



Review

Short chain fatty acids and its producing organisms: An overlooked therapy for IBD?



Sara Deleu^a, Kathleen Machiels^a, Jeroen Raes^{b,c}, Kristin Verbeke^a, Séverine Vermeire^{a,d,*}

^a Department of Chronic Diseases, Metabolism & Ageing [CHROMETA], Translational Research Center for Gastrointestinal Disorders [TARGID], KU Leuven, Leuven, Belgium

^b Center for Microbiology, VIB, Leuven, Belgium

^c Department of Microbiology and Immunology, Rega Institute, KU Leuven, Leuven, Belgium

^d Department of Gastroenterology and Hepatology, University Hospitals Leuven, KU Leuven, Leuven, Belgium

ARTICLE INFO

Article History:

Received 2 November 2020

Revised 1 March 2021

Accepted 7 March 2021

Available online 1 April 2021

Keywords:

Short chain fatty acids

Inflammatory bowel disease

Acetate

propionate

Butyrate

Gut

ABSTRACT

The gut microbiome and the intestinal immune system are driving contributors to inflammatory bowel diseases (IBD). Both have an important signalling factor in common: short-chain fatty acids (SCFAs). SCFAs (acetate, propionate and butyrate) are produced by bacterial fermentation in the gut and exert several effects on host metabolism and immune system. This review provides an overview of the current knowledge of these effects, with specific focus on energy metabolism, intestinal barrier, immune system, and disease activity in IBD. To conclude, more research is needed on the cross-feeding mechanisms in the gut microbiome, as well as on the therapeutic potential of SCFAs on different disease models. Also randomized controlled trials and prospective cohort studies should investigate the clinical impact of SCFA administration.

© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

1. Introduction

The human gut microbiota is a complex and dynamic microbial community of bacteria, fungi/yeasts, and viruses [1,2]. It is integral to the maintenance of health and the regulation of the host immune system [2]. Alterations in microbial composition have been linked to multiple pathologies, such as diabetes mellitus, obesity, colorectal cancer, and inflammatory bowel diseases (IBD) [2,3]. Especially bacterial species that feed on non-digestible dietary fibers play an important role in human health as they produce metabolites, such as SCFA. Next to being important cross-feeding products, these metabolites also beneficially affect the intestinal mucosa [4,5]. Due to their role in metabolic and immunologic homeostasis, and gut barrier integrity, they are of interest for therapeutic development, for instance in IBD which include Crohn's disease (CD) and ulcerative colitis (UC). These diseases are characterized by a complex interplay between the environment, the gut microbiome, and the host immune system in genetically susceptible individuals. However, the exact pathogenesis is still unknown. Consequently, therapeutics are mainly based on dampening the immune system [5] in a more or less targeted way. However,

results are not satisfying and only 30% of patients at most achieve and maintain clinical and endoscopic remission [6,7]. Considering the pivotal role of the microbiota in IBD, new therapeutic approaches manipulating or replacing the gut microbiome have been proposed to restore a normal interaction between the immune system and the gut microbiome [8]. However, therapies such as prebiotics, probiotics, antibiotics, and fecal microbial transplantation (FMT) have so far shown inconsistent results.

The increasing knowledge on the importance of the gut microbiome in health and disease has led to the quest for new or next-generation probiotics, seeking a better selection, and formulation of administrated strains [9]. For the reasons mentioned above, SCFAs and SCFA-producing organisms might be the missing piece of the puzzle. We here review the current knowledge of the effects of SCFAs on human metabolism, on intestinal barrier, and on inflammation in IBD patients, to assess the utility of such next-generation probiotics.

2. Bacterial fermentation and production of SCFA

Many members of the intestinal microbiota are able to obtain carbon and energy from the fermentation of complex carbohydrates, which are indigestible by the human host [10]. This process of fermentation leads to the production of SCFAs in the colon [10]. The 3 major SCFAs produced by gut bacteria are acetate, propionate, and

* Corresponding author at: Division of Gastroenterology & Hepatology, University Hospitals Leuven, Department of Chronic Diseases and Metabolism, KU Leuven, Belgium.

E-mail address: Severine.Vermeire@uzleuven.be (S. Vermeire).

butyrate, which are aliphatic carboxylic acids containing 1 carbon in the carboxylic function and respectively 1, 2 and 3 carbons in the aliphatic tail [5]. Recent advances in metagenomics enabled the description of bacteria responsible for this production [4]. After microbial hydrolysis (Fig. 1), pyruvate, the main precursor for SCFAs, is produced through the glycolytic pathway for (deoxy-) hexoses and the pentose phosphate pathway for pentoses [10]. The pathways for acetate production are commonly spread among bacterial classes and attains the highest concentration in the intestinal lumen [11], in contrast to propionate and butyrate pathways which are more conserved and substrate specific [4]. To produce propionate, two pathways are possible, namely, the succinate pathway used by Bacteroidetes and Negativicutes (Firmicutes phylum), and the propanediol pathway used by Lachnospiraceae [11]. Butyrate production is mediated by the key enzymes, butyrate kinase, or butyryl CoA:acetate CoA transferase [5]. The majority of the butyrate producers, amongst *Faecalibacterium prausnitzii*, *Eubacterium rectale* and *Eubacterium hallii* use the latter pathway whereas only a few butyrogenic taxa such as *Coprococcus comes* and *Coprococcus eutactus* are known to use the butyrate kinase route [4,12].

After fermentation, millimolar SCFA concentrations are available in the lumen from where they are absorbed by the colonic epithelium through passive and active transport [10]. Depending on the available substrates, SCFA concentrations [13] reach relative molar ratios for acetate-propionate-butyrates of approximately 60-20-20 respectively and for all human colonic regions (Fig. 1). Absolute concentrations are higher in the proximal colon due to higher availability of carbohydrates and SCFA uptake by the epithelium. After absorption, the proportion of butyrate decreases to 8% in the portal blood and the propionate proportion to 12%. Eventually, this drop was thought to show that the metabolism of propionate and butyrate is mainly restricted to the intestinal mucosa [5,13]. Contrarily, acetate concentrations remain measurable after uptake in the peripheral blood [13], pointing in the direction of systemic functions for acetate [5]. However, this should be nuanced as a recent randomized, cross-over study in healthy subjects exposed that during fermentation of carbohydrates labelled propionate and butyrate can double, as acetate may only increase with 10% [14]. Moreover, acetate does not exclusively originate from the colon, it is also produced endogenously from fatty acid oxidation [15].

Next to the butyrate producing commensals in the gut, a few gut pathogens also possess butyrogenic pathways [16]. However, they

have divergently evolved to produce butyrate using distinct pathways. All gut commensals ferment pyruvate for butyrate production, whereas the pathogenic bacteria, for example *Fusobacterium*, utilizes different pathways like those for Glutamate (4-aminobutyrate) and Lysine, which are associated with a release of harmful byproducts like ammonia [16].

3. Impact of the gut environment on bacterial growth and SCFA production

Several factors influence the production of SCFA in the gastrointestinal tract [11]. First, carbohydrates that lead to high SCFA production, lower the pH in the colon, which sequentially affects the microbial composition and because of this, the SCFA production [12]. Moreover, most SCFA are absorbed in the proximal colon in exchange for bicarbonate, which neutralizes the luminal pH. As a result, the pH in the cecum is lower (5.5) than the rectum (6.5) [12]. For example, *in vitro* studies exploiting single-stage fermentor systems showed restricted growth of *Bacteroides* species fed on soluble polysaccharide, in mildly acidic pH [17]. This phenomenon can be explained by the lower capacity of *Bacteroides* compared to *Firmicutes* species to tolerate the presence of SCFA at pH 5.5 than at pH 6.5 resulting in a microbial composition shift [17]. This shift limits propionate and stimulates butyrate production [17,18]. Another example are bacteria using the butyryl CoA – acetate CoA transferase pathway that display higher acetate consumption and butyrate production at mildly acidic pH in comparison to near neutral pH [18]. Moreover, the lower pH prevents overgrowth by pH-sensitive pathogenic bacteria [12].

Besides pH, the presence of growth factors is needed, more specifically, iron has proven to be essential for SCFA production [11]. In iron-deficient rats, significantly lower concentrations of faecal butyrate and propionate were retrieved [19] and the bacterial composition was strongly modified, involving a higher abundance of *Lactobacilli* and *Enterobacteriaceae* and a significant decrease of *Roseburia* species and *Eubacterium rectale*, which are major butyrate producers [19]. In the same study, repletion of iron by the administration of FeSO_4 led to increased caecal butyrate concentrations and partially restored microbial populations [19]. Similarly, the effects of iron on the production of SCFA have been examined in an *in vitro* model for the human gut [20]. Herein, very strong iron deficiency leads to a decreased production of butyrate and lower butyryl CoA: acetate CoA transferase gene expression. Conversely, under high iron conditions, *Ruminococcus intestinalis* displayed higher butyrate and higher gene expression [20]. Thus, iron depletion and repletion affect microbial composition and metabolic activity in the rodent gastrointestinal tract [19,20].

Third, intestinal gasses have an impact on SCFA production. Oxygen concentrations differ over different areas of the gastrointestinal tract and this is mainly caused by a different microbial composition with other oxygen sensitivity and metabolic capacity [11] and vice versa, the oxygen level affects the composition [21]. For example, *Faecalibacterium prausnitzii* is an obligate anaerobe which has a growth optimum at low, but not zero, oxygen levels [22]. Thus, oxygen levels influence the fermentation process and production of SCFA. In addition, bacteria consuming hydrogen affect the hydrogen partial pressure that by consequence influences the overall fermentation balance, and thus the SCFA production [11,23].

4. Effects of SCFA on the human metabolism

SCFA exert different beneficial effects on the human energy metabolism, including glucose, lipid, and cholesterol metabolism in several tissue types [4,12]. SCFA are transported through the colonocytes, which exploit butyrate as their main energy source after apical uptake [24]. The fraction of SCFAs that is not consumed by the colonic mucosa is transported through the basolateral membrane towards

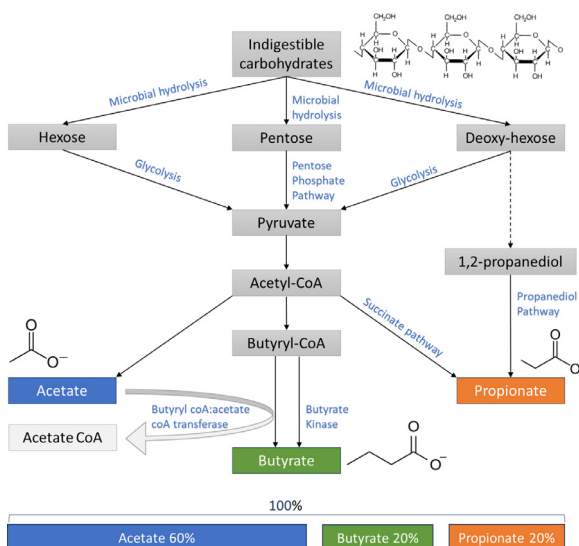


Fig. 1. Overview of the pathways of bacterial fermentation resulting in the production of SCFA hydrolysis, including molar ratios of SCFAs in the colon on a total of 100%.

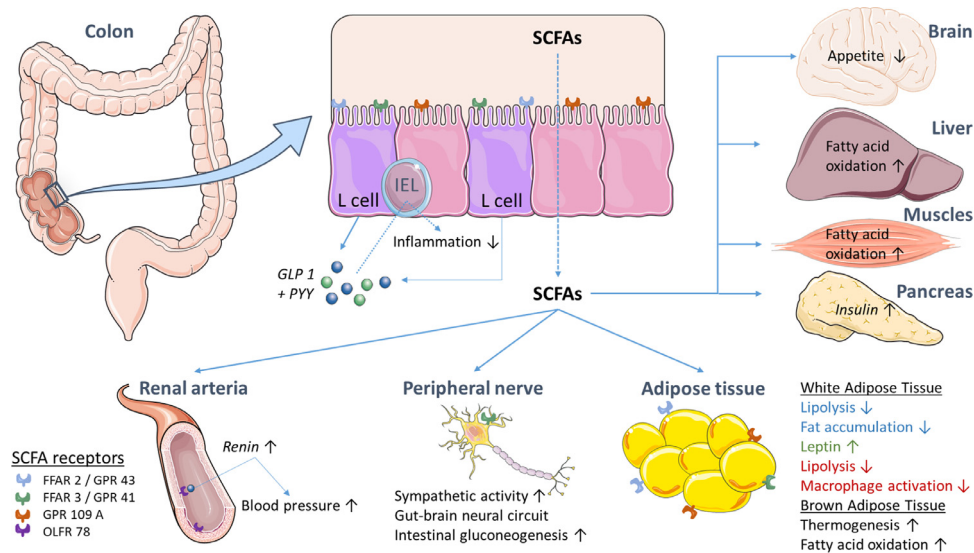


Fig. 2. Overview of the direct and indirect effects of SCFAs on different tissues and human metabolism. FFAR= Free Fatty Acid Receptor, GLP 1=Glucagon-like receptor, GPR= G-coupled protein receptor, IEL=Intraepithelial lymphocyte, OLFR= Olfactory receptor, PYY=Peptide tyrosine tyrosine.

the portal blood stream [12]. The transport mechanisms for SCFAs from the blood to different tissue types is still largely unknown [12]. SCFA have shown to be present throughout the human body as several SCFA receptors have been identified, however, these are not involved in transportation (Table 1 and Fig. 2).

The SCFA receptors (Table 1) include free fatty acid receptors (FFAR) which are G-protein coupled receptors (GPRs) [24,25]. FFAR 2 or GPR 43 has been recognized on intestinal endocrine L-cells and the receptor is activated mainly by acetate and propionate [24,26]. For example, intestinal IgA production is promoted and mediated by FFAR2 upon acetate activation [27]. In contrast, FFAR 3 or GPR 41 is preferably activated by propionate and butyrate over acetate [24,25]. Activation of both receptors leads to PYY and GLP 1 secretion by the L-cells, which affect several tissues including the cardiovascular system, the pancreas and the brain [24].

More recently, GPR 109A and Olfactory receptor 78 (OLF 78) have also been identified as SCFA receptors. These receptors exert different effects depending on their location [24]. GPR 109A is expressed by colonic epithelial cell and upon activation by (β -hydroxy-) butyrate, it mediates anti-inflammatory properties like IL10 production [24,28,29]. Contrarily, OLFR 78 is mainly expressed by renal blood vessels, and is involved in blood pressure regulation [30].

Together, SCFAs are able to affect the metabolism and energy homeostasis [24]. However, many of the biological roles of SCFA have been investigated *in vitro* and in *in vivo studies* whereas little is known about their impact in humans, mainly due to a lack of quantitative data on SCFA kinetics, and clinical trials [12]. More extensive reading on the specific effects of SCFA on the energy homeostasis and metabolism can be found in the paper of Canfora *et al* [31].

5. Effects of SCFA on epithelial barrier and immune system

The innate immune system consists of components which are important in the first defense against infections, including the intestinal barrier. These components prevent the entrance of micro-organisms and their multiplication in the body. Furthermore, these mechanisms are regulated by the adaptive immune system, which requires more time and is cell-specific, unlike the innate response [32].

5.1. Innate immune system

5.1.1. Intestinal barrier integrity

The intestinal barrier is a complex, multi-component system in which the epithelium and innate immune cells contribute to its integrity and hence prevent unwanted translocation of components from the lumen to the body [33]. When this barrier is disturbed, bacteria can infiltrate and cause a dysregulated immune response, which is thought to be an important primary event in the pathogenesis of IBD [34].

The mechanisms that disturb barrier integrity include the down-regulation of epithelial cadherin in tight junctions, a thinner mucus layer, abnormal goblet cell functioning including mucin 2 and resistin-like molecule β (RELM β); and dysfunctional Paneth cell associated mechanisms, including secretion of antimicrobial products, nucleotide binding oligomerization domain protein 2 (NOD2), and autophagy-related 16-like 1 (ATG16LI) gene associated functions [35].

Butyrate and other SCFAs provide abundant energy to the intestinal epithelial cells by β -oxidation in the mitochondrial tricarboxylic

Table 1

Overview of the SCFA-receptors, their expression, activation, production and function. (*)= Indirect effect, A= Acetate, Al= Anti-inflammatory, B= Butyrate, P= Propionate.

Receptor	Expression	Activation	Production	Function
FFAR 2/ GPR 43	IEL-cells	A > P > B	PYY, GLP 1	Glucose and lipid metabolism Appetite regulation*
	Adipose tissue		/	Regulation lipid homeostasis, Insulin regulation
	Immune cells		Al signals (Treg & IL10 \uparrow)	Anti-inflammatory properties
FFAR 3/ GPR 41	IEL-cells	B > P > A	PYY, GLP 1	Glucose metabolism, Appetite regulation*
	Sympatic ganglia	P	/	HR \uparrow , E expenditure \uparrow
GPR 109 A	Colonic epithelial cells	(β -OH-) B	Al-signals	Colonic inflammation \downarrow , carcinogenesis \downarrow
	Adipose tissue		/	Regulate lipid homeostasis
	Immune cells (M, DC)		Al-signals (Treg & IL10 \uparrow)	Anti-inflammatory properties
OLFR 78	Renal blood vessels	Microbial A and P	Renine*	Blood pressure regulation

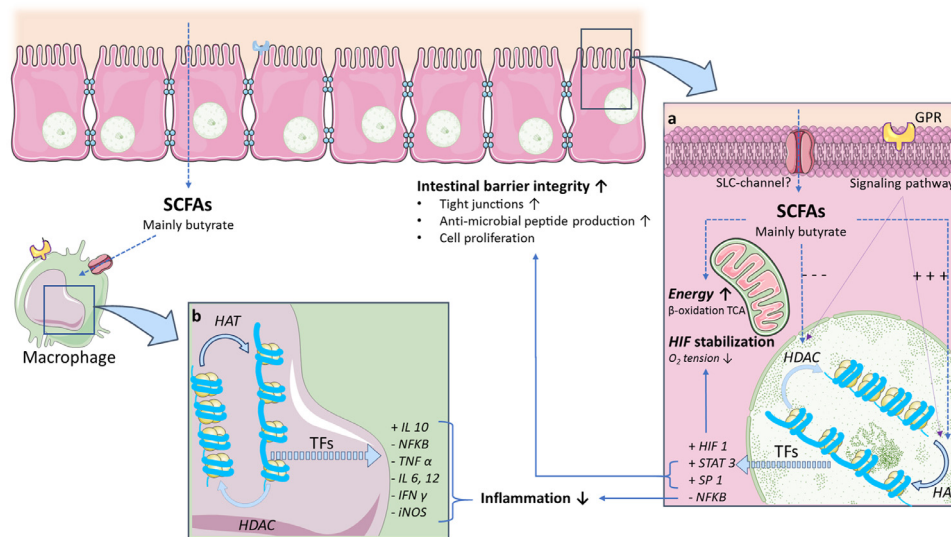


Fig. 3. Mechanisms of SCFA, mainly butyrate, to protect the intestinal barrier and control inflammation by inhibition of HDAC and activation of HAT in colonocytes (a) and macrophages (b). This inhibition leads to translation of the DNA and the production transcription factors resulting in HIF stabilization, increased intestinal barrier integrity and downregulation of inflammatory mediators. GPR= G-protein coupled receptor, HAT= histone acetyl transferase, HDAC= histone deacetylase, HIF= hypoxia inducible factor, SLC= solute carrier, TCA= tricarboxylic acid cycle, TF= transcription factor.

acid cycle (TCA cycle) [5,34] (Fig. 3 A). Second, butyrate also acts as a hypoxia inducible factor 1 (HIF 1) stabilizer, a transcription factor that coordinates barrier protection [5,36]. Butyrate encouraged barrier protection, unless HIF-1 β is absent [36]. Third, butyrate promotes barrier integrity through the activation of genes coding for tight-junction components and protein reassembly through transcription factors (Fig. 3A), such as STAT3 and SP1 [5]. This activation results in the maintenance and/or increased transepithelial electrical resistance (TEER) in human, rodent, and porcine experiments [37–39]; even after exposure to inflammatory conditions [40]. Similar effects were achieved through the supplementation of CD microbiota with a butyrate-producing candidate probiotic, namely *Butyrococcus pullicaecorum*. These *in vitro* observations provided evidence for the beneficial effects of butyrate on the intestinal barrier [41]. This is relevant for IBD as butyrate-producing bacteria are often depleted and intestinal barrier is proposed to be an important therapeutic target [5,42].

Although positive effects on the intestinal barrier have been noted in rodent, porcine, and human experiments, contradictory data has been achieved in some *in vitro* studies [72]. Vancamelbeke *et al.* demonstrated that butyrate (8mM, for 48h) – when applied to a human primary colonic monolayer – induced beneficial effects on TEER [42]. In the same experiments, butyrate did not improve epithelial barrier integrity in the presence of inflammatory stimulators and was even shown to be detrimental there [42]. Similar observations have been noted regarding the protection of colonic stem cells against microbial metabolites [43]. Thus, butyrate may have dual effects and even negatively affect the gut barrier, especially in the presence of inflammation. Therefore, additional studies are needed to provide a more nuanced explanation for the effects of butyrate on the intestinal barrier [42,43].

5.1.2. Effects of SCFAs on neutrophils, monocytes and macrophages

SCFAs also affect neutrophils, monocytes and macrophages (Fig. 3 B) by downregulating histone deacetylase (HDAC) [39,44] and nuclear factor- κ B (NF κ B) [24]. Consequently, these gut metabolites are able to manipulate gene expression. In neutrophils, this mechanism is thought to inhibit TNF production in the presence of lipopolysaccharides (LPS) [45]. Furthermore, high SCFA concentrations inducing IL8, IL6 and IL1 β in these cells, while lower concentrations do not [28]. Moreover, SCFAs affect chemotaxis of neutrophils,

induced by inflammatory mediators and chemokines, and their viability [29].

Many studies have determined the specific effects of SCFAs on monocytes and macrophages. A study by Cox *et al* showed the anti-inflammatory capacity of SCFA by regulating cytokine and prostaglandin E₂ (PGE₂) production [46]. Decreased IL10 was observed after LPS-stimulation, as well as a dose-dependent inhibition of cytokine CCL2. Similarly, in PBMC's a drop in LPS-induced TNF α and IFN γ have been noticed. SCFAs do appear to reduce the responsiveness of lamina propria macrophages to commensal bacteria via nitric oxide, IL6, and IL12 [47]. However, another study identified pro-inflammatory effects, as augmented concentrations of IL1 β , IL6, and IL8 were observed [48]. Yet, these different observations seem to be dependent on the studied tissue, the applied stimulations, and environmental conditions [28]. Nevertheless, it is clear that SCFAs affect monocytes and macrophages in their immune function [28].

5.1.3. Effects on dendritic cells

SCFAs may affect the differentiation and functions of dendritic cells (DC). In *In vitro* studies butyrate could inhibit the maturation of DCs when incubated with different inflammatory inducers [28]. The delay in human DCs maturation was explained by inhibition of dendrite formation and expression of surface markers⁸⁸. Moreover, butyrate altered the production of cytokines, such as IL10 and IL12 [49]. These pre-treated DCs with butyrate showed a lower capacity to stimulate T cells [49]. Also downregulation of DC development by butyrate has been reported, although the functional maturation was not affected after LPS stimulation [50]. Most likely butyrate and propionate can inhibit the histone deacetylase (HDAC) and thereby suppress the expression of transcription factors [50], which acetate cannot [51]. These observations point in the direction of unique characteristics for each SCFA.

5.2. Adaptive immune system

SCFAs are able to modulate the adaptive immune system by influencing the macrophages and dendritic cells as well as exerting direct effects on the adaptive immune system. First of all, the activation of GPR109A-receptor by butyrate in macrophages and DCs (Fig. 3B and Fig. 4) appears to be crucial for maintaining the equilibrium between pro- and anti-inflammatory T cells [52]. GPR109A-deficient KO mice

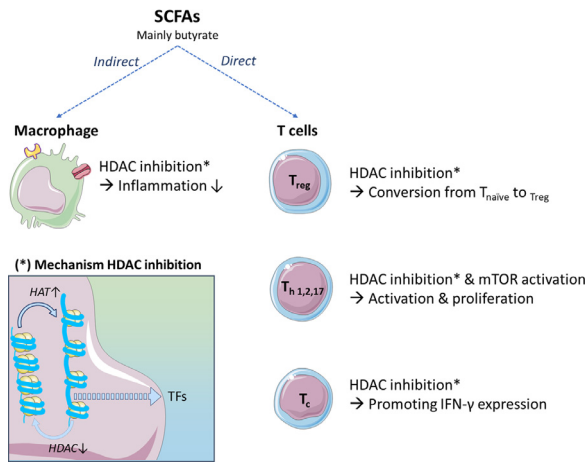


Fig. 4. Schematic overview of the (in)direct effects of SCFAs, mainly butyrate, on the adaptive immune system. The HDAC-inhibition mechanism is shown in the square (*) and promotes translation of transcription factors. HAT= histone acetyl transferase, HDAC= histone deacetylase, mTOR= mammalian target of rapamycin, TF= transcription factor.

exhibit a reduced IL10 and an increased IL17 production by T cells which increases the susceptibility to colitis [52].

Second, these microbial metabolites can potentiate the conversion of naïve T cells to regulatory T cells. This might be explained by taking together the effects on DCs and the results of *in vitro* studies that showed an increase of two enzymes, indoleamine 2,3-dioxygenase 1 (indoleamine) and aldehyde dehydrogenase 1A2, which are known to diminish immune activation through tryptophan depletion and generation of retinoid acid [53]. In this mechanism the SLC5a8 transporter might be involved, as well as the inhibition of HDAC [50].

5.2.1. Regulatory T cells

Increased numbers of regulatory T cells are thought to be caused by the inhibition of HDAC. For example, butyrate has been demonstrated to facilitate extrathymic peripheral polarization towards regulatory T cells *in vitro* and *in vivo* [51]. *Clostridia spp.* producing butyrate can induce colonic regulatory T cells in mice. The causal mechanism might be the enhancement of H3 acetylation in the promoter and the conserved non-coding sequence regions of the Foxp3 locus by butyrate, which increases the expression of this transcription factor [54]. The activation of FFAR2 might also be involved in this shift towards regulatory T cells. Contrarily, acetate does not directly show HDAC inhibition, therefore, the conversion towards butyrate by the microbiota might be an interesting target [50].

5.2.2. Helper T cells

Next to the polarization of regulatory T cells, SCFAs are also affecting polarization and activation of Th1, Th2, and Th17 by HDAC inhibition. For example, studying allergic airway inflammation, SCFAs have been shown to cause an impaired FFAR3 mediated Th2 polarization [55]. In this regard, a recent study demonstrated the protection of a high fiber diet and acetate administration against the development of allergic airways disease [56]. Acetate, propionate and butyrate also promote the generation of Th1 and Th17 *in vitro* [57]. Additionally, *in vivo* mice studies showed less inflammation after supplementing acetate in the drinking water in combination with anti-CD3 administration. This observation might be the consequence of simultaneous production of the anti-inflammatory cytokine IL10 [28,57]. These effects were again mediated by HDAC inhibition, nevertheless, mTOR (mammalian target of rapamycin) activation was also proposed to be involved [57]. Moreover, the same HDAC mechanism is exploited to induce Fas-mediated apoptosis of T cells by butyrate. *In vitro* studies showed that butyrate inhibits the proliferation of T lymphocytes at

lower concentrations whereas higher concentrations induce apoptosis in activated T cells [58].

5.2.3. Cytotoxic T cells

The effects of SCFAs, especially butyrate and propionate, on cytotoxic T cells (CTL) have been investigated [59]. An *in vitro* Study by Luu *et al.* (2018) showed independence of SCFA-receptors FFAR2 and -3 in the butyrate-mediated regulation of CTLs [59]. However, similarly to other T cells, HDAC inhibition by butyrate and propionate was identified to modulate the gene expression of CTLs and thereby promoting their IFN- γ expression. Acetate was suggested to enhance this IFN- γ production as well, conversely by operating as a metabolic substrate for CTLs [59]. Moreover, increased acetate concentrations are required for optimal memory CD8+ T cell function *in vitro* and *in vivo* during bacteremia in mice [60].

6. Relevance of SCFA-producing bacteria and SCFAs in Inflammatory Bowel Diseases: an update of recent work

IBD is characterized by an altered microbial composition, often mentioned as dysbiosis [5]. Generally, microbial dysbiosis in CD and UC patients is associated with fewer SCFA-producing bacteria. Many of the depleted bacteria belong to the Ruminococcaceae and Lachnospiraceae families of the Firmicutes phylum. Both families represent most of the butyrate-producing bacteria in the gut. The most repetitive finding is the reduced abundance of *F. prausnitzii* in patients with active IBD. In UC, alterations in *Roseburia* species have been observed. In CD, a higher abundance of *Ruminococcus gnavus*, and a lower abundance of *F. prausnitzii*, *Bifidobacterium adolescentis*, *Dialister invisus* and other SCFA-producing microbiota has been reported [5,61]. The presence of *Eubacterium* and *Roseburia* species, as well as SCFA biosynthesis and secondary bile acids has been associated with remission in fecal microbial transplantation studies in IBD patients [62]. Whereas, absence of remission has been linked to *Fusobacterium*, *Suterella*, and *Escherichia spp.*, as well as increased heme [62]. Moreover, acetate-producing bifidobacteria are known to exhibit various probiotic effects against enteropathogenic infections [63]. Furthermore, co-culturing studies with bifidobacteria and *Faecalibacterium spp.* demonstrated proven cross-feeding activities between both bacterial groups. Additionally, acetate is a crucial source for butyrate producing bacteria. This production has already proven its importance in the immune system and highlights the relevance of studies on acetate [24,44]. Therefore, cross-feeding is an important metabolic interaction mechanism and includes features such as the utilization of acetate by butyrate-producing bacteria [64].

Moreover, crossfeeding might explain the indirect effects of acetate on the human body, especially the immune system [64]. For example, although acetate did not show a direct inhibition of the HDAC mechanism [56], administration of acetate was shown to have protective effects, mediated by HDAC inhibition [55]. This points in the direction of its utilization by microbiota to produce butyrate. Yet, increasing acetate might not be enough, and it might be necessary to increase the abundance of the butyrate-producing bacteria, as well as fiber in the diet [5]. Therefore, more research on this topic is necessary.

Next to crossfeeding, different strategies might be pursued to obtain higher SCFA concentrations in the colon [65]. First, the administration of a prodrug, for example tributyrin, may increase serum butyrate level. However, up today, there are no reports of clinical trials with this compound in IBD [65]. Second, highly fermentable dietary fibers such as prebiotics can cause a significant rise in plasma SCFA levels. Moreover, administration on long term basis increases fecal levels of total SCFA and butyrate [65]. Although prebiotics might be good inducers of SCFA, they are often not well tolerated in IBD patients because of bloating and abdominal cramps [65]. Third, direct postbiotic delivery of SCFAs in the colon might be obtained by

enema-based administration or orally slow-release formulations [65]. Fourth, exploiting endogenous metabolic processes that produce SCFA to attain health benefits is another interesting possibility [65]. For example, low alcohol consumption creates a temporary increase in plasma acetate levels and is associated with a lower risk of heart disease and diabetes [65]. Different methods might be integrated, for example, combining dietary and postbiotic administration by esterification of SCFAs into resistant starch, protects them from release and absorption in the small intestine [65].

Furthermore, SCFAs measured in feces of IBD patients are lower compared to healthy controls. A decrease in acetate and propionate, yet not in butyrate concentrations was observed in UC patients [61], while another study showed decreased propionate and butyrate [66]. The reduction in SCFA in IBD has furthermore been associated with disease activity, and patients in remission have higher levels of butyrate than patients with severe active disease [67]. Recently, butyrate has been shown to promote the growth of SCFA-producing bacteria in IBD patients [68]. However, further investigation is needed to determine its clinical implication.

Supplementation of butyrate-producing bacteria, especially *Butyr-ococcus pullicaecorum*, improved epithelial barrier integrity in CD-based simulations [41]. Boesmans *et al.* positively assessed the safety and tolerance of this species in healthy individuals [69]. Nevertheless, addition of butyrate on biopsies of inflamed and non-inflamed UC patients showed that its potency to downregulate gene expression in inflammatory pathways was greater in non-inflamed controls [70]. Moreover, TNF α has been identified to reduce the responsiveness of the intestinal epithelium to butyrate, which might undermine its potential efficacy in IBD patients with active inflammation [71] and may rather suggest a prophylactic administration to prevent disease flares.

In this respect, *in vivo* mice experiments showed contradicting results, for example, oral administration of butyrate aggravates dextran sulphate (DSS)-induced colitis, while daily intraperitoneal butyrate injection mildly improved disease activity [72]. Another study by Tye *et al.* (2018) demonstrated an aggravation of DSS colitis in mice by limiting butyrate-producing organisms due to nucleotide-binding oligomerization leucine rich repeat and pyrin domain containing (NLRP1), which is an inflammasome sensor [73]. Loss of this inflammasome significantly ameliorated the induced colitis [73].

Recently a multi-omics study of the gut microbial ecosystem in IBD showed that SCFAs were generally reduced in dysbiotic patients [74]. The reduction of especially butyrate is consistent with the observed depletion of butyrate producers such as *F. prausnitzii* and *R. hominis* [74]. Most clinical studies are randomized trials, and only very limited numbers of cohort studies are available on this topic [74]. There is especially a lack of large prospective cohort studies [74].

Considering the differences in UC and CD presentation [3], there might indeed be a different response between these two clinical phenotypes upon SCFA administration. Although speculative, protective effect of SCFA administration may be more observed in UC or CD colitis compared to CD ileitis, or perhaps in general more in patients with a milder disease manifestation. However, to our knowledge, there are no studies available on this topic.

Up till now, most studies used butyrate and its potential to decrease symptoms, and inflammation. However, crossfeeding in IBD might be an interesting target as well. For example, acetate supplementation in colitis induced mice have been showed to play a crucial role in the response of the gut to insults and tissue repair [75]. Moreover, administration of butyrate may be more toxic than acetate when directly added to the intestinal cells [59]. Together, more research is needed to determine the nuanced effects of acetate, acetate-producing organisms and propionate when targeting inflammatory bowel disease.

7. Conclusion and research gaps

Microbial SCFAs exert several beneficial effects on the human metabolism by intervening in glucose homeostasis, lipid metabolism, and appetite regulation. Moreover, SCFAs affect intestinal barrier integrity and hence the host's immunity, mainly by inhibiting the HDAC-mechanism, and are therefore of increasing interest in therapy development. So far, most research has been performed on the effects of butyrate, pointing in the direction of a prebiotic and/or a probiotic treatment to support management in IBD. Restoring dysbiosis in CD and UC by raising colonization of butyrate-producing organisms seems promising, although acetate might be an alternative target.

To date, *in vitro* studies have been mainly executed in epithelial monocultures. The development of coculture models combining the microbiome and the immune system is mandatory to make progress. Additionally, most *in vivo* studies have been based on chemically induced colitis models in mice. Consequently, adding different models, like immune mediated models, instead of chemically induced ones would broaden the knowledge on this topic. Subsequently, more research is needed on the cross-feeding mechanisms in the gut microbiome, as well as on the therapeutic potential of SCFAs on different disease models, including determination of optimal dose and composition. Also randomized controlled trials and prospective cohort studies should investigate the clinical impact of SCFA administration.

8. Outstanding questions

It is unclear how SCFA administration might affect microbiome composition, and immune system in IBD patients. To bridge this knowledge gap, it is important to decipher the crossfeeding mechanisms, as well as the therapeutic potential of SCFA. Do acetate and propionate have the potential to be therapeutic targets, and what would be the optimal dose and indication? How does crossfeeding work, and how can it be used to support IBD management? Are there any differences in potential effects in CD vs UC? How relevant are disease activity and extent of the lesions? Given that a reduction of *Faecalibacterium prausnitzii* is associated with postoperative Crohn's recurrence, could SCFAs be indicated in the setting of Crohn's disease postoperative prevention of recurrence? A better fundamental understanding of these aspects will provide evidence for the support by pre-, pro-, or synbiotics in the management of IBD.

9. Search strategy and selection criteria

Data for this review were identified by searches of PubMed and references from relevant articles using the search terms "short chain fatty acids", "SCFA", "butyrate", "acetate", "propionate", "inflammatory bowel disease", "IBD", "gut microbiome", and any combinations of these terms. Only articles published in English between 2010 and 2020 were included, earlier articles were only added if highly relevant.

Contributors

SD has written the manuscript and SD, KM, KV, JR and SV had substantial contributions to all of the following: (1) drafting the article or revising it critically for important intellectual content and (2) final approval of the final version of the submitted manuscript.

Declaration of Competing Interest

KM reports employment as a Pfizer Medical advisor in inflammation and immunology since 5/01/2021. **JR** reports grants from J&J, Beneo, Colruyt, Prodigest, Apeha.Bio, Cargill, other from Yakult, Nutricia, GSK Vaccines, MRM health, Apeha.Bio, Janssen Pharmaceuticals,

Roche, Takeda, Tsumura, ABInbev, DSM, Nestle, Pfizer, BMS, Ferring, outside the submitted work; **KV** reports grants from Nestlé, personal fees from Yakult, personal fees from Biocodex, outside the submitted work; **SV** reports grants from AbbVie, J&J, Pfizer, and Takeda, other from AbbVie, Arena Pharmaceuticals, Avaxia, Boehringer Ingelheim, Celgene, Dr Falk Pharma, Ferring, Galapagos, Genentech-Roche, Gilead, Hospira, Janssen, Mundipharma, MSD, Pfizer, Prodigest, Progenity, Prometheus, Robarts Clinical Trials, Second Genome, Shire, Takeda, Theravance, and Tillots Pharma AG, outside the submitted work; The remaining authors disclose no conflicts.

Acknowledgements

This work was supported by the VIB Grand Challenges Program and European Research Council (ERC) [grant number 694679]; K.M. is a postdoctoral fellow and S.V. is senior clinical investigator of the Fund for Scientific Research-Flanders, Belgium (FWO-Vlaanderen). The funders had no role in review design, interpretation and writing of this work.

The figures have been created using Servier Medical Art from <https://smart.servier.com>.

References

- [1] Stange EF, Schroeder BO. Microbiota and mucosal defense in IBD: an update. *Expert Rev Gastroenterol Hepatol* 2019;13:963–76.
- [2] Vrancken G, Gregory AC, Huys GRB, Faust K, Raes J. Synthetic ecology of the human gut microbiota. *Nat Rev Microbiol* 2019. doi: 10.1038/s41579-019-0264-8.
- [3] Eisenstein M. A slow-motion epidemic. *Nature* 2016;540 S98–S99.
- [4] Morrison DJ, Preston T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes* 2016;7:189–200.
- [5] Venegas DP. Short chain fatty acids (SCFAs) mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front Immunol* 2019;10.
- [6] Pagnini C, Pizarro TT, Cominelli F. Novel pharmacological therapy in inflammatory bowel diseases: beyond anti-tumor necrosis factor. *Front Pharmacol* 2019;10:1–11.
- [7] Schreiner P. Mechanism-based treatment strategies for IBD: cytokines, cell adhesion molecules, JAK inhibitors, gut flora, and more. *Inflamm Intestinal Dis* 2019;4:79–96.
- [8] Pigneur B, Sokol H. Fecal microbiota transplantation in inflammatory bowel diseases: the quest for the holy grail. *Mucosal Immunol* 2016;9:1360–5.
- [9] El Hage R, Hernandez-Sanabria E, Van de Wiele T. Emerging trends in 'smart probiotics': Functional consideration for the development of novel health and industrial applications. *Front Microbiol* 2017;8:1–11.
- [10] Hugenholtz F, Mullaney JA, Kleerebezem M, Smidt H, Rosendale DI. Modulation of the microbial fermentation in the gut by fermentable carbohydrates. *Bioactive Carbohydr Dietary Fibre* 2013;2:133–42.
- [11] Louis P, Flint HJ. Formation of propionate and butyrate by the human colonic microbiota. *Environ Microbiol* 2017;19:29–41.
- [12] Den Besten G. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* 2013;54:2325–40.
- [13] Cummings JH, Pomare EW, Branch HWJ, Naylor CPE, MacFarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* 1987;28:1221–7.
- [14] Boets E. Systemic availability and metabolism of colonic-derived short-chain fatty acids in healthy subjects: a stable isotope study. *J Physiol* 2017;595:541–55.
- [15] Layden BT, Angueira AR, Brodsky M, Durai V, Lowe WL. Short chain fatty acids and their receptors: new metabolic targets. *Transl Res* 2013;161:131–40.
- [16] Anand S, Kaur H, Mande SS. Comparative in silico analysis of butyrate production pathways in gut commensals and pathogens. *Front Microbiol* 2016;7:1–12.
- [17] Walker AW, Duncan SH, Carol McWilliam Leitch E, Child MW, Flint HJ. pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. *Appl Environ Microbiol* 2005;71:3692–700.
- [18] Kettle H, Louis P, Holtrop G, Duncan SH, Flint HJ. Modelling the emergent dynamics and major metabolites of the human colonic microbiota. *Environ Microbiol* 2015;17:1615–30.
- [19] Dostal A. Iron depletion and repletion with ferrous sulfate or electrolytic iron modifies the composition and metabolic activity of the gut microbiota in rats. *J Nutr* 2012;142:271–7.
- [20] Dostal A. Iron modulates butyrate production by a child gut microbiota in vitro. *mBio* 2015;6:1–12.
- [21] Zheng L, Kelly CJ, Colgan SP. Physiologic hypoxia and oxygen homeostasis in the healthy intestine. A review in the theme: cellular responses to hypoxia. *Am J Physiol – Cell Physiol* 2015;309 C350–C360.
- [22] Khan MT. The gut anaerobe *Faecalibacterium prausnitzii* uses an extracellular electron shuttle to grow at oxic-anoxic interfaces. *ISME J* 2012;6:1578–85.
- [23] Wolf PG, Biswas A, Morales SE, Greening C, Gaskins HR. H2 metabolism is widespread and diverse among human colonic microbes. *Gut Microbes* 2016;7:235–45.
- [24] Kasubuchi M, Hasegawa S, Hiramatsu T, Ichimura A, Kimura I. Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. *Nutrients* 2015;7:2839–49.
- [25] Chambers ES, Morrison DJ, Frost G. Control of appetite and energy intake by SCFA: what are the potential underlying mechanisms? *Proc Nutr Soc* 2015;74:328–36.
- [26] Mohammad S. Role of free fatty acid receptor 2 (FFAR2) in the regulation of metabolic homeostasis. *Curr Drug Targets* 2015;16:771–5.
- [27] Wu W. Microbiota metabolite short-chain fatty acid acetate promotes intestinal IgA response to microbiota which is mediated by GPR43. *Mucosal Immunol* 2017;10:946–56.
- [28] Corrêa-Oliveira R, Fachi JL, Vieira A, Sato FT, Vinolo MAR. Regulation of immune cell function by short-chain fatty acids. *Clin Transl Immunol* 2016;5:1–8.
- [29] Ohira H, Tsutsui W, Fujioka Y. Are short chain fatty acids in gut microbiota defensive players for inflammation and atherosclerosis? *J Atheroscler Thromb* 2017;24:660–72.
- [30] Kotlo K. The olfactory G protein-coupled receptor (Olf1r-78/OR51E2) modulates the intestinal response to colitis. *Am J Physiol-Cell Physiol* 2020;318:C502–13.
- [31] Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol* 2015;11:577–91.
- [32] Peterson LW, Artis D. Intestinal epithelial cells: Regulators of barrier function and immune homeostasis. *Nat Rev Immunol* 2014;14:141–53.
- [33] van Hemert S, Skonieczna-Zydecka K, Loniewski I, Szredzki P, Marlicz W. Microscopic colitis—microbiome, barrier function and associated diseases. *Ann Transl Med* 2018;6 39–39.
- [34] Dong J-P. Protective effect of *Saccharomyces boulardii* on intestinal mucosal barrier of dextran sodium sulfate-induced colitis in mice. *Chin Med J* 2019;132:1951–8.
- [35] Ramos GP, Papadakis KA. Mechanisms of disease: inflammatory bowel diseases. *Mayo Clin Proc* 2019;94:155–65.
- [36] Kelly CJ. Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. *Cell Host Microbe* 2015;17:662–71.
- [37] Miao W. Sodium butyrate promotes reassembly of tight junctions in Caco-2 monolayers involving inhibition of MLCK/MLC2 pathway and phosphorylation of PKC β . *Int J Mol Sci* 2016;17:1–12.
- [38] Valenzano MC. Remodeling of tight junctions and enhancement of barrier integrity of the CACO-2 intestinal epithelial cell layer by micronutrients. *PLoS One* 2015;10.
- [39] Zheng L. Microbial-derived butyrate promotes epithelial barrier function through IL-10 receptor-dependent repression of claudin-2. *J Immunol* 2017;199:2976–84.
- [40] Yan H, Ajuwon KM. Butyrate modifies intestinal barrier function in IPEC-J2 cells through a selective upregulation of tight junction proteins and activation of the Akt signaling pathway. *PLoS One* 2017;12:1–20.
- [41] Geirnaert A. Butyrate-producing bacteria supplemented in vitro to Crohn's disease patient microbiota increased butyrate production and enhanced intestinal epithelial barrier integrity. *Sci Rep* 2017;7:1–14.
- [42] Vancamelbeke M. Butyrate does not protect against inflammation-induced loss of epithelial barrier function and cytokine production in primary cell monolayers from patients with ulcerative colitis. *J Crohn's Colitis* 2019;13:1351–61.
- [43] Kaiko G. The colonic crypt protects stem cells from microbiota-derived metabolites. *Cell* 2016;165:1708–20.
- [44] Ratajczak W. Immunomodulatory potential of gut microbiome-derived short-chain fatty acids (SCFAs). *Acta Biochim Pol* 2019;66:1–12.
- [45] Vinolo MAR. Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils. *J Nutr Biochem* 2011;22:849–55.
- [46] Cox MA. Short-chain fatty acids act as anti-inflammatory mediators by regulating prostaglandin E2 and cytokines. *World J Gastroenterol* 2009;15:5549–57.
- [47] Liu L. Butyrate interferes with the differentiation and function of human monocyte-derived dendritic cells. *Cell Immunol* 2012;277:66–73.
- [48] Mirmonsef P. Short-chain fatty acids induce pro-inflammatory cytokine production alone and in combination with toll-like receptor ligands. *Am J Reprod Immunol* 2012;67:391–400.
- [49] Nastasi C. Butyrate and propionate inhibit antigen-specific CD8+ T cell activation by suppressing IL-12 production by antigen-presenting cells. *Sci Rep* 2017;7:1–10.
- [50] Singh N. Blockade of dendritic cell development by bacterial fermentation products butyrate and propionate through a transporter (Slc5a8)-dependent inhibition of histone deacetylases. *J Biol Chem* 2010;285:27601–8.
- [51] Arpaia N. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 2013;504:451–5.
- [52] Singh N. Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity* 2014;40:128–39.
- [53] Oliveira LDM, Teixeira FME, Sato MN. Impact of retinoic acid on immune cells and inflammatory diseases. *Mediators Inflamm* 2018 2018.
- [54] Cao T. The epigenetic modification during the induction of Foxp3 with sodium butyrate. *Immunopharmacol Immunotoxicol* 2018;40:309–18.
- [55] Trompette A. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med* 2014;20:159–66.
- [56] Thorburn AN. Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. *Nat Commun* 2015;6.

- [57] Park J. Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. *Mucosal Immunol* 2015;8:80–93.
- [58] Bailón E. Butyrate in vitro immune-modulatory effects might be mediated through a proliferation-related induction of apoptosis. *Immunobiology* 2010;215:863–73.
- [59] Luu M. Regulation of the effector function of CD8+ T cells by gut microbiota-derived metabolite butyrate. *Sci Rep* 2018;8:1–10.
- [60] Balmer ML. Memory CD8+ T cells require increased concentrations of acetate induced by stress for optimal function. *Immunity* 2016;44:1312–24.
- [61] Machiels K. A decrease of the butyrate-producing species *roseburia hominis* and *faecalibacterium prausnitzii* defines dysbiosis in patients with ulcerative colitis. *Gut* 2014;63:1275–83.
- [62] Paramsothy S. Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis. *Gastroenterology* 2019;156 1440-1454.e2.
- [63] Fukuda S. Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* 2011;469:543–9.
- [64] Rios-Covian D, Gueimonde M, Duncan SH, Flint HJ, De Los Reyes-Gavilan CG. Enhanced butyrate formation by cross-feeding between *Faecalibacterium prausnitzii* and *Bifidobacterium adolescentis*. *FEMS Microbiol Lett* 2015;362: 1–7.
- [65] Gill PA, van Zelm MC, Muir JG, Gibson PR. Review article: short chain fatty acids as potential therapeutic agents in human gastrointestinal and inflammatory disorders. *Aliment Pharmacol Ther* 2018;48:15–34.
- [66] Huda-Faujan N. The impact of the level of the intestinal short chain fatty acids in inflammatory bowel disease patients versus healthy subjects. *Open Biochem J* 2010;4:53–8.
- [67] Kumari R, Ahuja V, Paul J. Fluctuations in butyrate-producing bacteria in ulcerative colitis patients of North India. *World J Gastroenterol* 2013;19:3404–14.
- [68] Facchin S. Microbiota changes induced by microencapsulated sodium butyrate in patients with inflammatory bowel disease. *Neurogastroenterol Motil* 2020;13–25. doi: 10.1111/nmo.13914.
- [69] Boesmans L. Butyrate producers as potential next-generation probiotics: safety assessment of the administration of *butyricococcus pullicaecorum* to healthy volunteers. *mSystems* 2018;3:1–11.
- [70] Magnusson MK, Isaksson S, Öhman L. The anti-inflammatory immune regulation induced by butyrate is impaired in inflamed intestinal mucosa from patients with ulcerative colitis. *Inflammation* 2020;43:507–17.
- [71] Ferrer-Picón E. Intestinal inflammation modulates the epithelial response to butyrate in patients with inflammatory bowel disease. *Inflamm Bowel Dis* XX 2019;1–13.
- [72] Berndt BE. Butyrate increases IL-23 production by stimulated dendritic cells. *Am J Physiol – Gastrointestinal Liver Physiol* 2012;303:1384–92.
- [73] Tye H. NLRP1 restricts butyrate producing commensals to exacerbate inflammatory bowel disease. *Nat Commun* 2018;9.
- [74] Lloyd-Price J. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* 2019;569:655–62.
- [75] Laffin M. A high-sugar diet rapidly enhances susceptibility to colitis via depletion of luminal short-chain fatty acids in mice. *Sci Rep* 2019;9:1–11.