethane, without affecting aberrant crypt foci and colonic proliferation. *Nutrition and Cancer*, *30*, 175–181.

Cassidy, A., Bingham, S. A., & Cummings, J. H. (1994). Starch intake and colorectal cancer risk: an international comparison. *British Journal of Cancer*, *69*, 937–942.

Champ, M., Langkilde, A. M., Brouns, F., Kettlitz, B., & Le Bail-Collet, Y. Advances in dietary fibre characteristics. *Nutritional Research Reviews* (in press).

Chapman, M. A. S., Grahn, M. F., Boyle, M. A., Hutton, M., Rogers, J., & Williams, N. S. (1994). Butyrate oxidation is impaired in the colonic mucosa of sufferers of quiescent ulcerative colitis. *Gut*, *35*, 73–76.

Conway, P. L. (2001). Prebiotics and human health : the state-of-the-art and future perspectives. *Scandinavian Journal of Nutrition*, *45*, 13–21.

Cummings, J. H., *et al*. (1996). Digestion and physical properties of resistant starch in the human large bowel. *British Journal of Nutrition*, *75*, 733–747.

Cummings, J. H. (1997). Short-chain fatty acid enemas in the treatment of distal ulcerative colitis. *Journal of Gastroenterology and Hepatology*, *9*, 149–153.

Cummings, J. H. (1981). Short chain fatty acids in the human colon. *Gut*, *22*, 763–779.

Cummings, J. H., & Englyst, H. N. (1991). Measurement of starch fermentation in the human large intestine. *Canadian Journal of Physiology and Pharmacology*.

D'Argenio, G., Consenz, V., Cave, M. D., Iovino, P., Valle, N. D., Lombardi, G., & Mazzacca, G. (1996). Butyrate enemas in experimental colitis and protection against large bowel cancer in a rat model. *Gastroenterology*, *110*, 1727–1734.

Ellerhorst, J., Nguyen, T., Cooper, D. N. W., Estroy, Y., Lotan, D., & Lotan, R. (1999). Industion of differentiation and apoptosis in the prostate cancer cell line LNCaP by sodium butyrate and galectin-1. *International Journal of Oncology*, *14*, 225–232.

Emenaker, & Basson (1998). Short chain fatty acids inhibit human (SW116) colon cancer cell invasion by reducing urokinase plasminogen activator activity and stimulating TIMP 1 and TIMP 2 activities rather than via MMP modulation. *Journal of Surgical Research*, *76*, 41–46.

Fuchs C. S., Giovannucci, E. L., Coldlitz, G. A., Hunter, D. L., Stampfer, M. J., Willett, W. C. (1999). Dietary fiber and the risk of colorectal cancer and adenoma in woman. *The New England Journal of Medicine*, 340, number 3 p.p. 169–176

Gamet, L., Daviaud, D., Denis-Pouxviel, C., Remesy, C., & Murat, J. (1992). Effects of short chain fatty acids on growth and differentiation of the human colon-cancer cell line HT29. *International Journal of Cancer*, *52*, 286–289.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition*, *125*, 1401–1412.

Gibson, G. R., Willems, A., Reading, S., & Collins, M. D. (1996). Fermentation of non-digestible oligosaccharides by human colonic bacteria. *Proc. Nutr. Soc.*, *55*, 899–912.

Glitsø, L. V., Mazur, W. M., Adlercreutz, H., Wähälä, K., Mäkelä, T., Sandströöm, B., & Knudsen, K. E. B. (2000). Intestinal metabolism of rye lignans in pigs. *British Journal of Nutrition*, *84*, 429–437.

Goodlad, R. A., Ratcliffe, B., Fordham, J. P., *et al*. (1989). Does dietary fibre stimulate intestinal eptihelial cell proliferation in germ free rats? *Gut*, *30*, 820–825.

Goodlad, R. A., & Wright, N. A. (1995). Epithelial kinetics, control and consequences of alterations in disease. In R. Whitehead (Ed.), *Gastrointestinal and oesophageal pathology* (pp. 97–116). Edinburgh: Churchill Livingstone.

Heerdt, B. G., Houston, M. A., Anthony, G. M., & Augenlicht, L. H. (1999). Initiation of growth arrest and apoptosis of MCF-7 mammary carcinoma cells by tributyrin, a triglyceride analogue of the short-chain fatty acid butyrate, is associated with mitochondrial activity. *Cancer Research*, *59*, 1584–1591.

Hylla, S., Gostner, A., Dusel, G., *et al*. (1998). Effects of resistant starch on the colon in healthy volunteers: possible implications for cancer prevention. *American Journal of Clinical Nutrition*, *67*, 136–142.

Jacobasch, G., Schmiedl, D., Kruschewski, M., & Schmehl, K. (1999). Dietary resistant starch and chronic inflammatory bowel disease: review. *International Journal of Colorectal Disease*, *14*, 201–211.

Johnson, T. T. (2001). New Food Components and gastrointestinal health. *Proceedings of the Nutritional Society*, *60*, 481–488.

Kamath, P. S., Philips, S. F., & Zinsmeister, A. R. (1988). Short-chain fatty acids stimulate ileal motility in humans. *Gastroenterology*, *95*, 1496–1502.

Kleesen, B., Stoof, G., Prol, J., Schmiedl, D., Noack, J., & Blaut, M. (1997). Feeding RS affects fecal and cecal microflora and short-chain fatty acids in rats. *Journal of Animal Science*, *75*, 2453–2462.

Langkilde, A. M., Philipsson, H., Andersson, H., & Brouns, F., (2001). Report to Cerestar: in vivo measurement of RS and oligo-saccharides. Göteborg University, Gotenbrug, Sweden

Lebet, V., Arrigoni, E., & Amado, R. (1998). Measurement of fermentation products and substarte disappearance during incubation of dietary fiber sources with human fecal flora. *Lebensmittel Wiss. Technol.*, *31*, 473–479.

Lee, A., Storey, D., Bornet, F., & Brouns, F. The gastrointestinal responses and adaptation of young adults following consumption of resistant Starch (RS3). *American Journal of Clinical Nutrition* (in press)

Mariadason, J. M., Barkla, D. H., & Gibson, P. R. (1997). Effect of short chain fatty acids on paracellular permeability in CACO2 intestinal epithelium model. *American Journal of Physiology*, *272*, G705–G712.

Martin, L. J. M., Dumon, H. J. W., Lecannu, G., & Champ, M. (2000). Potato and high amylose maize starches are not equivalent producers of butyrate for the colonic mucosa. *British Journal of Nutrition*, *84*, 689–696.

McIntyre, A., Gibson, P. R., & Young, G. P. (1993). Butyrate production from dietary fibre and protection against large bowel cancer in a rat model. *Gut*, *34*, 386–391.

Ménanteau, J., Perrin, P., Pierre, F., Champ, M., Pradal, G., Bornet, F., & Meflah, K. (1998). Non-digestible carbohydrates interfere with colon cancer in two different animal models In *Functional properties of non-digestible carbohydrates* (pp. 225–226). INRA Nantes. European Air Concerted Action AIR3CT94–2203

O'Keefe, S. J., Kidd, M., Espitalier-Noel, G., & Owira, P. (1999). Rarity of colon cancer in Africans is associated with low animal product consumption, not fiber. *American Journal of Gastroenterology*, *94*, 1373–1380.

Perrin, F., Pierre, F., Patry, Y., Berreur, M., Pradal, G., Bornet, F., Meflah, K., & Menanteau, J. (2001). Only fibres promoting a stable butyrate producing colonic ecosystem decrease the rate of aberrant crypt foci in rats. *Gut*, *48*, 53–61.

Perrin, P., Cassagnau, E., Burg, C., Patry, Y., Vavasseur, F., Harb, J., Le Pendu, J., Douillard, J. Y., Galmiche, J. P., Bornet, F., & Meflah, K. (1994). An interleukin Sodium butyrate combination as immunotherapy for rat colon cancer peritoneal carcinomatosis. *Gastroenterology*, *107*, 1697–1708.

Pierre, F., Perrin, P., Champ, M., Bornet, F., Meflah, K., & Ménanteau, 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.

Roediger, W. E. W. (1980). Role of the anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut*, *21*, 793–798.

Roediger, W. E. W. (1990). The starved colon:diminshed mucosal nurition, diminished absorption, and colitis. *Dis. Colon Rectum*, *33*, 858–862.

Rosignoli, *et al*. (2001). Protective effect of butyrate on hydrogen peroxide induced DNA damage in isolated human colonocytes and HT29 tumour cells. *Carcinogenesis*, *22*, 675–680.

Scheppach, W., Bartram, H. P., & Richter, F. (1995). Role of short-chain fatty acids in the prevention of colorectal cancer. *European Journal of Cancer*, *31A*, 1077–1080.

Scheppach, W., Sommer, H., Kirchner, T., Papanelli, G. M., Bartram, P., Christl, S., Richter, F., Dusel, G., & Kasper, H. (1992). Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. *Gut*, *38*, 886–893.

Scheppach, W. (1994). Effects of short chain fatty acids on gut microbiology and function. *Gut* (Suppl. 1), S35–S38.

Smith, J. G., Yokoyama, W. H., & German, B. G. (1998). Butyric acid from the diet: actions at the level of gene expression. *Clinical Reviews in Food Science*, *38*, 259–297.

Stein, J., Schröder, O., Milovic, V., & Caspary, W. (1995). Mercaptoproprionate inhibits butyrate uptake in isolated apical membrane vesicles of the rat distal colon. *Gastroenterology*, *108*, 673–679.

Silvester, C., Bingham, S., & Pollock, J., *et al*. (1995). Ileal recovery of starch from whole diets containing resistant starch measured in vitro and fermentation of ileal effluent. *American Journal of Clinical Nutrition*, *62*, 403–413.

Thornton, J. R., Dryden, A., Kelleher, J., & Losowsky, M. S. (1987). Super-efficient starch absorption. A risk factor for colonic neoplasma? *Digestive Disease and Sciences*, *32*, 1088–1091.

Topping, D. L., & Bird, A. R. (1999). Foods, nutrients and digestive health. *Australian Journal of Nutrition and Dietetics*, *56*(Suppl. 3), S22–S34.

Trock, B., Lanza, E., & Greenwald, P. (1990). Dietary fiber, vegetables, and colon cancer: critical review and meta-analysis of the epidemiological evidence. *Journal of the National Cancer Institue*, *82*, 650–661.

Velàzquez, O. C., Howard, M., & Rombeau, J. L. (1996). Butyrate and the colonocyte. *Implications for neoplasia*. *Digestive Disease and Sciences*, *41*, 727–739.

Wasan, H. S., & Goodlad, R. A. (1996). Fibre-supplemented foods may damage your health. *Lancet*, *348*, 319–320.

Further reading

Arrigoni, E., Naef, C., Ruolet, I., & Amado, R. (2002). In vitro fermentability of indigestible oligo-and polysaccharides. Poster (+ proceedings). *Nutrition*, *26*, 53–57.

Bowling, T. E., Raimundo, A. H., Grimble, G. K., & Silk, D. B. A. (1999). Reversal by short-chain fatty acids of colonic fluid secretion induced by enteral feeding. *Lancet*, 342.

Brandtzaeg, P., Halstensen, T. S., Kett, K., Krajci, P., Kvale, D., Rognum, T. O., Scott, H., & Sollid, L. M. (1989). Immunobiology and immunopathology of human gut mucosa: humoral immunity and intraepithelial lymphocytes. *Gastroenterology*, *97*, 1562–1584.

Campbell, J. M., Fahey, G. C., Wolf, J., & Wolf, B. W. (1997). Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. *Journal of Nutriton*, *127*, 130–136.

Cummings, J., & Mcfarlane, G. (1991). The control and consequences of bacterial fermentation in the human colon. *Journal of Applied Bacteriology*, *70*, 443–459.

Perrin, P., Pierre, F., Champ, M., Bornet, F., Méflah, K., & Ménanteau, 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.

Pories, S. E., Ramchurren, N., Summerhayes, I., & Steele, G. (1993). Animal models for colon carcinogenesis. *Arch. Surg.*, *128*, 647–653.

Thomson, I. T. (2001). Mechanisms and anticarcinogenic effects of diet-related apoptosis in the intestinal mucosa. *Nutritional Research Review*, *14*, 229–256.