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The physiology of colonic metabolism. Possibilities for interventions with pre- and probiotics

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Summary The awareness is increasing that in the colon many metabolic processes take place in relation to the fermentation of our food, which might be relevant for health and disease. However, the relation between food, colon metabolism and health or disease is far from clear. In this overview, the physiology of colonic metabolism and possibilities for its modification by the use of pre- and probiotics are discussed. Results of *in vitro* and animal studies indicate a beneficial impact of probiotics on adverse metabolic processes in the colon, but confirmation in human studies has to be extended. The administration of prebiotics seems to be promising with regard to their capacity to modulate the bacterial composition in the colon and there are indications that prebiotics can beneficially influence colonic metabolism. Whether these modulations brought about by pre- or probiotics have an effect on the health of the host, however, needs to be established in most cases.

Key words probiotics – prebiotics – colonic metabolism – intestinal microflora – non-digestible oligosaccharides

Introduction

The relationship between colonic metabolism and health has not been defined very well. This is partly due to the relative inaccessibility of the colonic epithelium and the diversity of the colonic bacterial metabolism. Due to the development of new methods to identify and quantify human bacterial flora, new challenges are created to establish the link between colonic metabolism and health. The colonic flora can be manipulated by prebiotics and probiotics as well as by indigestible food components (also discussed elsewhere in this supplement). In the following, the physiology of the colonic metabolism will be described and possibilities for its modulation by the use of pro- and prebiotics discussed.

Colonic metabolism

Components available for fermentation

The major part of our food is digested in the stomach and small intestine, facilitated by a large number of digestive enzymes. However, some of the food components escape digestion and become available for colonic metabolism. The efficiency of the digestion in the small intestine is influenced by many factors like the pH of the luminal content and the presence of competitive substrates and enzyme inhibitors. Also motility, affecting the time of interaction between substrates and enzymes/transporters, is variable and influenced by a number of factors (e. g., stress, physical activity) [1].

Normally, fats are digested by 90–95 %, which means that from a load of 70 g/day 3.5 g of fat enter the colon. The digestion of proteins is less complete; it is estimated that about 3–9 g pass into the colon daily [2]. The various carbohydrates are digested to different degrees. Monosaccharides are absorbed efficiently (> 90 %) when consumed in physiological amounts. The absorption of fructose is limited when it is present in excess of glucose [3, 4]. Most disaccharides are digested well. Lactose, an important energy source for new-born and young infants, is digested and absorbed efficiently during the first few years. In adults with lactase non-persistence this is reduced to about 10 % – 50 % of the level normally found in infants [5, 6]. Oligosaccharides present in daily food are galacto-oligosaccharides (e. g., raffinose and stachyose in beans) and fructo-oligosaccharides (e. g., in wheat, onion, bananas). They are not digested due to their chemical composition and are fermented in the colon. Recent studies have shown that human milk contains fucosylated and sialyated lactose-derived oligosaccharides (e. g., lacto-*N*-fucopentaoses, sialyl-lacto-*N*-tetraose) that are apparently not digestible and seem to beneficially contribute to the health of breast-fed infants [7, 8]. A number of factors affect the biological availability of the polysaccharides. The availability of starch (α-glycosides) depends on its pre-treatment (baking, cooking, subsequent cooling) and its physical form or chemical structure [9]. It is estimated that in a Western diet about 10 % of all starch is not digested. This means that approximately 10–30 g of resistant starch is passing into the colon daily [2]. Sources of resistant starch are cereals, legumes and potatoes that are cooled after cooking. Polymers of the β-glucosides (dietary fiber or non-starch polysaccharides) are not digestible because they cannot be broken down by pancreatic α-amylase. Dietary fiber is a normal constituent of most foods derived from plants. Other food components becoming available as substrates in the colon are minerals/trace elements, vitamins and bioactive components (redox influencing factors) which also might influence colonic metabolism. Besides food components, digestive proteins (enzymes), mucus and desquamated intestinal cells enter the colon [10]. The amount of enzymes and mucus entering the colon is estimated to be 4–6 g/day and 2–3 g/day, respectively [2].

Colonic microflora

A gradient of bacterial density exists along the gastrointestinal tract. The effects of the fermentative activity of bacteria in the small intestine are generally neglected. This might be justified because of the relatively small number, but with respect to the more strategic position of the bacteria towards substrates this might be a misjudgement.

The bacterial mass is the highest in the caecum (10^{11} cells per gram of contents) [2, 11, 12] where the undigested material enters the colon. Therefore the caecum is the most important part of the colon with respect to bacterial metabolism, followed by the colon ascendens, colon transversum and the colon descendens. Due to differences in substrate availability, the bacterial populations in the various sections of the colon differ with respect to its diversity and numerical importance of the diverse species [13]. To fully understand the role of the colonic microflora in health and disease, a number of questions need to be addressed: which bacteria are present in the human colon, how do they interact and what are their activities?

For detailed characterization of the colonic microflora, molecular techniques are now available.

The classical method to determine the quantitative composition of bacteria from colonic contents is culturing on suitable growth media. The sample is diluted and plated on a specific medium. The bacterial count of the original sample is then determined by multiplying the number of colonies that develop by the degree of dilution. There are two important problems using this technique. First, the bacterial count depends on the culturability of a bacterial species. Not all bacteria can be cultured and therefore this will lead to an underestimation of the quantitative contribution of certain genera. Second, specific media are not truly specific and certain bacterial species may be counted more than once on different specific growth media. This may lead to an overestimation of the quantitative contribution of certain genera. The net result is an inaccurate picture of the composition of the gut flora and this method is therefore not suitable to study population dynamics in the intestinal tract.

Advances in the field of molecular phylogeny have made it possible to study bacterial populations by a culture-independent approach. The methodology is based on the 16S ribosomal RNA (16S rRNA) [14]. Comparison of sequences of different bacterial 16S rRNAs (approximately 1500 nucleotides in length) shows that the molecule contains segments with different degrees of variability. This allows to construct phylogenetic trees which reveal evolutionary relationships between species [15, 16]. The different degree of variability also leads to another application of the sequence information. Currently, more than 20,000 16S rRNA sequences are available and this allows the design of DNA probes, usually consisting of 18–27 nucleotides, that hybridize with a particular sequence in the 16S rRNA molecule. Probes are available directed at different phylogenetic levels (Domain, Family, Genus, Species) of the bacterial kingdom. Fluorescent *in situ* hybridization (FISH) with fluorescently labeled 16S rRNA targeted oligonucleotides [17–21] is one of the techniques that can be employed to accurately determine the quantitative composition of the bacterial microflora of the colon. Quantification of positively hybridized bacteria can be performed by means of visual counting. To improve the speed and ac-

European Journal of Nutrition, Vol. 41, Supplement 1 (2002)

curacy, an automated microscopic counting procedure was developed [22].

Table 1 gives an overview of the quantitative contribution of various aerobic and anaerobic strains of bacteria to the microflora of the human colon determined by culturing and by FISH.

A second technique to study changes in microflora composition is denaturing gradient gel electrophoresis (DGGE). It is based on sequence-specific separation of 16S rDNA amplicons [23]. First, a variable part of the 16S rDNA is amplified by PCR with primers specific for all bacterial 16S rRNA (or rDNA). These amplicons are separated on a polyacrylamide gel containing a gradient of DNA-denaturing agents (urea/formamide) or on a temperature gradient. In this way, the amplicons are separated on the basis of their melting behavior and GC content. After electrophoresis the gel is stained, thus, producing fingerprints of the community present within the sample. Individual amplicons (bands) can be identified by comparing those with PCR products of pure cultures or, by excising the band from the gel, reamplifying it and analyzing its sequence. This technique has been applied successfully to study microbial populations in human fecal samples over time [24]. This study showed that the 16S rDNA-derived banding patterns were highly constant over a period of approximately half a year. Only some slight differences in the intensities of the bands were observed in individual patterns. This indicates that for healthy individuals the dominant microbial composition remains quite constant over time. The technique was also applied on fecal samples to identify bifidobacterial populations [25] and lactobacilli populations [26] using group-specific 16S rDNA primers. Besides these studies confirming the stability of the microbial compositions on a species level, they also show that with DGGE the changes in microflora composition due to modulation with pre- and probiotics can be monitored [27]. Although DGGE shows that the species composition is relatively stable, it does not give information on the relative numbers of the species present. It has been shown that these can fluctuate over time [17]. However, the advantage of the DGGE technique is that there can be a high throughput of samples, which can thus be screened for changes in the total microbial flora as a result of administering pre- or probiotics, even when these micro-organisms are not known yet.

The possibilities and limitations of these techniques are still not fully exploited. Development and application of these techniques might have a major impact on understanding colon food physiology and its modulation by pre- and probiotics.

Table 1 The normal fecal microflora of healthy adult humans

Genus	Bacteria per gram wet feces by culturing[a]		by FISH[b]
Non-sporing anaerobes			
Bacteroides spp.	10^{10}–10^{11}	including Prevotella	4×10^9–2×10^{10}
Bifidobacterium spp.	10^{10}–10^{11}		$< 10^7$–4×10^9
Atopobium group	nd[c]		8×10^8–1×10^{10}
Eubacterium spp.	10^9–10^{10}	E.cylindroides group	1×10^7–2×10^9
		E. rectale-C. coccoides group	3×10^9–1×10^{10}
		Eubacterium low G + C2	3×10^8–8×10^9
Propionibacterium spp.	10^9–10^{11}		nd
Veillonella spp.	10^5–10^8		$< 10^7$–1×10^8
Ruminococcus group	nd		2×10^8–1×10^{10}
Phascolarctobacterium group	nd		$< 10^7$–7×10^8
Sporing anaerobes			
Clostridium spp.	10^5–10^9	C. histolyticum group	$< 10^7$–2×10^8
		C. lituseburense group	$< 10^7$–4×10^7
Sporing aerobes			
Bacillus spp.	10^4–10^6		nd
Microaerophiles			
Lactobacillus spp.	10^7–10^9	lactobacilli-enterococci group	$< 10^7$–3×10^7
Streptococcus spp	10^7–10^9		$< 10^7$–5×10^7
Enterococci	10^5–10^7		nd
Facultative organisms			
Coliforms	10^7–10^9	Enterobacteriaceae	$< 10^7$–5×10^8
other Enterobacteria	10^5–10^9		

[a] from Role of Gut Bacteria in Human Toxicology and Pharmacology (M. J. Hill, Ed.) Taylor & Francis, London, 1995. [28]
[b] from Harmsen AEM 2002 [81], Franks 1998 [17] and unpublished results
[c] *nd* not determined

Fate of components in the colon

The undigested material entering the colon will be (partly) fermented leading to several metabolites. Fermentation products of resistant starch and water soluble fibers are gases like H_2, CO_2 and CH_4 and the short chain fatty acids (SCFAs) acetate, propionate and butyrate. An increased production of those acids leads to a decreased luminal pH. Both acetate and propionate are absorbed from the colonic lumen. Propionate is transported to the liver for gluconeogenesis and acetate to various tissues as a fuel. Butyrate is oxidized by the colonic epithelium. Proteins are fermented in the more distal part of the colon and are broken down to SCFAs, branched chain fatty acids (isobutyrate, methylbutyrate, isovalerate) and also to potential toxic agents like ammonia, amines, phenols and indoles [29–32]. Part of these metabolites are re-used again as a nitrogen source for bacterial growth. Another part of these products, however, will be taken up by colonocytes and transported into the blood stream. Little is known about the first pass metabolic capacity of the colonocytes and the clearance capacity of the liver of these metabolites. Fats are only partly metabolized in the colon. Toxic metabolites can be formed like hydroxy fatty acids from unsaturated fatty acids, diacylglycerol from triglycerides and lysophospholipids from phospholipids. Various phytohormones and phytosterols are metabolized in the colon leading to bioactive components which can be absorbed by the colonocytes.

It can be concluded that a variety of substrates, dependent on the diet consumed, become available for fermentation and affect the metabolic processes that take place in the colon. What are the consequences of this for the colon and ultimately for the health of the host? How can possible adverse effects be modified?

Effects of colonic metabolism on health and disease

Table 2 gives an overview of a number of metabolic processes resulting from the interaction of the bacterial population with the substrates present in the colon. These processes can have a beneficial or detrimental effect on the health of the host. However, the scientific evidence for these health- or disease-related effects is in many cases not (yet) well established.

Many studies are conducted to examine the possible beneficial effects of the formation of butyrate. 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 [33, 34]. This might have implications for the prevention of colon cancer and for nutrition in early life. Optimal levels of butyrate are not yet defined. Another example of a metabolic process with a possible health-promoting effect is the conversion and (re)absorption of phyto-estrogens (lignans and isoflavones) [35]. Bacterial metabolism of bile acids (deconjugation or dehydroxylation) might

Table 2 Metabolic processes in the colon and their disease- (**A**) or health-related (**B**) effects

A. Metabolic processes →	Harmful effects
Production of toxic metabolites Production of osmotic active substances	Induction of diarrhea
Formation of sulphide	Induction of colitis ulcerosa
Formation of toxic agents Formation of secondary bile acids Formation of hydroxy fatty acids and diacylglycerol Formation of nitrite	Induction of colon cancer
B. Metabolic processes →	Beneficial effects
Formation of short-chain-fatty acids	– Prevention of colon cancer – Proliferation of colonocytes – Contribution to energy metabolism and reduction of malnutrition
Vitamin production (B-complex, K)	Improving vitamin status
Nitrogen incorporation and fecal excretion	Treatment of hepatic encephalopathy
Phosphate incorporation and fecal excretion	Treatment of chronic kidney failure
Production of osmotic active substances	Treatment of constipation
Degradation of oxalate	Prevention of development of kidney stones
Removal of bile acids/neutral sterols	Reduction risk factors of cardiovascular disease
Conversion and (re)absorption of phyto-estrogens	Role in breast cancer and fertility

have impact on bile acid/cholesterol metabolism and is also linked to colorectal carcinogenesis [36]. An adverse effect is, for instance, also the changed extent of microbial degradation of taurine due to an alteration of the intestinal microflora as a consequence of a canned heat-processed diet in cats and dogs. This can lead to a deprivation of taurine in the animal [37, 38]. Various detrimental effects are related to the formation of toxic bacterial metabolites. Examples of potentially pathogenic processes are the formation of secondary bile acids and the conversion of nitrate to nitrite. Toxic bacterial metabolites can have an effect on the epithelial membrane (cytolytic effect) and effects inside the cell for instance the formation of DNA adducts.

Several factors determine whether colonic processes result in adverse health effects.

The production of potentially toxic metabolites. The substrate determines to a great degree which metabolites are produced (protein leading to amines, sulphur, etc.). To what extent the bacterial composition of the colonic flora plays a critical role in this process is not clear. In addition, harmless substances present in the colon (e. g., plant glycosides) can be converted to potentially toxic agents by colonic bacteria or the toxicity of a compound (e. g., nitrate, bile acids) can be increased by bacterial conversion [35].

Luminal concentration of toxic metabolites. A high flux of (non-fermented) dietary fiber increases fecal weight and decreases transit time which results in a decreased concentration of toxic metabolites and a reduced exposure of the colonic mucosa to the toxins.

Luminal removal of toxic metabolites. A higher rate of bacterial growth increases the demand on N- and S-sources and thereby these metabolites will be removed effectively. Little is known about the rate-limiting factors of bacterial growth in the colon. There is some evidence that removal of certain toxic compounds could be accomplished by binding to intestinal bacteria [39, 40].

Epithelial response to toxic metabolites. This comprises many processes like regulating uptake and release of toxic agents, intracellular biotransformation as well as apoptotic mechanisms.

An increased intake of dietary fiber is associated with a reduced risk of colorectal cancer [41] which is likely to be due to its effect on the luminal concentration of toxic metabolites, as stated above. A more direct effect on metabolic processes involved in toxin production or removal could be expected from interventions with pro- or prebiotics.

Manipulation of colonic metabolism

Interventions with probiotics

The most widely used definition of a probiotic is "a live microbial feed supplement which beneficially affects the host (animal) by improving its intestinal microbial balance" [42]. Recently probiotics were defined as "microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host" [43]. We prefer this extended definition because it also allows beneficial effects that are not necessarily related to an improved microbial balance to be regarded as probiotic effects. Furthermore, it includes effects brought about by microorganisms that do not survive the passage through the gastrointestinal tract. Microorganism mediated immune modulation, decrease in unfavorable metabolites and alleviation of symptoms of lactose intolerance are examples of effects that with this extended definition can now be termed correctly probiotic.

In two ways the proposed beneficial effects of probiotic microorganisms can be exerted:

- Bacteria or components of bacteria, that are not resistant to the gastrointestinal conditions (low pH, presence of bile, proteases) exert its effect in the small-intestinal tract (proposed mechanism involved in, e. g., improved lactose digestion [44]).
- Bacteria that are resistant to the digestive conditions arrive in the colon and compete with the endogenous flora.

Because we are dealing with positive and negative aspects for the host, it is tempting to classify the bacteria as "good" and "bad" [45]. Lactic acid producers as lactobacilli and bifidobacteria are considered to be "good" and clostridia to be "bad" bacteria. Enterococci, *E. coli* and bacteroides can belong to both categories. For categorizing bacteria, it is necessary to define the desired effect first. When butyrate production is required, butyrate producers can be considered as positive. For reducing the net ammonia production, other bacterial groups are most likely effective. Thus, good and bad might overlap considerably. Also, one has to be aware that many qualities might be strain- and not species-specific. Until now mainly lactic acid bacteria are considered as important probiotic microorganisms in food and nutrition [46]. But also other microorganisms might have probiotic properties, which was shown for, e. g., the yeast *Saccharomyces boulardii* [47, 48]. *Oxalobacter formigenes* is another probiotic candidate and the possibility to treat calcium-oxalate kidney stone disease by interventions with this microorganism is currently under investigation [49, 50].

Effects of probiotic interventions are commonly thought to depend on the adherence capacity of the administered strain to the colonic epithelium as prerequisite for the ability to colonize the human colon [51]. However, a discussion is ongoing whether there is a permanent bacterial flora with specific adherence sites to epithelial membranes or to biofilms and a transient flora susceptible to the flux of substrates. Recent results do not confirm the existence of a mucus or epithelial cell-adherent flora in the human colon [52]. Furthermore, the general concept at the moment is that the colonic flora tends to develop to a dynamic balance; within this balance only minor changes seem to take place [24]. Also, a well-balanced flora is thought to be able to prevent colonization by exogenous bacteria (colonization resistance) [53]. In line with this, there is currently no evidence that administration of probiotics results in colonization in humans, despite the apparent property of certain probiotic microorganisms to adhere to intestinal cells in vitro [54, 55]. However, some data suggest that colonization might not be required to exert probiotic effects [54, 55].

For an exact evaluation of probiotic effects, the techniques used to identify and quantify the colonic microflora are important: techniques with an increased sensitivity will reveal more subtle changes. Furthermore, the question as to what extent of change in bacterial numbers is relevant with respect to aimed health effects needs to be answered.

So far, it seems that administration of probiotics is most effective in cases of disturbed microbial balance. The risk and duration of antibiotic associated diarrhea is shown to be decreased by interventions with *S. boulardii* [44, 55] and *Lactobacillus rhamnosus* GG is able to reduce the duration of diarrhea due to rotavirus infection [57]. The beneficial impact of probiotics on human colonic metabolism and their colon cancer-preventing potential is less clear. Numerous studies were conducted to investigate the anti-carcinogenic activities of probiotic microorganisms. Their results were recently evaluated by several authors [58–60]: There is evidence from experimental and animal studies that lactic acid bacteria have the property to bind and degrade potential carcinogens and to produce antitumorigenic or antimutagenic compounds. Also, the metabolism of foreign compounds or endogenously produced agents could be altered by certain probiotics since a decrease of enzymes that are implicated in the carcinogenic process (e. g., β-glucuronidase, nitroreductase) in the human colon was observed. Whether this effect can contribute to a reduction of cancer rates has to be established. Furthermore, mechanisms observed *in vitro* or in animal models do not necessarily take place in humans [59, 35]. Therefore, despite the apparent beneficial impact of probiotics on certain adverse metabolic processes, there is no evidence yet that this could inhibit colon cancer development in humans. Possible effects on the immune response are discussed elsewhere in this supplement.

Interventions with prebiotics

Prebiotics are commonly defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, that can improve the host health" [45]. Screening of potential prebiotics is focused on the selective stimulation of the growth of lactic acid producing bacteria (especially bifidobacteria) because of their potential positive effects on human health [45]. Indeed, predominance of bifidobacteria in the colonic microflora of breast-fed infants is associated with prevention of diarrhea due to increased colonic resistance to pathogens [55]. However, health benefits of an increased bifidobacterial population in adults are less clear [61, 62]. Mostly short-chain carbohydrates (lactulose, fructo-, galacto-oligosaccharides, etc.) are examined for their prebiotic potential, but also saccharides with a higher degree of polymerization (inulin and resistant starches) [63, 64] are studied. The effects of lactulose are discussed elsewhere in this supplement. Inulin and fructooligosaccharides are most widely used and have been shown to stimulate the growth of bifidobacteria [65–67]. Since candidate prebiotics vary in molecular structure and chain length it is unlikely that they are fermented at equal rates and/or in the same part of the colon (proximal, distal). This could lead to specific metabolic and biological effects and might offer possibilities for modulating colonic metabolism at selected sites. Furthermore, some general effects on colonic processes of the various substrates are possible, like a change of the luminal pH due to an increased production of SCFAs or stimulation of the bacterial growth rate. A more acidic environment can result in a decreased activity of various pro-carcinogenic enzymes (e. g., 7α-hydroxylase, nitroreductase) [68]. Stimulation of colonic bacterial growth might lead to a reduction of toxic metabolites because nitrogen- and sulphur-sources are needed for new bacterial cell mass [69, 65]. Besides those general detoxifying effects, there is some evidence from animal studies that interventions with prebiotics might be able to suppress colon tumorigenesis. As result of administration of inulin and fructooligosaccharide the number of preneoplastic lesions (aberrant crypt foci: ACF), which are early indicators of risk of tumor development, was reduced in azoxymethane-treated rats [70, 71] and apoptosis in the colon of rats treated with 1,2-dimethylhydrazine was stimulated [72]. About the underlying mechanisms of these observations can so far only be speculated. It is suggested that the reduced ACF formation could be due to an increase in the number of bifidobacteria that may be able to bind carcinogens and to decrease the intestinal pH by the production of lactic acid [73] with the above mentioned positive effects on enzyme activity. Upregulation of apoptosis could be related to an increased concentration of butyrate. However, until now there are no human studies conducted to confirm the suggested inhibitory effects of inulin and fructooligosaccharides on carcinogenesis. Resistant starches were originally not considered prebiotics because of their alleged quality to stimulate the growth and/or metabolic activity of bacterial species "that are both potentially harmful and beneficial" [61]. In the meantime limited data suggest a prebiotic potential of resistant starches [64, 74]. Especially the capacity of certain types of resistant starch to stimulate butyrate production *in vitro* [75, 76] and *in vivo* [77–80] looks promising but certainly needs to be studied more extensively.

Thus, there are indications that administration of prebiotics could positively influence processes in the colon that are potentially harmful to the host. To what extent this modification impacts the health of the host still needs to be established in most cases.

Relevance for food physiology and health

It has become clear that in the colon many metabolic processes relevant for health and disease take place. These processes can be influenced by food components, but they can also be a target for pharmacological treatment and pre- and probiotics. Not only prevention of, for instance, diarrhea, constipation and colon cancer, could be achieved by manipulating colonic processes but also the treatment of various diseases (hepatic encephalopathy, diarrhea, constipation, atopic eczema) seems feasible. Beneficial effects of dietary fiber are known, but intake in the general population is lower than recommended. Therefore, the significance of modifying colonic processes by other means might be increased. Especially in situations of unbalanced microflora or in which genetically determined deviations of the normal large intestinal metabolism occur leading to an increased risk of disease, may the well-targeted manipulation of colon metabolism be relevant.

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European Journal of Nutrition, Vol. 41, Supplement 1 (2002)

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