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REVIEW

The impact of pre- and/or probiotics on human colonic metabolism: Does it affect human health?

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Since many years, the role of the colonic microbiota in maintaining the host's overall health and well-being has been recognized. Dietary modulation of the microbiota composition and activity has been achieved by the use of pre-, pro- and synbiotics. In this review, we will summarize the available evidence on the modification of bacterial metabolism by dietary intervention with pre-, pro- and synbiotics. Enhanced production of SCFA as a marker of increased saccharolytic fermentation is well documented in animal and *in vitro* studies. Decreased production of potentially toxic protein fermentation metabolites, such as sulfides, phenolic and indolic compounds, has been less frequently demonstrated. Besides, pre-, pro- and synbiotics also affect other metabolic pathways such as the deconjugation of secondary bile acids, bacterial enzyme activities and mineral absorption. Data from human studies are less conclusive. The emergence of new analytical techniques such as metabolite profiling has revealed new pathways affected by dietary intervention. However, an important challenge for current and future research is to relate changes in bacterial metabolism to concrete health benefits. Potential targets and expected benefits have been identified: reduced risk for the metabolic syndrome and prevention of colorectal cancer.

Keywords:
Carbohydrate and protein fermentation / Metabolomics / Prebiotics / Probiotics

1 Introduction

The available evidence on the impact of the gut microbial activity on human health is rapidly expanding and several host–microbiota interactions, positive as well as negative, have been reported [1, 2]. The human large intestine is recognized as one of the most metabolically active organs of the human body and the resident microbial ecosystem is extremely complex and dynamic with high densities of living bacteria [3]. The presence and metabolic activities of this specific bacterial community play an important role in maintaining the host's overall health and well-being, and has been shown to respond to metabolic challenges and dietary factors. Dietary modulation of the composition and activity of the microbiota has been achieved by the use of pre-, pro- and synbiotics. The potential health effects related to these substrates and the underlying mechanisms responsible for most effects have been extensively investigated. They can be roughly classified as modification of the host immune response and interference with the colonic microbial ecosystem, resulting in a modulation of the colonic bacterial metabolism.

This review focuses on the available evidence from animal and human studies, and *in vitro* models on the modification of bacterial metabolism by dietary intervention with pre-, pro- and synbiotics. Most often, a hypothesis-driven targeted approach, for example quantification of selected metabolites from carbohydrate (*e.g.* SCFA), has been used. More recently, metabolic profiling strategies

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Abbreviations: AXOS, arabinoxylan oligosaccharides; **FOS,** fructo-oligosaccharides; **HAMS,** high-amylose maize starch; **HBM,** human baby microbiota; **OF-IN,** oligofructose-enriched inulin; **RS,** resistant starch; **SHIME,** simulator of the human intestinal microbial ecosystem; **TIM,** TNO-intestinal model; **WBF,** wheat bran fiber

have been applied to characterize the metabolic effects of nutritional intervention.

2 Metabolic activity of the colonic microbiota

The intestinal microbiota comprises a diverse collection of microbial species that are mostly bacterial. It encompasses more than 400 different bacterial species and strains *per* individual, in a total concentration up to 10^{11} or 10^{12} cells/g luminal contents [4]. Metagenomic analyses show that in adults, the major constituents of the colonic microbiota are represented by the phyla *Bacteroidetes, Firmicutes, Actinobacteria* and *Proteobacteria* [5].

A number of factors influence the composition and the metabolic activity of the colonic microbiota (Table 1) [6]. Nutrient availability is believed to be the most important regulator of bacterial metabolism. Especially, the ratio of available carbohydrate to nitrogen determines the degree of saccharolytic *versus* proteolytic fermentation [7].

Dietary components that escape digestion by endogenous enzymes in the upper gastrointestinal tract become available for fermentation in the large intestine. It is estimated that about 20–60 g/day dietary carbohydrates (resistant starches (RS), dietary fibre, oligosaccharides and plant cell wall material), and 5–20 g/day protein pass into the colon [8, 9]. The digestion of fat is more complete (90–95%), which means that from a load of 70 g/day, 3.5 g fat enters the colon [10]. Besides food components, also pancreatic enzymes, mucus and desquamated intestinal cells enter the colon [11]. Overall outcomes of this complex metabolic activity are recovery of metabolic energy and absorbable substrates for the host, and supply of energy and nutritive products for bacterial growth and proliferation [12, 13].

In the proximal colon, substrates are abundantly available. The microbiota will preferentially ferment carbohydrates since it is energetically more favorable to produce ATP from carbohydrates than from proteins [14]. As a consequence, saccharolytic species are predominant in the proximal part of the colon. Along the length of the large intestine, the ratio of available carbohydrate to nitrogen progressively declines and bacterial composition changes towards a more proteolytic, methanogenic and sulfate-reducing type of microbiota [7, 8].

Table 1. Factors affecting metabolic activity and composition of the microbiota

Nutrient availability
Physicochemical properties of nutrients
Colonic transit time
Luminal pH
Age of the host
Local immunity
Individual bacterial fermentation strategies
Production of bacterial metabolites

2.1 Carbohydrate fermentation

The metabolic endpoint of carbohydrate fermentation is the generation of SCFA, predominantly acetate, propionate and butyrate [15]. Most of the SCFA formed by the intestinal bacteria are absorbed and metabolized, thereby contributing towards the host energy gain. Propionate is transported to the liver for gluconeogenesis and acetate to various tissues as a fuel. Butyrate is mainly oxidized by the colonic epithelium. Increased SCFA synthesis creates a more acidic environment in the gut [16], which enhances the colonization resistance against pathogens [17], reduces the formation of secondary bile acids [18] and impairs the activity of specific enzymes such as proteases [19]. Another important role of SCFAs on colonic physiology is their trophic effect on the intestinal epithelium. All three major SCFA stimulate epithelial cell proliferation and differentiation in the large and small bowel *in vivo* [20]. In addition, butyrate inhibits cell proliferation and stimulates cell differentiation in epithelial cell lines of neoplastic origin *in vitro* [21]. Furthermore, SCFAs have been shown to posses anti-inflammatory capacities, affect satiety hormones and play a role in insulin resistance [22]. A role for SCFA in prevention of some human pathological conditions such as ulcerative colitis and colon carcinogenesis has been presumed although conclusive evidence is still lacking.

2.2 Protein fermentation

Although the end products of carbohydrate metabolism are benign and of benefit to the host, some metabolites from proteolysis are potentially toxic. Proteolysis mainly occurs in the distal part of the colon where less substrate is available and the pH is close to neutral [7]. Anaerobic metabolism of peptides and proteins (putrefaction) by the microbiota produces SCFA and branched-chain fatty acids (isobutyrate, methylbutyrate and isovalerate) but, at the same time, it generates a series of potentially toxic substances including ammonia, amines, phenols, thiols and indoles [23, 24]. Some of these metabolites are reused as nitrogen source for bacterial growth, whereas others are taken up by colonocytes and transported into the blood stream. For instance, phenols and indoles are breakdown products of aromatic amino acids, tyrosine and tryptophan, respectively. Generally, these compounds enter the hepatic circulation to be detoxified in the liver and eventually excreted in urine.

3 Modulation of the colonic metabolism: pre-, pro- and synbiotics

Pre- and probiotics, as well as their combination, have been shown to influence the growth and/or metabolic activity of the gut microbiota and, thereby, its composition and functions [25]. Because of the beneficial effects of carbohydrate

fermentation and the generation of potentially toxic end products of protein fermentation, most dietary interventions are focused on enhancing the saccharolytic activity in the colon, while declining proteolysis. More specific, dietary factors may shift the balance of the gut microbiota away from potentially harmful or pathogenic bacteria such as clostridia, sulfate-reducers and certain *Bacteroides* species towards a predominance of potentially beneficial or health-promoting bacteria such as lactobacilli and bifidobacteria.

Probiotics are live organisms, which provide a benefit to the host when provided in adequate quantities [26]. Various lactic acid-producing lactobacilli, a number of bifidobacteria strains, the Gram-negative *Escherichia coli* strain Nissle 1917 and *Saccharomyces boulardii* are the primary micro-organisms classified and studied as probiotic agents. An alternative strategy to improve the balance of the microbiota lies in the use of prebiotics. A prebiotic is defined as a food ingredient that is not hydrolyzed by the human digestive enzymes in the upper gastrointestinal tract and beneficially affects 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 [27]. Examples are inulin-type fructans [28], RS [29] and arabinoxylan oligosaccharides (AXOS) [30]. For a dietary substrate to be classified as a prebiotic, the substrate must not be hydrolyzed or absorbed in the stomach or small intestine, it must be selectively fermented by beneficial commensal bacteria in the colon such as bifidobacteria and the fermentation of the substrate should induce beneficial luminal/systemic effects within the host [31]. 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) and thus various effects on colonic processes of the various substrates are possible. Hence, rather than introducing exogenous strains into an individual's gastrointestinal tract, prebiotics aim to stimulate the proliferation and activity of advantageous indigenous bacteria. A synbiotic is a mixture of probiotics and prebiotics that improves the survival and implantation of live microbial dietary supplements in the gastro-intestinal tract [6]. Survivability, colonization and the beneficial effects of feeding an exogenous probiotic may be enhanced and extended by simultaneous administration of a prebiotic which can be used by the probiotic within the intestinal tract.

4 Evidence of pre- pro- and synbiotics effects on metabolic pathways in the colon

4.1 Saccharolytic *versus* proteolytic fermentation

A series of human, animal and *in vitro* studies investigating the effect of dietary interventions on carbohydrate and protein fermentation have been carried out in recent years. Because of the inaccessibility of the human colon, *in situ* studies are difficult and most information about the luminal bacterial processes has been obtained from the analysis of breath (carbon dioxide, hydrogen and methane), urine and mainly faeces. Since faecal samples form the end product of colonic fermentation, their analysis probably reflects only the metabolic processes occurring in the distal colon rather than in the more proximal parts. In most human studies, targeted hypothesis-driven approaches have mainly focused on the quantification of SCFA in fecal samples.

4.2 Prebiotic intervention studies

Numerous studies investigated the production of SCFA, as a marker of enhanced saccharolytic fermentation, in response to prebiotic treatment. The production rate of SCFA is often considered as an indication of beneficial bacterial activity [32].

In vitro SCFA production can be measured using fecal or digesta homogenates, or using models of the large intestine, such as the simulator of the human intestinal microbial ecosystem (SHIME, University of Gent, Belgium) [33] or the TNO-intestinal model (TIM-2, University of Wageningen, The Netherlands) [34]. The SHIME consist of a series of five temperature and pH-controlled vessels that simulate the stomach, small intestine, ascending, transverse and descending colon, respectively. The SHIME harbors a microbial community resembling that from the human colon both in fermentation activity and in composition. This model has been applied to investigate the effects of prebiotics on microbiota composition and fermentation properties. The TIM-2 model is a more sophisticated *in vitro* model of fermentation in the proximal large intestine. It consists of a series of linked glass vessels containing flexible walls, which allows stimulation of peristalsis. The vessels are further equipped with a hollow fibre membrane in the lumen to stimulate absorption of water and SCFA. However, the relationship to the situation *in vivo* is equivocal. *In vivo* studies need to be performed to definitively demonstrate that the compound under investigation confers a health benefit to the host.

Using fecal inocula incubation, Cardelle-Cobas *et al.* [35] and Hernot *et al.* [36] demonstrated an increased SCFA production after addition of galactooligosaccharides or different fructans, respectively. Grootaert *et al.* and Van de Wiele *et al.* found comparable increases in SCFA production using the SHIME after fermentation of inulin and AXOS, respectively [37, 38]. Application of the TIM-2 to study the effect of five maize-based fibres demonstrated higher yields of SCFA compared with placebo [39]. *In vitro* studies have shown that different substrates yield various SCFA patterns. Starches yield high proportions of butyrate [40], whereas oligofructose results in higher molar ratios of propionate and butyrate than lactulose [35].

Animal studies, principally in rats, pigs, chickens and dogs, have shown that either total SCFA concentrations or butyrate concentrations are increased in the cecal contents or feces by provision of fermentable carbohydrates such as RS [36, 41, 42], oligosaccharides [25, 43], inulin [41, 44–47], AXOS [47] and wheat or oat bran [48, 49].

Controlled human studies are generally limited to fecal measurements of SCFA. Increased fecal SCFA concentrations have been observed after consumption of RS [25, 50, 51]. However, several other studies did not reveal changes in SCFA concentrations upon fermentable carbohydrate consumption [52–56]. The difference can be explained by the fact that fecal samples mainly represent metabolic processes in the distal colon since more than 90% of SCFA produced in the colonic lumen are directly absorbed through the colonic wall [8]. Changes in luminal SCFA concentrations might reflect the rate and/or the site of fermentation rather than the total SCFA production. Stable isotope technology, in which labeled carbohydrates are consumed and metabolites monitored in blood, urine and/or breath may form a suitable and noninvasive alternative to evaluate SCFA production *in vivo*. Recently, Verbeke *et al.* [57] evaluated the kinetics of SCFA appearance in the systemic circulation of ^{13}C-labeled barley.

In contrast to the number of studies measuring the increase in SCFA production, only a few studies focused on the capacity of prebiotics to decrease the proteolytic fermentation. Mechanisms leading to reduced protein fermentation can be explained by (i) the rapid fermentation of the substrate in the colon resulting in a lower colonic pH which reduces the hydrolysis of proteins, peptides and amino acids by bacterial proteases which have a neutral pH optimum [23], (ii) a process of so-called catabolite repression which implicates that the transcription of genes involved in the amino acids metabolism is repressed in the presence of carbohydrates resulting in an inhibition of the deamination of amino acids [58] and (iii) an enhanced uptake of amino acid and intermediary metabolites for bacterial biosynthesis [59]. The impact of dietary interventions on protein fermentation has been mainly evidenced from the excretion of phenolic compounds or ammonia.

Studies in human volunteers demonstrated that the presence of nondigestible carbohydrates resulted in a lower urinary excretion of phenols [60–63]. Acarbose, a substrate that decreases carbohydrate assimilation in the small intestine through inhibition of small intestinal α-glucosidase inhibitors, decreased serum levels and urinary excretion of *p*-cresol [64]. Other studies have reported only on the effect of fermentable fiber on the fecal excretion of phenols [65, 66]. However, contradictory results were noted on the effect of dietary intervention in human and animal studies on the fecal concentrations of phenols [44, 55, 65, 66]. In a recent phase I/II trial, *p*-cresol generation and *p*-cresyl sulfate serum concentrations were lowered in hemodialysis patients by the ingestion of oligofructose-enriched inulin (OF-IN) [67]. Chronic kidney disease is a progressive loss in renal function over a period of months or years. As renal function declines, substances that are either excreted or metabolized by the kidney accumulate, resulting in increased blood concentrations of numerous molecules [68]. A number of these retention solutes originate from colonic bacterial metabolism. In addition, small intestinal assimilation of proteins is impaired in renal failure, resulting in an increased availability of proteins for fermentation in the colon [69]. Accumulation of some of those metabolites in serum has been associated to increased morbidity and mortality [70]. As a consequence, a dietary strategy, such as prebiotics, that contributes to a lower generation of protein fermentation metabolites might constitute a significant improvement in the management of those patients.

Studies carried out *in vitro* [71] and *in vivo* in both animals [46, 72, 73] and humans [74] showed that the presence of fermentable carbohydrates was able to lower fecal ammonia concentrations. Further evaluation of the effect of fermentable carbohydrates on the ammonia–nitrogen metabolism in the colon in animals [75, 76] and humans [77] demonstrated a shift of nitrogen excretion from urine to feces, which was explained by increased bacterial protein synthesis and a subsequent decrease in colonic absorption of nitrogen in the form of ammonia. The removal of ammonia from the colonic lumen might be considered as a health benefit since it prevents ammonia from damaging colonocytes [78]. Initial studies found an increase in fecal nitrogen excretion without a concomitant decrease in urinary nitrogen excretion [65, 79]. Recently, using lactose-[^{15}N,^{15}N]-ureide, a stable isotope-labeled biomarker to study the fate of ammonia *in vivo*, it was shown that prebiotic intake decreased the urinary ^{15}N excretion, which was correlated with a significant increase in fecal ^{15}N excretion. Further fractionation of the fecal samples into fiber, soluble and bacterial fractions demonstrated that the increased fecal ^{15}N excretion was due to an increased incorporation of the biomarker into the bacteria [60, 80–82]. In general, fermentable carbohydrates stimulate bacterial proliferation which leads to incorporation of nitrogen (from ammonia and other sources) into bacterial cell walls and consequent excretion in feces [83].

4.3 Probiotic intervention studies

Compared with the well-documented prebiotic effects on colonic saccharolytic activity, and to a lesser extent on proteolytic activity, the number of *in vitro* and *in vivo* studies, addressing the effects of probiotics on colonic microbial activity, is limited. Probiotic administration may also influence the formation of SCFA. Analogously to prebiotics, probiotics may stimulate bacterial activity in the gut, resulting in an increased uptake of nitrogen, amino acids or metabolic products into the bacterial fraction [84]. More studies on the effect of probiotics on saccharolytic and proteolytic processes in the colon are warranted. Probiotics

mainly exert probably an indirect effect on carbohydrate and protein fermentation. Probiotics enrich the population of gut microbiota species that preferentially ferment carbohydrates and have little proteolytic activity. As compared with prebiotics, the extent of the probiotic effects on the colonic saccharolytic and proteolytic activity is generally less pronounced.

Recently, Meimandipour *et al.* [85] assessed *in vitro* in cecal broiler contents, the probiotic effects of *Lactobacillus agilis* JCM 1048 and *L. salivarius* ssp. salicinius JCM 1230 on the microbiota and metabolic end products. The supplementation of both lactobacilli significantly increased the production of lactate, propionate and butyrate in a 24-h batch-culture incubation. In the study of Sakata *et al.* [86], four different probiotic combinations were added to diluted pig cecal contents for batch-culture and continuous-culture incubation. SCFA production was increased as well as lactate and succinate. They attribute the effect to the increased breakdown of available carbohydrate in the presence of probiotic bacteria.

In humans, the effects of probiotics on proteolytic and saccharolytic processes have been inconclusive. Two placebo-controlled, double-blinded, randomized crossover trial in healthy subjects, investigating the effect of *L. plantarum* 299v and a combination of *L. acidophilus* 74-2 and *Bifidobacterium animalis* subsp *lactis* DGCC 420, respectively, reported no changes in fecal SCFA [87, 88]. Treatment with the probiotic yeast *S. boulardii* for 6 days did not change fecal SCFA concentrations in healthy subjects [89], but increased the fecal SCFA levels in patients on long-term enteral nutrition. Very little is reported on the use of probiotics to lower metabolites of protein fermentation in humans. De Preter *et al.* [60] demonstrated in a randomized, placebo-controlled, crossover study in healthy subjects that *L. casei* Shirota and *B. breve* (Yakult) significantly decreased urinary *p*-cresol and favorably affected the ammonia metabolism. Also, 4-wk *Lactobacillus GG* intake decreased the urinary *p*-cresol excretion [90].

4.4 Synbiotic intervention studies

The addition of *L. reuteri* to *in vitro* batch fermentation enhanced the SCFA production of inulin, but not of wheat dextrin and psyllium [91].

Bird *et al.* [92] compared the effect of high-amylose maize starch (HAMS) and fructo-oligosaccharides (FOS) on SCFA in pigs fed *B. animalis* for 7 days. A higher fecal excretion of SCFA was seen in pigs fed HAMS, whereas no effect was seen when fed FOS.

In a 4-wk randomized, blind, placebo controlled trial, premature infants were treated with either a dietary supplement containing two *Lactobacillus* species plus FOS, a supplement containing several species of lactobacilli and bifidobacteria plus FOS, or placebo twice daily. Neither weight gain nor stool SCFA content was different between groups [93]. A synbiotic combination of 2×10^9 CFU *L. acidophilus* NCFM and lactitol (5 g) tested in a randomized, double-blind placebo-controlled study in healthy elderly subjects did not significantly change the fecal SCFA levels [94].

Only one study evaluated the effect of a synbiotic preparation on metabolites of protein fermentation. A reduction in urinary *p*-cresol and ^{15}N, originating from lactose-[^{15}N,^{15}N]-ureide, was found in a 4-wk randomized, blind, placebo-controlled trial in healthy volunteers after treatment with *L. casei* Shirota ($2 \times 6.5 \times 10^9$ CFU) combined with OF-IN (2×10 g) [60]. Obviously, a higher dose of the prebiotic constituent was administered in this study as compared with the previously mentioned studies, which might explain the positive results.

4.4.1 Bacterial enzyme activity

Complementary information on the effect of dietary intervention on the modulation of the gut microbiota may be provided by the analysis of bacterial enzyme activities in the colon [95]. Hydrolysis of glycosidic bonds is one of the best-known examples of bacterial enzyme activity. The principal glycosidases are β-glucosidase and β-glucuronidase which hydrolyze the glycosidic bond of glycoside and glucuronide conjugates, respectively, to release aglycones, of which many are carcinogenic [96].

A number of animal and human studies have investigated the ability of pre- and probiotics to modulate the bacterial enzyme activity in the colon. In most studies, probiotic intervention significantly decreased the β-glucuronidase activity [90, 95, 97–99]. In two other studies, no effects of probiotic treatment of β-glucuronidase were observed [87, 100]. Concerning β-glucosidase, inconclusive results were found. Spanhaak *et al.* [98] found decreased levels of this enzyme after *L. casei* Shirota administration, whereas De Preter *et al.* [99] observed no changes. Differences in the effect of probiotic treatment may be explained by (i) the use of different lactobacilli or bifidobacteria strains as probiotic, (ii) the amount or (iii) the duration of the probiotic intake.

On the other hand, prebiotics have been shown to increase intestinal bifidobacteria concentrations, which produce low levels of β-glucuronidase, and to suppress fecal activities of carcinogen metabolizing enzymes in humans and rats [99, 101–103].

4.4.2 Bile acid metabolism

Bile acids are natural amphipathic detergents that emulsify and allow for the uptake lipids and fat-soluble vitamins. The human liver synthesizes the primary bile acids cholic acid and chenodeoxycholic acid by cytochrome p450-mediated oxidation of cholesterol and conjugates to either glycine or

taurine (bile salts). The primary bile salts are secreted in bile from the gallbladder into the small intestine during digestion. They are actively absorbed in the ileum and returned to the liver *via* the portal vein, a process known as the enterohepatic circulation. About 5% of bile salts escape the enterohepatic circulation and enter the colon where it is subject to bacterial biotransformation reactions. When cholate and chenodeoxycholate reach the colon, they are first deconjugated and successively undergo other enzymatic reactions, the most important being the dehydroxylation by bacterial 7α-dehydroxylase to form the secondary bile acids deoxycholic and lithocholic acid [104]. Secondary bile acids have been shown to be cocarcinogenic and tumor promotors [18, 105].

A number of *in vitro* studies on the effects of pre- and probiotics on bile acid metabolism have been performed. The formation of secondary bile acids by bacteria in batch cultures of fecal samples of human volunteers was inhibited when starch was simultaneously fermented [106]. Also, Zampa *et al.* [18] demonstrated in a semi-continuous culture with human fecal inoculum that incubation with starch and xylo-oligosaccharides decreases the rate of bile acid conversion. A negative correlation was observed between butyrate production and bile acid metabolism. In addition, the supplementation of the human fecal microbiota with lactobacilli and bifidobacteria also decreased the conversion rate.

In vivo, similar effect were observed in rats with other fermentable carbohydrate such as lactose, lactulose, amylomaize or potato starches demonstrating an inhibition of the dehydroxylation of primary bile acids [107]. In humans, a reduction of fecal deoxycholic acid concentration together with a significant increase in fecal excretion of butyrate and acetate has been observed in subjects whose standardized diets were supplemented with a HAMS [108]. The observed decreases are mediated through acidification of the large bowel by production of SCFA which inhibits conversion of primary to secondary bile acids.

However, in a clinical phase III trial investigating the effect of wheat bran fiber (WBF) for the prevention of adenomatous polyp recurrence, high WBF intake did not reduce the aqueous-phase concentrations of secondary bile acids in stool, although their concentrations in solid-phase stool were repressed. The inability of the high WBF intervention to reduce colorectal adenoma recurrence may be a consequence of its lack of effect on fecal aqueous-phase secondary bile acid concentrations [109].

4.4.3 Mineral absorption

Several studies in animals and humans have shown positive effects of prebiotics (inulin, oligofructose, FOS, galactooligosaccharides and RS) on mineral absorption. Increased uptake of minerals, in particular calcium and magnesium, has been observed in rat models [45], mice models [110] and healthy subjects [111, 112]. Several mechanisms may be responsible for the increased mineral absorption. Improved absorption may be indirectly caused by the lower luminal pH which improves the solubility of minerals [113, 114]. In an experimental animal study, the addition of FOS to the diet has been shown to increase the length of the villi and depth of the crypts, resulting in a larger absorptive surface of the colonic mucosa [115]. The underlying mechanism for this change in mucosal structure is probably the production of butyrate, which fuels the mucosal cells. A third possible reason for improved calcium absorption in the large intestine after FOS intake is an increased production of mucosal calcium-binding proteins [116]. Less evidence is available for a probiotic effect on facilitating mineral absorption.

4.4.4 Analysis of the metabolic activity with metabolomics

New techniques such as metabolomics allow to evaluate the colonic metabolism from a top-down approach bypassing the need for an *a priori* hypothesis. Metabolomics has been defined as ''the quantitative measurement of the multiparametric metabolic responses of a living system to pathophysiological stimuli or genetic modification'' [117]. With the emergence of ''meta-omics'' analyses in recent years, new insight in metabolic pathways in relation to health and disease will evolve. Metabolic profiling is a recently applied approach that might reveal new affected metabolic pathways and hold promise for the assessment of pre-, pro- and synbiotic functionality. In this way, new potential biomarkers of metabolic activity may be discovered.

The first article describing the effects of a probiotic combination using integrative metabolite profiling and modeling was by Martin *et al.* [118]. They described the probiotic modulation of the gut microbial–host metabolic interactions in a humanized microbiome mouse model. Mice inoculated with a human baby microbiota (HBM) received either 10^8 CFU/day *L. paracasei* or 10^8 CFU/day *L. rhamnosus* or placebo for 2 wk. Probiotic treatments decreased fecal acetate and butyrate production, whereas fecal concentrations of isobutyrate and isovalerate, and urinary excretion of phenolic and indolic compounds were increased. The probiotics supplementation of HBM mice was associated with a specific amino acid pattern that was linked to *L. paracasei* proteolytic activities. Furthermore, changes in lipid profiles, gluconeogenesis and amino acid and methylamine metabolism associated to fermentation of carbohydrates by different bacterial strains were observed. A similar study described the impact of 2 wk supplementation of a synbiotic containing 10^8 CFU/day *L. paracasei* and galactosyl-oligosaccharide prebiotics in HBM mice. Metabolic profiling of the mice feces showed higher levels of 5-aminovalerate, choline and acetate, whereas levels of valine, ornithine, glutamate and glycine decreased over time [119].

Vitali *et al.* [120] studied the impact of a 4-wk intake of the synbiotic combination of 500 mg FOS and the probiotic strains 10^9 CFU/day *L. helveticus* Bar13 and 10^9 CFU/day *B. longum* Bar33 on the total gut metabolite profile of healthy subjects. They found a significant increase in SCFA, ketones, carbon disulfide and methyl acetate. De Preter *et al.* [121] incubated fecal slurries with different doses of OF-IN in an *in vitro* model. Although a significant increase in SCFA levels was observed, this was not the key feature in distinguishing control samples from OF-IN-incubated samples. A more discriminatory factor for the prebiotic effect was an increase in the concentration and number of esters. Increased acid production might be the origin of a higher presence of esters. The relevance of esters to health is yet unknown. In addition, incubation of fecal slurries with OF-IN prevented the production of *S*-containing compounds and phenols in a dose- and time-dependent way. Similar metabolic effects have been observed *in vivo* (De Preter *et al.*, submitted).

5 Colonic metabolism: relevance for health

An important challenge for current and future research is to relate changes in bacterial metabolism to the improvement of human health. Although there is convincing evidence that pre- and probiotics can modulate colonic bacterial metabolism, the concrete health benefits associated to this modulated metabolism remain to be established. Most studies use changes in metabolic activity as a surrogate biomarker or end point for a health benefit, being either an improvement of a clinical condition or a reduction in disease risk.

An example of a clinical condition that might be prevented or improved by modulating bacterial colonic metabolism is the metabolic syndrome. The metabolic syndrome is a cluster of metabolic abnormalities characterized by abdominal obesity, impaired fasting glucose, dyslipidemia and elevated blood pressure, which confers an increased risk of developing type 2 diabetes mellitus and cardiovascular disease [122]. There is emerging evidence that SCFA not only exert effects in the colonic epithelial cells but also enter the circulation and can influence metabolic processes in other tissues and organs [123]. SCFA may provide further benefits for the systemic metabolism. For example, it has been shown that acetate and propionate can modify hepatic lipid metabolism. Propionate serves as a substrate for gluconeogenesis and may inhibit cholesterol synthesis, whereas acetate is utilized as a substrate for the synthesis of longer chain fatty acids [123]. Furthermore, SCFAs have been identified to be ligands for the orphan G protein-coupled receptor GPR41 which is primarily expressed in adipose tissue [124] and stimulation of leptin production in adipocytes by propionate (not acetate) has been described [125]. Since adipose tissue is known to secrete various signalling peptides influencing among others insulin sensitivity, feeding behaviour and inflammation [126], it could constitute an important link between SCFA production and peripheral metabolic effects [127]. In the case of colon cancer, there is evidence that the metabolic activity in the colon plays a role in risk of disease [128]. Increased production of butyrate, decreased production of protein fermentation metabolites and secondary bile salts and reduced activity of bacterial β-glucuronidase activity are all possible mechanisms that might contribute to reduced colon cancer risk [105]. Nevertheless, epidemiological studies have not been able to demonstrate an association between pre- and probiotics intake and a reduced risk. Also, patients with chronic kidney disease might benefit from a modulated colonic metabolism. Chronic renal failure is characterized by progressive retention of a number of microbial metabolic end products which are not eliminated due to kidney failure and which can hardly be removed using dialysis techniques because of their high protein binding [68]. As a consequence, strategies that contribute to a lower generation of protein fermentation metabolites might constitute a significant improvement in the management of those patients [67].

In future studies, biomarkers of colonic metabolism should be combined with the assessment of hard clinical end points, such as cardiovascular events, development of type 2 diabetes, chronic renal failure and incidence or recurrence of colorectal cancer.

6 Concluding remarks

The results described in this review indicate a beneficial impact of pre- and/or probiotics on the metabolic processes in the colon, but confirmation in human studies has to be extended. Furthermore, it needs to be established whether these changes confer a health benefit to the host.

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