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Relationship of prebiotics and food to intestinal microflora

Summary Dietary carbohydrates that escape digestion in the small intestine, undergo bacterial fermentation in the colon. This process affects the microbial ecology of the gastrointestinal tract and influences gut metabolism and function. Prebiotics are non-digestible but fermentable oligosaccharides that are specifically designed to change the composition and activity of the intestinal microbiota with the prospect to promote the health of the host. Dietary fiber and non-digestible oligosaccharides are the main growth substrates of gut microorganisms. Their fermentation results in the acidification of the colonic contents and the formation of short chain fatty acids which serve as fuels in different tissues and may play a role in the regulation of cellular processes. Prebiotics specifically stimulate the growth of endogenous microbial population groups such as bifidobacteria and lactobacilli which are perceived as being beneficial to human health. In spite of the interesting nutritional properties of prebiotics it is questionable whether a wholesome diet rich in fruit and vegetables needs to be supplemented with prebiotics for optimal health effects.

Key words prebiotics – dietary fiber – intestinal microflora – non-digestible oligosaccharides

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Introduction

Epidemiological studies indicate that diet has a major impact on human health: a diet low in fat and high in fruit and vegetables has been correlated with a decreased incidence of so-called Western diseases such as coronary heart disease and colon cancer [1]. Such a diet contains not only nutrients that are readily absorbed in the small intestine but also components that escape digestion by pancreatic and small bowel enzymes. The latter are the principal substrates of the bacteria resident in the human intestinal tract. Since a number of nutritional health effects are mediated by the intestinal microflora, diet is key in influencing their composition and activity. It has been increasingly recognized that the bacterial community in the intestine influences human health and well-being [2]. Consequently, nutrition may be considered as a tool for influencing the intestinal microbiota in such a way that harmful bacteria are suppressed and beneficial bacteria are stimulated. Dietary strategies that serve to support health-promoting effects of the intestinal microflora include the ingestion of probiotics [3] and/or prebiotics [4], as well as a diet rich in fiber [5].

Prebiotics

A prebiotic has been defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" [4]. Prebiotics are intended to modify the intestinal microbiota in such a way that bacterial activities advantageous to the host are stimulated and bacterial activities adverse to host health are suppressed. The concept of prebiotics arose from the observation that inulin and fructooligosaccharides selectively stimulate the growth of bifidobacteria [6, 7] which are considered to be beneficial for human health [4]. Although most research has been done on inulin and fructooligosaccharides, other non-digestible oligosac-

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charides (NDO) including xylooligosaccharides, galactooligosaccharides and isomaltooligosaccharides have also been tested for their prebiotic effect [8]. The majority of candidate prebiotics are oligosaccharides but also include polysaccharides (Table 1). To serve as a bacterial substrate in the colon, a prebiotic may not be hydrolyzed or absorbed in the upper part of the gastrointestinal tract.

Dietary fiber

Dietary fiber is a normal constituent of most foods derived from plants. However, the proportion of dietary fiber in a wholesome diet is higher than in a typical Western diet. Dietary fiber escapes digestion in the small intestine and passes into the colon. While certain dietary fibers undergo hydrolysis and fermentation by colonic bacteria, others remain unfermented and are subsequently excreted in the feces. Dietary fibers undergoing bacterial degradation include polysaccharides such as resistant starch, pectin, inulin, guar gum and oligosaccharides [9]. In contrast, structural polysaccharides such as cellulose and lignin, which are usually components of a complex fiber structure, are insoluble in water and not or hardly degraded by intestinal bacteria during their passage through the colon. Wheat bran, for instance, belongs to the latter category because it is composed of a mixture of complex polysaccharides combined in a supramolecular structure. In general, both fermentable and non-fermentable fibers have a bulking effect, which results in an increased fecal output. In case of non-fermentable fiber, the extent of bulking depends on the inherent mass or water-holding capacity [10]. In contrast, fermentable fibers result in an increase of the bacterial biomass which leads to an increase in stool fecal output.

Epidemiological studies indicate that a high intake of vegetables and fruits is associated with a decreased risk of colorectal cancer [1]. Besides micronutrients (vitamins C, D, E, calcium, selenium and β-carotene), glucosinolates, lignans and flavonoids, dietary fiber has been implicated as protective agent in fruit and vegetables against colorectal cancer [10]. Based on these data the German Nutrition Society (Deutsche Gesellschaft für Ernährung: DGE) recommends the daily intake of at least 30 g of dietary fiber. This recommendation has been upheld in spite of seemingly conflicting results from the so-called "Nurses Health Study" [11].

Intestinal microflora

Throughout lifetime the microorganisms in the gastrointestinal tract affect the host in many ways including the modulation of the immune system, the production of short-chain fatty acids and gases (H_2, CO_2 and CH_4) [12], the transformation of bile acids [13], the formation of vitamins [14], but also the potential formation of toxic, mutagenic and carcinogenic substances [15, 16]. The microbial populations in the intestine are thought to protect the host from adherence and subsequent invasion by pathogenic bacteria [17]. Moreover, the intestinal flora is a major determinant for the development of the immune system [18]. Although the major bacterial population groups can be found in every individual there are distinct differences at the species level between different persons. Thus it can be stated that each individual has his/her unique microflora.

The bacterial populations in the gastrointestinal tract differ from section to section because the environmental conditions change along this route [14]. In healthy subjects the stomach and the duodenum are only sparsely populated (10^1 and 10^3 cells/ml) because the secretion of HCl into the stomach may result in a pH as low as 2 in the stomach and 4 in the duodenum. The bacterial population density increases in the more distal parts of the small intestine (10^4 to 10^8) and even further in the colon reaching a maximal concentration of up to 10^{12} cells/g contents. The bacterial population groups encountered in the various sections of the gastrointestinal tract also differ with respect to their diversity and numerical importance [19]. It can be assumed that the bacteria have adapted optimally to the conditions prevail-

Table 1 Prebiotics and candidate prebiotics

Compound	Composition	DP*
Xylo-oligosaccharides	β (1→4) linked xylose moieties	2–4
Soybean oligosaccharides	Raffinose (F-Gal-G) and stachyose (F-Gal-Gal) mixtures	3–4
Transgalactosylated oligosaccharides	6′ Galactosyllactose	2–8
Palatinose condensates	Enzymatically rearranged sucrose molecules	2–7
Isomaltooligosaccharides	Transgalactosylation of maltose	2–8
Inulin	β (2→1) Fructans	2–65
Oligofructose	β (2→1) Fructans	2–8
Lactulose (Bifiteral®)	Galactosyl-β (4→1) fructose	3–5

*DP** Degree of polymerization

ing in the respective section. The colon is the preferred site of bacterial fermentation.

For the gut microflora, non-digestible carbohydrates of plant origin are the main substrates and include resistant starch as well as non-starch polysaccharides such as cellulose, hemicellulose, pectin and inulin [20] which are referred to as dietary fiber. However, the extent of microbial breakdown of dietary fibers differs: It depends on the matrix and the type of polysaccharides. For example, purified wood cellulose is not broken down by the intestinal microflora, while a considerable proportion of the cellulose in cabbage is degraded [21]. Moreover, considerable amounts of dietary protein escape digestion and also pancreatic enzymes and other gastrointestinal secretions serve as bacterial substrates [22]. Other principal substrates of the gut microflora include mucus, sloughed epithelial cells, and lysed bacteria (Table 2). These substrates provide carbon and energy for growth of the gut microorganisms. The degradation of these and a large variety of other substrates is brought about by the concerted action of an estimated number of several hundred bacterial species. This complex assemblage of diverse microorganisms has a great metabolic potential. The fermentation of carbohydrates occurs primarily in the proximal colon, while proteins are fermented preferentially in the distal colon. The latter results in the formation of the characteristic branched chain fatty acids such as isobutyrate, methylbutyrate and isovalerate [23]. The amount of branched chain fatty acids formed has been used as a measure for the amount of protein fermented by intestinal bacteria [24]. Some metabolites resulting from the bacterial breakdown of proteins in the large bowel may be considered as potentially adverse to health: NH_3, skatol, indol, cresol and H_2S [25–27]. The intestinal microflora may also be involved in the generation of toxic, mutagenic and carcinogenic substances: Numerous bacterial species in the human intestinal tract are capable of hydrolyzing sulfated or glucuronated compounds that are secreted into the intestinal tract via the bile, thus resulting in the enterohepatic circulation of potentially toxic or mutagenic compounds [28]. Bacterial activity may also be responsible for the hydrolysis of plant glycosides in the gut [29]. The resulting products may be more toxic or mutagenic than the mother compound. However, the transformation by intestinal bacteria of secondary plant metabolites may also result in compounds with possible health-promoting properties. For example, the plant lignans secoisolariciresinol or matairesinol need to be transformed by intestinal bacteria to the so-called mammalian lignans enterodiol and enterolactone to be effective [30].

Table 2 Substrates available for fermentation in the human colon [9]

Substrates	Amount (g/day)
Carbohydrates:	
Resistant starches	8–40
Non-starch polysaccharides (= dietary fiber)	8–18
– Cellulose	
– Hemicellulose	
– Pectin	
– Inulin	
Unabsorbed sugars and sugar alcohols	2–10
Chitin and amino sugars	1–2
Synthetic carbohydrates	*
– Lactulose	
– Lactitol	
– Polydextrose	
Nitrogenous compounds:	
Dietary protein	3–12
Pancreatic enzymes and other GI secretions	4–6
Urea, nitrate	0.5
Others:	
Mucus	2–3
Bacterial recycling	?
Sloughed epithelial cells	?

* Small on average but variable in individuals

The bacterial metabolism in the human colon is primarily anaerobic, because more than 99 % of the bacteria encountered in an adult's fecal flora are strict anaerobes [31]. The available substrates are broken down to the short chain fatty acids acetate, propionate, butyrate and the gases hydrogen (H_2) and carbon dioxide (CO_2). Formate, valerate and caproate are formed in small amounts only. The total concentration of short chain fatty acids in the colon ranges between 70 and 100 mmol/l [32]. Lactate, ethanol and succinate are intermediates which are also converted to short chain fatty acids [33]. While acetate and propionate are absorbed and transported to the liver for gluconeogenesis (propionate) or to various tissues as a fuel (acetate), butyrate is oxidized by the colonic epithelium [32]. Butyrate is not only the preferred fuel of the colonic epithelial cells but is also presumed to play a major role in the regulation of cell proliferation and differentiation [34, 35].

H_2 is a major electron sink product of the colonic microbiota. It is formed during the breakdown of fermentable substrates [33]. The amount of hydrogen formed depends on the diet consumed. In a systematic comparison fecal slurries were incubated with various carbohydrates and the formation rate of hydrogen was quantified and decreased in the following sequence (in mmol $\times$ [g feces $\times$ h]$^{-1}$): lactose: 3.3; lactulose: 2.8; mucin: 0.7; starch: 0.7; pectin: 0.3 [36]. The rate of H_2 formation is an indirect measure of the speed of fermentation of the respective carbohydrate. The H_2 excreted in breath or flatus is only 10 % of the amount originally formed [37]. The rest is utilized by methanogenic, acetogenic or sulfate-reducing microorganisms [38]. Methanogenic archaea catalyze the reduction of CO_2 with H_2 to methane (CH_4) [39] while acetogens reduce CO_2 with H_2 to acetate [40]; sulfate-reducing bacteria re-

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duce sulfate with H_2 to hydrogen sulfide [41]. All of these processes afford a considerable reduction in the amount of hydrogen gas formed during fermentation. Which of these three processes is responsible for H_2 oxidation differs. In only 30–40 % of the Europeans does methanogenesis play a role as a H_2 sink, while the rate in South Africans is 80 % [42, 43].

Relationship of prebiotics, microflora and health

Based on the important role of the intestinal microflora for human health, there is currently considerable interest in altering the composition and metabolic activity of this microbial community in such a way that beneficial activities are enhanced and detrimental activities are suppressed [44, 45]. Attempts have been made to increase bifidobacteria and lactobacilli which many consider to be health-promoting bacterial groups. One strategy to meet this objective is the consumption of fermented milk products that contain bacterial strains which have been selected for certain beneficial properties and are called probiotics [3]. Another dietary strategy is aimed to stimulate the growth of potentially health-promoting bacterial populations that are already resident in the colon. Since it was first demonstrated that inulin and oligofructose selectively stimulate bifidobacteria [6], the term prebiotics was coined and a number of mostly non-digestible oligosaccharides have been proposed to be prebiotics (Table 1). The classification of a food ingredient as a prebiotic requires that 1) it is neither hydrolyzed nor absorbed in the upper part of the intestinal tract, 2) is selective for a limited number of endogenous, and thereby 3) is able to alter the intestinal microbiota in favor of a healthier composition, and 4) induce luminal or systemic effects that are beneficial to the host health (Gibson and Roberfroid 1995). The stimulation of bifidobacteria and lactobacilli has been considered as advantageous because of their purported properties which include the action as immunomodulaters, the inhibition of the growth potential of pathogens, the reduction of ammonia formation, the lowering of blood cholesterol levels and the restoration of the normal flora during antibiotic therapy [3].

Non-digestible oligosaccharides such as oligofructose or lactulose have a number of common properties [8]. They highly stimulate bacterial fermentation and growth in the proximal colon resulting in the formation of short chain fatty acids and the decrease in the luminal pH. A low pH may stimulate the growth of lactobacilli and bifidobacteria which are well adapted to low pH. While a low pH suppresses undesirable bacteria, short chain fatty acids may play an important role for the optimal functioning of the colonic epithelium and the absorption of various cations including Ca^{2+}, Mg^{2+} and Fe^{2+}. There are also indications for a role of SCFA in the regulation of lipid and cholesterol metabolism. The effects postulated for non-digestible oligosaccharides have been assessed recently in a consensus paper (Table 3). Recent animal experiments have shown that the application of lactulose or preparations of *Lactobacillus reuteri* prevent colitis in interleukin 10 gene-deficient mice [46].

Conclusions

Approximately 10^{14} bacterial cells make up the bacterial community in the human gastrointestinal tract. The modulation of this microbial community through prebiotics may be a great opportunity to support human health and well-being. A number of preliminary results from investigations on prebiotic effects are promising. However, some health claims made for prebiotics need to be verified by human trials [47]. In view of the fact that a diet rich in fruit and vegetables contains a wide range of dietary fiber, it is questionable whether people consuming such a diet require an additional intake of prebiotics to obtain optimal health effects. Many open questions have to be answered before any final statements on the value of prebiotics can be made.

Table 3 Consensus on effects of non-digestible oligosaccharides [8]

Effect	Evidence (in human subjects)
Prebiotic and interaction with intestinal flora	Strong
Regulation of bowel habit, stool bulking and increase in stool frequency	Strong
Increased mineral absorption	Promising
Impact on lipid metabolism	Preliminary, data still inconsistent
Colon cancer	No human data available (experimental animal; preliminary)

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