Beyond butyrate

Fagundes, Raphael R; Belt, Saskia C.; Bakker, Barbara M; Dijkstra, Gerard; Harmsen, Hermie J M; Faber, Klaas Nico

Published in:
Trends in Microbiology

DOI:
10.1016/j.tim.2023.07.014

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Version created as part of publication process; publisher's layout; not normally made publicly available

Publication date:
2023

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 16-09-2023
Human gut bacteria produce metabolites that support energy and carbon metabolism of colonic epithelial cells. While butyrate is commonly considered the primary fuel, it alone cannot meet all the carbon requirements for cellular synthetic functions. Glucose, delivered via circulation or microbial metabolism, serves as a universal carbon source for synthetic processes like DNA, RNA, protein, and lipid production. Detailed knowledge of epithelial carbon and energy metabolism is particularly relevant for epithelial regeneration in digestive and metabolic diseases, such as inflammatory bowel disease and type 2 diabetes. Here, we review the production and role of different colonic microbial metabolites in energy and carbon metabolism of colonocytes, also critically evaluating the common perception that butyrate is the preferred fuel.

Introduction

All animals, including humans, harbor a sophisticated machinery for the digestion, uptake, and processing of food. Microorganisms in the digestive system, collectively called microbiota, play a crucial role in food processing. Simple sugars are obtained from the diet as monomers, dimers, or polymers, such as starches and structural carbohydrates. Polymeric carbohydrates derived from the diet need to be depolymerized to be absorbed by intestinal epithelial cells and enter systemic circulation (Figure 1). The human genome encodes 17 glycoside hydrolases to digest food-derived glycans, such as starch, sucrose, and lactose [1]. However, humans do not harbor endogenous enzymes capable of degrading many plant-specific complex carbohydrates, such as cellulose, hemicellulose, lignin, pectin, mucilage, and gum [2]. Fortunately, a large variety of microbial enzymes break down diet-derived indigestible carbohydrates, providing carbon and energy sources to the colonic mucosa.

Recent studies unraveling the metabolism of colonocytes revealed intriguing insights into the energy and carbon metabolism of these cells. Energy metabolism is the process of harvesting energy in the form of adenosine triphosphate (ATP) as a result of intracellular nutrient metabolism, such as aerobic respiration (oxidative phosphorylation), anaerobic respiration (glycolysis), and the metabolism of fatty acids and amino acids. Conversely, central carbon metabolism refers to the process in which sugars undergo a series of intricate enzymatic steps to form metabolic precursors, which are responsible for producing the biomass of a cell [3]. The intestinal epithelium has high energy demands for fast cell turnover and needs sugars for cellular functions like synthesizing DNA, RNA, proteins, and lipids. Butyrate is not a precursor for most of these building blocks; instead, colonic epithelial cells require other carbon sources from the blood or microbial metabolism in the gut.

The human microbiota consists of trillions of microbes, with high individual variation [4]. While the genomes of individual bacterial species encode many fewer genes than the human genome, the...
great diversity of the colonic bacteria harbors at least 100-fold more unique genes than the human genome [5]. Besides cross-feeding to the intestinal epithelium, the establishment of this symbiotic host–microbe relationship also plays an important role in immune development and host defense against pathogens [6,7]. An unbalanced gut microbiota composition (i.e., dysbiosis) is associated with a spectrum of diseases, including inflammatory bowel disease (IBD), colon cancer, metabolic-associated fatty liver disease, and diabetes mellitus, as well as cardiovascular and neurological diseases, often presenting a microbiome signature that is independent of co-morbidities [8–11]. Though causal relationships between dysbiosis and disease development still need to be established, it strongly links gut microbial health to human health.

Bacterial metabolites represent the majority of the fecal metabolome; these include alcohols, amino acids and derivatives, phenols and polyphenol derivatives, indoles and sulfides [12–14]. Short-chain fatty acids (SCFAs) are the most abundant bacterial metabolites in human feces and can be used by human cells as an energy source, but they also have anti-inflammatory effects, improve barrier function, and harbor anticarcinogenic properties, as reviewed in detail elsewhere [15]. To a lesser extent, lipids, such as phosphatidylcholine and acylcarnitine, and secondary bile acids (such as tauroursodeoxycholic acid and tauroliothocholic acid) are also part of the human fecal metabolome [16,17]. Furthermore, monosaccharides, the bilirubin metabolite stercobilin, and hundreds of soluble metabolites such as glycoside- and sulfate-conjugates have been reported [18,19]. Although the composition of the fecal metabolome has been extensively studied, it remains unclear how these metabolites contribute to the energetic demands of intestinal epithelial cells. In this review, we discuss the impact of metabolites from bacterial digestion of dietary fibers in the human gastrointestinal (GI) tract on intestinal epithelial energy metabolism and cellular proliferation, in particular SCFAs and monosaccharides.
Bacterial metabolism and cross-feeding in the intestine

Primary degraders
Enzymes that break down glycosidic bonds in complex carbohydrates are collectively named carbohydrate-active enzymes or CAZymes. Gut microbiome CAZymes can be subdivided into evolutionarily conserved families: glycoside hydrolases, polysaccharide lyases, carbohydrate esterases, and glycosyltransferases, the first two representing the vast majority of the microbial-derived CAZymes [20]. Numerous glycoside hydrolases are identified with different catalytic specificities, however, no single bacterial species encodes all enzymes to break down all polymeric carbohydrates [21]. In most cases, dietary fibers are first extracellularly degraded and the resulting pentoses and hexoses are subsequently taken up by bacteria and converted to pyruvate via the Embden–Meyerhof pathway and, in the case of bifidobacteria, the pentose phosphate pathway (Figure 1). Both of these pathways eventually produce pyruvate via fructose-6-phosphate. Pyruvate can then be converted into lactate, succinate, and/or acetyl-CoA. The latter yields CO₂ and formate as byproducts and, potentially, is further converted into acetate [21,22]. These steps are predominantly executed by primary degraders: bacterial species that can use polymeric carbohydrates as growth substrates. Despite the limitations to the concept of primary and secondary degraders, it presents a helpful framework for grasping the intricate network of bacterial interactions in the human colon.

It is important to note that complex carbohydrates in the colonic lumen originate not only from the diet. Mucins are produced by goblet cells in the intestinal epithelial lining and are composed of large complex glycoproteins containing a dense array of O-linked carbohydrates (70% of their mass) [23]. Akkermansia muciniphila, a member of the phylum Verrucomicrobia, is well known to degrade mucins in the human colon [24], encoding almost 200 CAZymes in its genome [25]. Several CAZymes with mucin-degrading activity have been reported [26], and glycoside hydrolases (GH16) were recently identified to be likely involved in mucin degradation by A. muciniphila [27]. The end-product of mucin degradation by glycoside hydrolases is acetate that, in turn, fuels the metabolism of secondary degraders, such as the butyrate-producer Faecalibacterium prausnitzii.

Secondary degraders
Other end-products from primary fermentation have been described, such as acetate, lactate, succinate, propionate, formate, CO₂, and H₂. These metabolites support the growth and metabolism of other bacterial species, which are collectively referred to as secondary degraders [28,29], and are generally subdivided into four groups: acetogens, sulfate-reducers, methanogens, and butyrate-producing bacteria. End-products of primary digestion can undergo secondary fermentation, yielding new molecules that become available in the colonic lumen, including, but not exclusively, SCFAs. Other described end-products from secondary fermentation are alcohols, CO₂, methane, H₂S, and H₂ [30]. The behavior of primary and secondary degraders is heavily affected by substrate availability and bacterial community diversity, as extensively reviewed elsewhere [31].

Acetogens generate acetate as end-products of their metabolism, generally applying the Wood–Ljungdahl pathway for the production of acetyl-CoA by reduction of CO₂ molecules [32]. Examples of acetogens include Blautia hydrogenotrophica and Marvinbryantia formatexigens [33]. Sulfate-reducers obtain energy from reduction of sulfates, generating H₂S as end-product. Sulfate-reducers identified in the human gut are Desulfovibrio species, and exacerbated production of H₂S is often associated with inflammation of the intestinal mucosa [34]. Methanogens reduce CO₂ in the presence of H₂, producing methane. Examples of prominent gut methanogens are Methanobrevibacter smithii, Methanosphaera stadtmanae and Methanomassiliicoccus luminyensis [35–38]. Butyrate-producing bacteria, including F. prausnitzii, Anaerostipes caccae and A. hallii, generate butyrate as an end-product, derived from digestion of food components in the digesta and metabolites produced by
fiber-degrading bacteria [39–41]. Butyrate production has also been shown to be enhanced by combining such primary and secondary degraders in vitro cocultures.

Many butyrate-producing bacteria require acetate for their growth, often an end-product from primary or acetogenic fermentation. Interestingly, in a study with 12 healthy individuals receiving colon delivery capsules containing defined amounts of $^{13}$C-labelled SCFAs, approximately 24% of the $^{13}$C-acetate in the colon is incorporated into butyrate. This was the most significant of all SCFA interconversions detected [42], which was also confirmed in a mouse model [43]. Another study on the production of butyrate from oligofructose, acetate, and lactate observed that most of the carbon in butyrate is derived from acetate [44]. Importantly, some butyrate-producing bacteria, such as *F. prausnitzii*, can also perform primary degradation of complex carbohydrates like inulin [45]. Furthermore, many intestinal bacterial species are not well-described, or not yet cultured in vitro, and their role as primary or secondary degraders remains obscure.

**Intestinal epithelial metabolism**

The abundance of monosaccharides in the human colon is often overlooked, as it is assumed that those are effectively absorbed in the small intestine or directly fermented by the bacteria [46]. Nevertheless, a recent study analyzed the luminal content metabolome along the human GI tract using an ingestible sampling device and found 31 metabolites >100-fold more abundant in the intestine, compared to stool samples. These included sugars, among other substances such as glycinated lipids and microbially conjugated bile acids [47]. Thus, microbial metabolism of indigestible fibers yields, besides SCFAs, an excess of sugars that may support epithelial growth. In the next subsections, we describe the capacity of intestinal epithelial cells to take up and utilize metabolites available in the gut lumen, specifically fatty acids and monosaccharides.

**SCFAs**

The majority of dietary lipids form an insoluble fraction and are composed of triacylglycerols, also called triglycerides (making up to 95% of lipids in the diet), with smaller amounts of phospholipids and sterols [48]. Insoluble lipids are mainly processed in the small intestine by pancreatic lipases and absorbed by small intestine enterocytes as free fatty acids and monoacylglycerols. These are used by enterocytes for fatty acid oxidation to generate ATP or are re-esterified to triacylglycerols again for storage or secretion into lymphatic circulation for redistribution to peripheral tissues [49,50]. In fact, high levels of dicarboxylic acids are detected along the upper human GI tract, pointing to a higher lipid catabolism of epithelial cells in the small intestine, compared to the colon [47]. In contrast, the lipid content in the colonic lumen is mostly composed of SCFAs.

The vast majority of the SCFAs produced in the colon are absorbed by the intestinal epithelium, and only the 5–10% remaining is excreted in feces [51]. Up to 70% of acetate and 50% of propionate produced in the human gut is transported and taken up by the liver and utilized to produce ATP via beta(β)-oxidation, or in the synthesis of cholesterol, ketone bodies, glutamate, glutamine, and glucose [52,53]. Butyrate concentrations in the colon vary from 10 and 25 mmol/l, and in the intestinal mucosa it peaks at the superficial epithelium and decreases towards the crypt base [54,55]. Butyrate concentrations in the peripheral blood were shown to be approximately 0.1% of the detected concentrations in the lumen of the large intestine [56,57]. SCFAs can enter the cell via passive diffusion [51,58], but the uptake is greatly increased by carrier-mediated anion transporters. The proton-coupled monocarboxylate-transporter-1 (MCT1/SLC16A1) and the sodium-coupled monocarboxylate transporter-1 (SMCT1/SLC5A8) are high-affinity transporters for butyrate [59,60]. The expression of MCT1 increases along the human intestine, with the highest mRNA and protein levels in the distal colon [61]. The levels of SLC5A8 mRNA are also highest in the human colon [62]. Furthermore, *in situ* hybridization analysis in mice showed that Slc5a8 mRNA
expression in the small intestine was restricted to the ileum [63]. Levels of Slc5a8 mRNA and SMCT1 protein are markedly reduced in the ileum and colon of germ-free (GF) mice and returned to normal levels after recolonization, suggesting a strong dependence of bacterial metabolism [64]. In line, butyrate enhances SLC16A1 expression in in vitro-cultured intestinal epithelial cells [65]. Finally, butyrate, and other SCFAs, are ligands for the G-protein-coupled receptors GPR41, GPR43, and GPR109A, activating anti-inflammatory signaling pathways in the intestinal epithelial, as well as immune cells and other tissues, as extensively reviewed elsewhere [15].

Experiments using stable isotopes showed that only a fraction (2–3%) of butyrate absorbed from the colon is detected in the plasma of healthy subjects [42]. By entering β-oxidation, butyrate increases oxygen consumption through oxidative phosphorylation [66–68]. Butyrate is transported into the mitochondria and converted to acetyl-CoA, which is then converted to acetyl-CoA and subsequently incorporated into the TCA cycle as citrate and to the electron transport chain to generate ATP (Figure 2). Butyrate has historically been considered a major ATP source for colonocytes. Nevertheless, the metabolism of butyrate yields acetyl-CoA as an intermediate, which per se is not adequate for the biosynthesis of the main building blocks of human cells, for example proteins, nucleic acids, and glycans, as we discuss later.

Monosaccharides
Colonocytes use fuel substrates from both luminal and blood origin. As discussed before, butyrate is often described the major luminal-derived substrate to the intestinal epithelial. Conversely, L-glutamine, L-glutamate, L-aspartate, and D-glucose are fuels for colonocytes coming from the arterial blood flow [69]. Though not much attention is given to gut-luminal monosaccharides as possible fuel for colonic epithelial cells, these cells express relevant uptake transporters for monosaccharide uptake throughout the colon. Apical transport of glucose and galactose is carried out by the Na+/glucose cotransporter 1 (SGLT1/SLC5A1) and glucose transporter type 2 (GLUT2/SLC2A2) [70]. These two transporters are mainly expressed in the human proximal small intestine for the absorption of simple sugars, but are also detected in the cecum, colon, and rectum, albeit at lower levels [71–73]. The uptake of monosaccharides by colonic intestinal epithelial cells is supported by in vivo studies. Colonic expression of Sglt1 is reduced in GF mice, and levels return back to normal after recolonization [64]. Together with knowledge that the expression of SGLT1 is directly regulated in response to the sugar content in diet [74], this suggests the possible uptake of glucose available from bacterial metabolism by colonic intestinal epithelial cells. In line, the uptake of glucose and 2-deoxyglucose was reduced twofold in GF mice compared to control mice [75]. Gene expression of SCL2A1, the gene encoding glucose transporter GLUT1 and predominantly present in erythrocytes, is also detected in the mouse and human colon and rectum [73,76].

The relevance of fructose transporters in the human colon is still unclear. Fructose transport is mainly carried out by GLUT5 (SLC2A5) and GLUT2 (SLC2A2) [77–79]. GLUT2 is a basolateral transporter mostly found in the small intestine, and its expression is not responsive to luminal fructose [76,80,81], although studies have shown that butyrate upregulates SLC2A2 mRNA expression in differentiated Caco2-BBBe cells in a dose-dependent manner [82]. Conversely, GLUT5 is situated at the apical membrane of intestinal epithelial cells and it has been shown to be upregulated by fructose [83,84]. The physiologic relevance of GLUT5 is illustrated by in vivo studies in which GLUT5 knockouts completely blocks transepithelial fructose transport [85,86]. Data publicly available at the Human Protein Atlas project shows SLC2A5 protein expression (Figure 3A,B) at the apical membrane of a subset of colonic intestinal epithelial cells and gene expression (Figure 3C), although low, is detectable (data obtained from https://v22.proteinatlas.org/ENSG00000142583-SLC2A5 and [87]). Moreover, we have recently found that GLUT5 expression is enhanced in intestinal epithelial cells as a consequence...
Fructose can also be transported via GLUT7 transporter (encoded by the gene $\text{SLC2A7}$), a hexose transporter found to be expressed in the colon of rats and upregulated by feeding a high-carbohydrate diet [88,89]. These observations support the notion that the colonic epithelium is adapted to absorb monosaccharides like glucose and fructose from the gut lumen.

Animal studies have reported luminal accumulation of monosaccharides in the distal intestines. Luminal glucose levels in the cecum of rats are low, but detectable (approximately 0.5–1.0 mmol/l) [84]. Pigs on a diet high in resistant starch showed increased colonic concentrations of monosaccharides, with levels around 15-fold higher than in pigs on a control diet low in resistant starch [90]. Moreover, many rapidly dividing cells change their metabolism from mitochondrial TCA cycle and

**Figure 2.** Schematic representation of host–bacterial cross-feeding in the human colon. Dietary fibers are firstly digested by primary degraders into monosaccharides that can be used as growth source for secondary degraders, such as the butyrate-producer Faecalibacterium prausnitzii. Glucose available in the lumen may enter intestinal epithelial cells by facilitated transport, after which it undergoes rapid glycolytic metabolism yielding pyruvate that can either be anaerobically metabolized into lactate or converted to acetyl-CoA in the mitochondria. Fructose is also transported into the cytoplasm, entering the glycolytic pathway as fructose-6-phosphate. Butyrate, however, is transported to the mitochondria where it undergoes $\beta$-oxidation, yielding acetyl-CoA. The latter may fuel the tricarboxylic acid (TCA) cycle and, subsequently, oxidative phosphorylation to generate energy in the form of ATP. Abbreviations: PEP, phosphoenolpyruvate; SCFAs, short-chain fatty acids.
oxidative phosphorylation to anaerobic lactic acid fermentation. This phenomenon is known as the Warburg effect, in which these cells rely mainly on glycolysis [91], a more efficient way to generate precursors for macromolecule biosynthesis to support cell growth and proliferation [92]. Butyrate treatment enhanced maximal glycolytic capacity by upregulating the gene expression of SLC2A4 and SLC16A1 in differentiated myotubes [93]. In contrast, absence of gut bacteria only minimally decreased glucose metabolism in colonocytes from GF rats [94], perhaps due to a shift towards consumption of systemic glucose by these cells. Furthermore, butyrate and propionate induced intestinal gluconeogenesis in rodents and Caco-2 cells [7], pointing to the relevance of glucose metabolism in the large intestine in the presence of SCFAs. Although more studies are needed to fully understand energy metabolism of colonocytes, these findings highlight the significance of glycolysis as a major pathway for metabolism in the colonic epithelium under control conditions.

Intestinal epithelial proliferation

Little is known about possible carbon sources that may arise from bacterial metabolism in the colonic lumen to support the high energetic demands for colonocyte growth and proliferation. However, the effects of microbial fermentation products on colonic epithelial growth are exemplified in several germ-free in vivo models, as illustrated in Figure 1 (in Box 1). In this section, we describe the impact of metabolites of colonic microbial fermentation on the proliferation of intestinal epithelial cells.

It is important to stress that, theoretically, butyrate alone cannot support human cell growth and proliferation. Human cells, as in other animals, do not have a glyoxylate cycle, hence these cells cannot convert acetyl-CoA (intermediate of butyrate metabolism) to glucose, which is the central carbon source for biosynthesis of macromolecules [95]. In the TCA cycle, acetyl-CoA reacts with oxaloacetate to form citrate, which in turn loses two other carbons to form succinyl-CoA. The latter can be converted back to oxaloacetate, which per se is a gluconeogenesis precursor. In fact, heavy isotopes from [2,4-13C2]butyrate were observed in glucose molecules [52,96]. However, this interconversion does not yield net glucose into the system since oxaloacetate re-enters the
TCA cycle. Thus, while carbons derived from fatty acids can become part of the metabolite pool of the TCA cycle, the net synthesis of glucose is accomplished through the entry of other intermediates, such as amino acids or lactic acid [95]. Lipids with an uneven number of carbons, such as propionate, have been shown to be precursors of gluconeogenesis in some tissues, like the liver [97]. In other words, we cannot produce carbohydrates from metabolism of lipids containing an even number of carbon atoms, such as butyrate. This effect is not colonocyte- or butyrate-specific, as it holds for all human cell types. Thus, it is inferred that butyrate does not support cell proliferation as sole carbon source, and numerous in vivo and in vitro models support this notion. Rats fed a fructooligosaccharide (FOS)-rich diet, which significantly enhanced fecal butyrate levels, did not show altered macroscopic or microscopic colonic morphology [98]. Moreover, butyrate inhibits cell growth in noncancerous (NCM460) cell lines by 47% in a process that involves the ERK1/2 signaling pathway [99]. In the same study, butyrate inhibited the growth of HTC116 cancer cell line by 84% compared to control. Increasing concentrations of butyrate, propionate, or acetate also inhibited proliferation and induced apoptosis of HT-29 cells, compared to untreated cells [98]. Moreover, recent studies using colonic crypts generated in vitro in differentiated intestinal organoids showed that butyrate treatment (0.5 or 5 mmol/l) suppressed cell proliferation and induced colonic cell senescence. These observations included a lowering of the number of cells in S-phase and reduction of expression levels of genes involved in cell proliferation and cell survival [100–102].

The detection of monosaccharides in human stool indicates an excess of these carbon sources in the intestinal lumen [13,18,19,45,84], which are evidently not fully utilized by bacteria and thus are

---

**Box 1. Metabolic changes in the intestinal mucosa of GF animals**

Mice subjected to oral treatment with a cocktail of antibiotics for 17 days become effectively depleted of intestinal microbiota and present shortening of the crypt-villus axis and crypt-based Ki67-positive (proliferative) cells, when compared to control mice. Moreover, antibiotic-induced microbiome depleted (AIDM) mice showed altered mitochondrial activity and reduced expression of genes involved in cellular growth, cell cycle progression, and lipid biosynthesis in the intestinal mucosa. Colonocytes from GF mice showed a reduced level of cell cycle progression and downregulation of 27 histone genes, followed by increased percentage of colonocytes in G1-phase and decreased percentage of colonocytes in S-phase, suggesting a blocked cell division [112–114]. A summary of the metabolic changes in the intestinal mucosa of GF animals is presented in Figure I.

---

**Colonic mucosal metabolism in germ-free in vivo models**

<table>
<thead>
<tr>
<th>Intestinal mucosa:</th>
<th>Colonocytes:</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Altered mitochondrial activity</td>
<td>- ↑ glycolysis</td>
</tr>
<tr>
<td>- ↓ expression of cell growth, cell cycle and lipid biosynthesis</td>
<td>- ↑ cells in G1-phase</td>
</tr>
<tr>
<td>- Shortening of crypt-villus axis</td>
<td>- ↓ cell cycle progression</td>
</tr>
<tr>
<td>- ↓ cell proliferation (Ki67+ cells)</td>
<td>- ↓ expression of histone genes</td>
</tr>
<tr>
<td></td>
<td>- ↓ cells in S-phase</td>
</tr>
<tr>
<td></td>
<td>- ↓ cell division</td>
</tr>
</tbody>
</table>

Figure I. Summary of the metabolic changes in the intestinal mucosa of GF (germ-free) animals.
available for colonic epithelial cells. Glucose metabolism yield a more diverse set of products than lipids (e.g., acetyl-CoA, ATP, NADPH, ribose and other metabolites), fulfilling the needs for the biosynthesis of cellular components [103]. For example, in the murine model, glucose was shown to undergo glycolysis in the intestinal epithelium and increase crypt proliferation, in part by activating the hypoxia-inducible factor 1α (HIF-1α) pathway [104]. Moreover, cell proliferation was, in turn, reduced if glycolysis is inhibited. Recently, experiments with mannose-fed mice showed a significant decrease in colonocyte apoptosis and increase in proliferative Ki67+ cells in mannose-fed mice [105]. Moreover, in vivo and in vitro studies showed that dietary fructose improved the survival of intestinal cells, also increasing intestinal villus length [106,107]. Finally, we recently obtained evidence of human colonocyte uptake of fructose derived from microbial digestion, which, in turn, sustained the growth and proliferation of these cells [45]. A very different effect, however, was found when butyrate was given as a source of ATP in colonocytes: the growth of these cells was nearly abolished by up to 10 mmol/l butyrate, especially in glucose-depleted conditions.

Still, the concept of butyrate inhibiting cell proliferation remains divisive. For instance, in vivo treatment with butyrate in piglets increased intestinal cell proliferation and correlated with a decrease in histone acetylation [108,109]. Moreover, in vitro incorporation rates of 14C-butyrate, but particularly 14C-acetate, in lipids were higher than 14C-glucose, 14C-glutamine, or 14C-propionate in rat colonocytes, pointing to butyrate and acetate as major carbon sources for lipid biomass in colonic intestinal epithelial cells [110]. Therefore, these observations illustrate the complex metabolic network that these highly specialized cell types carry out in the intestine, which likely involves the metabolism of multiple carbon and ATP sources from the intestinal microenvironment.

Concluding remarks
In this review, we summarized the mechanisms by which colonic bacteria process indigestible food components, especially fibers, in multiple steps involving a synergistic interaction between primary and secondary degraders. The products from this multistep mechanism directly affect the metabolism and proliferation of intestinal epithelial cells. In fact, the colonic intestinal epithelium can be considered a degrader of bacterial metabolites. We described how butyrate, although well known for its bioenergetic proprieties to colonocytes, cannot promote, or even sustain, the rapid proliferation of epithelial cells, and several studies point to an inhibitory effect of butyrate on cell proliferation. Additionally, we described how monosaccharides, widely produced by bacterial enzymatic decomposition of dietary fibers, can be absorbed by the intestinal epithelium via a variety of dedicated sugar transporters, and thereby provide carbon for biomass production.

We postulate that monosaccharides derive from the degradation of indigestible fibers, becoming available to neighboring bacteria and the intestinal epithelium. However, analysis of the metabolite composition in feces has inherited limitations as it does not represent the dynamic nature of metabolite production and consumption in the colon. In fact, recent effort on sampling luminal content along the GI tract has shown significant differences regarding microbiome composition, gene class abundance, metabolome and host proteome, compared to stool samples [47,111]. This underscores that the degradative, fermentative, and absorptive activity in the gut is extremely dynamic, and what spills over in the feces may represent what is left unused rather than a snapshot of the luminal content of the gut. Furthermore, there is a need to analyze fiber digestion by complex mixtures of bacteria in more detail to understand what becomes available for the epithelium (see Outstanding questions). We show that butyrate is an important energy source for the epithelium, but there are certainly other metabolites that may be used for epithelial growth and repair.

Outstanding questions
How do bacteria-derived metabolites fuel the energy needs of intestinal epithelial cells?

What are the differences between epithelial cell metabolism in the gut, compared to other tissues in the human body like the skin and lung?

How can oversimplifying the understanding of fiber degradation by the gut microbiome be avoided, considering the complexities involved in the concerted action of various bacteria, especially when dealing with complex dietary structures?

How do unculturable bacteria and microorganisms contribute to cross-feeding in the gut and provide metabolic fuels to intestinal epithelium?

What is the contribution of systemic circulating sugars versus bacterial-derived monosaccharides to intestinal epithelial metabolism?

What are the effects of SCFAs on diverse populations of intestinal epithelial cells, such as Paneth cells, Goblet cells, and enteroendocrine cells?

Can bacterial-derived monosaccharides promote mucosal healing in barrier-defective diseases like IBD or metabolic disorders?
This potentially represents new targets to study, especially in relation to intestinal diseases. Together, the work we presented in this review sheds light onto the dynamic mechanisms of cross-feeding between commensal gut bacteria and the intestinal epithelium.

Declaration of interests
No interests are declared.

References
45. Figuieras, P.R. et al. (2021) Inulin-grown Faecalibacterium prausnitzii cross-feed fructose to the human intestinal epithelium. Gut Microbes 13, 193592
47. Foltz, J. et al. (2021) Human metabolome variation along the upper intestinal tract. Nat. Metab. 5, 777–788
77. Wilker-Smith, O.C. et al. (2014) Fructose transporters GLUT5 and GLUT2 expression in adult patients with fructose intolerance. United European Gastroenterol. J. 2, 14–21
96. Weinman, E.O. et al. (1957) Conversion of fatty acids to carbohydrate: application of isotopes to this problem and role of the Krebs cycle as a synthetic pathway. Physiol. Rev. 37, 252–272
100. Wang, Y. et al. (2018) Formation of human colonic crypt array by application of chemical gradients across a shaped epithelial monolayer. CMGH 5, 113–130