Trends in Microbiology

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Review

Microbial Regulation of Host Physiology by Short-chain Fatty Acids

Bart van der Hee[1] and Jerry M. Wells[1,*]

Our ancestral diet consisted of much more nondigestible fiber than that of many societies today. Thus, from an evolutionary perspective the human genome and its physiological and nutritional requirements are not well aligned to modern dietary habits. Fiber reaching the colon is anaerobically fermented by the gut bacteria, which produce short-chain fatty acids (SCFAs) as metabolic by-products. SCFAs play a role in intestinal homeostasis, helping to explain why changes in the microbiota can contribute to the pathophysiology of human diseases. Recent research has shown that SCFAs can also have effects on tissues and organs beyond the gut, through their circulation in the blood. SCFAs not only signal through binding to cognate G-protein-coupled receptors on endocrine and immune cells in the body but also induce epigenetic changes in the genome through effects on the activity of histone acetylase and histone deacetylase enzymes. Furthermore, epigenetic imprinting likely occurs *in utero*, highlighting the importance of the maternal diet in early life. Here we review current understanding of how SCFAs impact on human and animal physiology and discuss the potential applications of SCFAs in the prevention and treatment of human diseases.

Highlights

Short chain fatty acids (SCFAs) contribute to intestinal homeostasis and the regulation of energy metabolism.

SCFAs circulating in the blood influence tissue-specific acetylation of histones 3 and 4 in a tissue-specific fashion.

Delivery of SCFAs to the colon, using specialized diets, prevents onset of diabetes in nonobese diabetic (NOD) mice.

During gestation, SCFAs can cause epigenetic imprinting *in utero* and protect against allergic airway disease.

SCFAs regulate the blood–brain barrier and neuroimmunoendocrine functions.

SCFAs Are the Main Players in the Interplay between Diet, Microbiota, and Health

SCFAs have fewer than six carbons in the aliphatic tail, and the most abundant in the intestine are acetate (C2), propionate (C3), and butyrate (C4). SCFAs are a metabolic by-product of microbial fermentation of complex polysaccharides not digested, or only partly digested, in the human small intestine. These nondigestible polysaccharides (NDPs) are found in plant cell walls and are further classified into soluble and nonsoluble dietary fibers. The soluble NDPs are highly fermentable and typically generate greater quantities of SCFAs in the colon than do soluble fibers.

Currently there are three well characterized human SCFA-sensing G-protein-coupled receptors (GPCRs) which are differentially expressed on different sets of immune cells as well as epithelium and endocrine cells central to the regulation of metabolism (Table 1). Studies in GPCR-gene-deficient mice have established the importance of SCFA signaling through these metabolite receptors in the control of inflammation and intestinal homeostasis but the functional roles of these receptors on different cell types is still not fully understood.

Over the past century fiber intake by humans has decreased substantially as compared to the communities of populations eating traditional high-fiber diets [7]. This is especially the case in high-income countries where allergy, type 1 diabetes, inflammatory bowel disease (IBD), and autoimmune diseases have steadily increased over the past 60 years [8]. The importance of nondigestible fiber to health has recently been highlighted by a systematic review and meta-analyses of prospective studies and randomized controlled trials [9]. The results suggest a 15–30% decrease in all causes of cardiovascular-related mortality, type 2 diabetes, and colorectal cancer when comparing high- and low-fiber consumers [9].

[1]Host-Microbe Interactomics, Animal Sciences Group, Wageningen University, Wageningen, The Netherlands

*Correspondence: jerry.wells@wur.nl (J.M. Wells).

Table 1. Summary of Currently Recognized SCFA-binding GPCRs, Their Ligands, Associated G-protein-effector Mechanisms, and Likely Expression in Different Cell Types[a]

Receptor	Alternative	Carbons	Fatty acid	Transduction	Tissue	Cell type	Refs
GPCR41	FFAR3	C2-C5	Acetate, propionate, butyrate, formate	Gi/o, β-gastductin	Intestine, adipose tissue, spleen, immune cells, pancreas	Enteroendocrine L-cells, monocytes, neutrophils, mdDC, adipocytes,	[1]
GPCR43	FFAR2	C2-C5	Acetate, propionate, butyrate, formate, pentanoate	Gi/o, Gq, β-arrestin-2	Intestine, adipose tissue, skeletal muscle, immune cells, spleen, pancreas	Enteroendocrine L-cells, beta-cells, adipocytes, B/T-cells, myeloid cells, monocytes	[2,3]
GPCR109A	HCAR2, NIACR1	C4	Butyrate, niacin	Gi/o, β-arrestin-1	Immune cells, intestine (lumen), adipose tissue	Dendritic cells, macrophages, epithelial cells, mdDC, DC, macrophages, monocytes	[2,3]
OLFR78 (m), OR51E2 (h)	PSGR	C2-C3	Acetate, propionate	Gαs, unknown	Prostate, colon, lung	Enteroendocrine cells, prostate epithelium, airway smooth muscle cells, melanocytes	[4–6]

[a]Abbreviations: DC, dendritic cells; mdDC, monocyte-derived DC.

SCFAs are now taking center stage as key players in the interactions with the host that impact on health and disease, especially given recent evidence for their capacity to modify the epigenome and effects on tissues and organs beyond the gut. Here we review the proposed mechanisms by which specific SCFAs may impact on host physiology and the *in vivo* evidence for their health benefits. Finally, we highlight the major findings and outstanding questions which will help to exploit SCFAs for the prevention and treatment of human diseases.

SCFAs – The What, the Where, and the How

Early studies in human cases of sudden death showed that SCFAs are produced in high amounts by the gut microbiota, reaching concentrations of around 13 ± 6 mmol/kg content in the terminal ileum and 80 ± 11 mmol/kg content in the descending colon [10]. In all parts of the colon acetate is at least twofold higher in concentration than propionate or butyrate. Measurements of acetate, propionate, and butyrate in the ascending colon, where most saccharolytic fermentation occurs, varies depending on the geographic origin of the cohort, but acetate typically accounts for about 60–75% of the total fecal SCFAs [11].

Nutritionally specialized bacteria in the phyla Firmicutes and Actinobacteria are considered to be important in initiating the degradation of NDPs [12]. The continued breakdown of complex carbohydrates is attributed to certain abundant species within the phylum Bacteroidetes (Figure 1). Acetate production is common to many bacterial groups in the phylum Bacteroidetes, one of the largest groups in the intestine [10]. Propionate is produced by a few dominant genera, including the cornerstone species *Akkermansia muciniphila* [13]. *Bacteroides vulgatus* and *Bacteroides thetaiotaomicron* are also producers of propionate through the succinate pathway [14]. *Coprococcus catus* has been reported to consume lactate and utilize the acrylate pathway for propionate production [14]. Butyrate can be synthesized through four different pathways: acetyl-CoA, glutamate, lysine, and succinate [12]. Butyrate is produced by clostridial clusters I, III, IV, XI, XIVa, XV, and XVI of obligate anaerobes, of which cluster XIVa and cluster IV bacteria, related to *Faecalibacterium prausnitzii*, are the most abundant groups in humans. Members of clostridial cluster IX are also propionate producers via the lactate pathway. Small amounts of other SCFAs are also produced in the gut, namely caproate, formate, and valerate. Valerate can be formed by elongation of propionate in the presence of methanol as an electron donor [15]. Caproate can be formed by butyrate or acetate, or directly from lactate as an electron

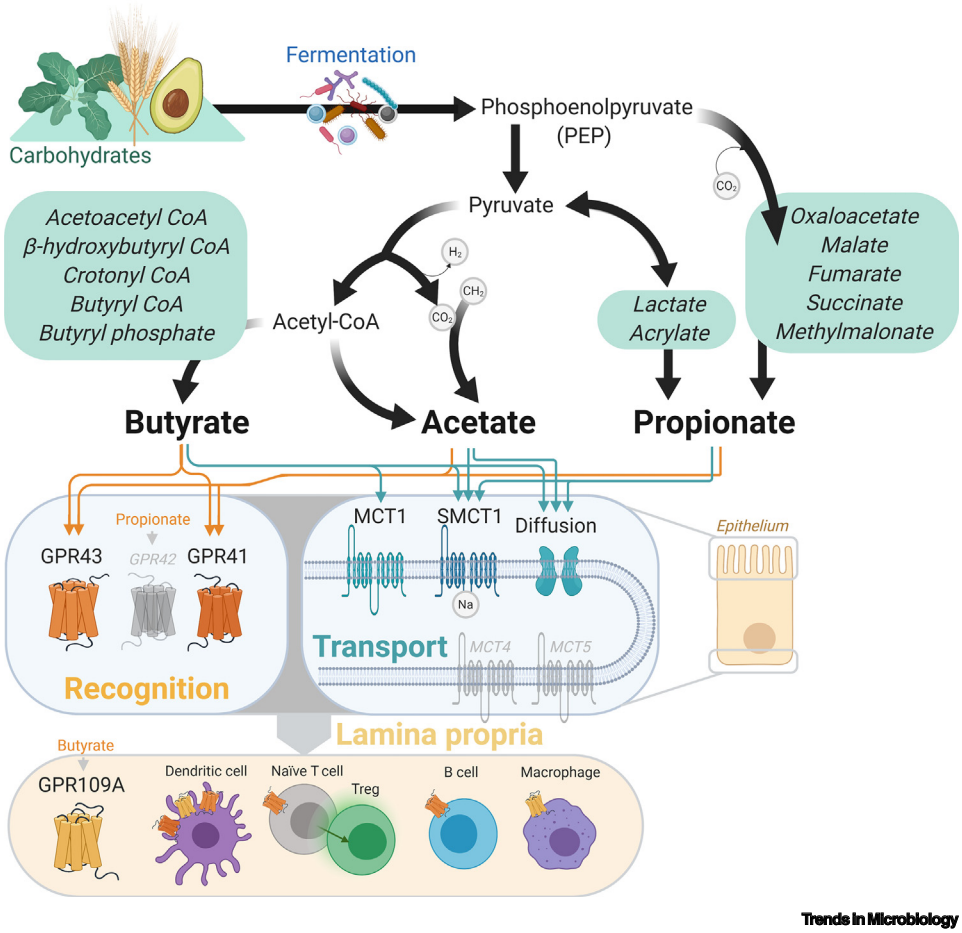

Figure 1. Gut Bacterial Pathways for Acetate, Propionate, and Butyrate Production by Fermentation of Nondigestible Fibers. Transporters MCT1 and SMCT1 take up short-chain fatty acids (SCFAs) from the lumen, and basolateral MCT4 and MCT5 are proposed to transport SCFAs out of the cell (light gray). SCFA receptors GPCR43 and 41 are expressed on intestinal epithelial cells and specific immune cells in the lamina propria and mucosal lymphoid tissue as indicated. GPCR42 (light gray) was a suspected pseudogene but was recently reclassified as a functioning gene, exhibiting sequence and copy number polymorphisms [22]. The figure was created using BioRender software. Abbreviation: Treg, T regulatory cell.

donor [16]. However, there are metabolic links between different types of bacteria, for example acetate produced by Bacteroidetes species can be utilized by species of Firmicutes to produce butyrate.

SCFAs can be passively taken up by epithelial cells but in greater amounts by active transport via the monocarboxylate transporter 1 (MCT-1) and to a lesser extent the sodium-coupled monocarboxylate transporter 1 (SMCT-1) [17,18]. SCFAs, especially butyrate, can be metabolized by colonocytes, providing 60–70% of their energy supply [19]. The remaining SCFAs are transported out of the cell across the basolateral membrane via an unknown HCO_3^- exchanger, suggested to be monocarboxylate transporter (MCT) 4 or 5 [20]. In the mucosa, SCFAs can enter the blood capillaries and reach the liver via the portal vein. The liver clears a major part of propionate and butyrate from the portal circulation, but acetate can reach 200 μM in the venous serum of humans and pigs [18,21].

The rapid absorption of SCFAs, and their metabolism by intestinal epithelium and liver, means that they can make a substantial contribution to the caloric requirements of humans and other

animals. In herbivorous ruminants, about 70% of the caloric requirement comes from SCFAs, and about 10% in omnivorous humans and pigs [23]. In patients with short-bowel syndrome (SBS), who lack a functional small intestine, NDPs have been used as a dietary strategy to overcome malabsorption because the SCFAs produced by bacterial fermentation in the colon can provide up to 1000 kcal per day [24,25]. SCFAs are also important for stimulating sodium and water absorption in the colon through the regulation of nutrient and ion transporters which can also help against diarrhea associated with SBS [26].

SCFA Signaling through Host GPCRs

Many common dietary metabolites are sensed by host GPCRs as a mechanism for the host to optimize responses and for survival with limiting nutrients (Figure 1 and Table 1). The same holds true for SCFAs which signal through three main GPCRs (Table 1). SCFA signaling through GPCRs on enteroendocrine cells, pancreatic cells and adipocytes plays an important role in the regulation of host metabolism [27–29] – for example, via butyrate- and propionate-stimulated secretion of glucagon-like peptide 1 (GLP-1) and the appetite-regulating hormone PYY in intestinal enteroendocrine cells [30–32]. Enteroendocrine cells also release GLP-2 in response to parenteral nutrition with butyrate, increasing plasma concentrations of GLP-2 [26,33]. GLP-2 increases the surface area of epithelial in the small intestinal by enhancing proliferation and inhibiting apoptosis in both humans and piglets [26,34]. GLP-2 also upregulates expression of the transporters for glucose, dipeptides, and amino acids in intestinal epithelial cells [35]. These effects of SCFAs most likely explain the intestinal enteropathy associated with starvation and the beneficial effects of prebiotic fiber on intestinal function.

GPCRs binding SCFAs are also expressed on intestinal enterocytes and other cell types in organs and tissues such as the liver, muscle, enteric neurons, and also in immune cells – indicating the breadth of their potential interactions throughout the body [36,37]. GPCR41 is highly expressed on sympathetic neuronal ganglia – in particular, the superior cervical ganglion (SCG) which controls energy expenditure via neural and hormonal effects on glucose and fat metabolism. GPCR41 is most abundantly expressed on the SCG during embryonic (E13.5 and E15.5) and postnatal (P1) stages but also in the sympathetic nervous system (SNS) of adult mice and humans [38]. GPCR41$^{-/-}$ mice have no growth differences to wild-type (WT) mice or abnormalities in metabolic parameters and hormones, but during development the SCG volume is significantly smaller than it is in WT mice, indicating that GPCR41 may be involved in sympathetic nerve growth [38]. These authors found that propionate promotes GPCR41-mediated SNS activation whereas β-hydroxybutyrate, a major ketone body produced during starvation, depresses activation of sympathetic neuronal ganglia. These findings indicate that SCFAs and ketone bodies control energy balance by directly regulating GPCR41-mediated sympathetic activation.

Fatty acid signaling through GPCRs expressed on immune cells is also important in immune regulation. GPCR43-deficient mice have exacerbated or unresolving inflammation in experimentally induced models of colitis, arthritis, and asthma [39]. GRPR41 but not GRP43, was also shown to be necessary for the protective effect of propionate in an induced allergic airway disease mouse model [40]. In the colon mucosa, vitamin B3 or butyrate binding to GPCR109A on antigen-presenting cells (APCs) induces an anti-inflammatory expression program in colonic APCs, which, in turn, induces differentiation of interleukin-10 (IL-10)-producing T regulatory cells (Tregs) [41].

SCFAs Modify the Host Epigenome

The epigenome describes the modifications to the genome that do not affect the DNA sequence but lead to altered gene expression. Epigenetic modifications include DNA methylation and histone modification which alter how the DNA is packaged into chromatin (Box 1) [42,43].

Box 1. Post-translational Modifications in the Histone Tails Enable Epigenetic Regulation

Chromatin comprises nucleosomes in which the DNA is wound around a central histone H3/H4 tetramer sandwiched between two histone H2A/H2B dimers. Adjacent nucleosomes are joined by a stretch of free linker DNA [92]. Further compaction is possible through the linking interaction of histone 1 proteins between nucleosomes. Condensed chromatin is generally limiting access of the transcription machinery to the DNA but can undergo relaxation in response to specific cellular and environmental signals to allow for DNA replication and transcription [93–95] (Figure I).

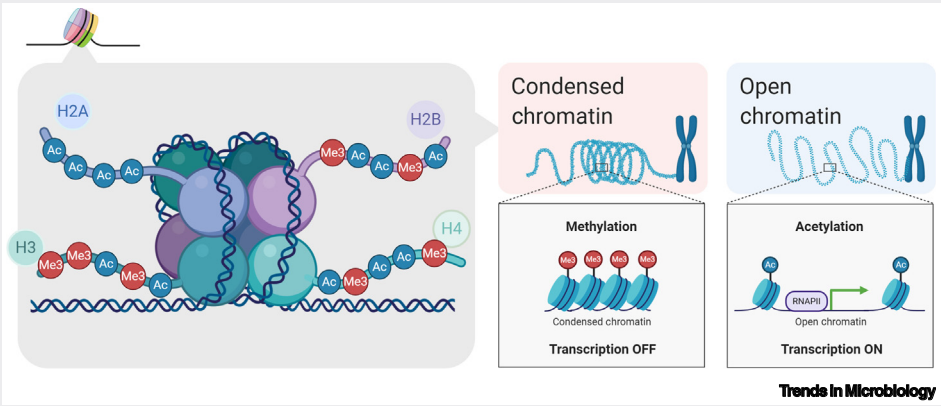

Figure I. Schematic Representation of DNA Wound Around a Central Histone H3/H4 Tetramer and Two Histone H2A/H2B Dimers. The accessible regions of histones that undergo post-translational modifications, such as acetylation and methylation, are depicted. The other panels depict the consequences of condensed or more open chromatic structures for gene transcription.

The accessible regions of histones, called histone tails, protrude from the nucleosome and undergo post-translational modifications (PTMs) such as acetylation, methylation, ubiquitination, and other modifications generating what is commonly referred to as 'the histone code'. The PTMs determine whether the chromatin is repressing or activating transcription. Acetylation of histone tails is carried out by histone acetyltransferase (HATs [43]) and reversed by histone deacetylases (HDACs). Acetylation of histone tails causes relaxation of chromatin through disruption of the DNA histone interaction, potentially activating transcription [96,97]. Conversely, removal of the acetyl groups by HDACs is considered to promote stronger histone–DNA associations. There are 18 known HDACs that are classified into four groups based on homologies and location. HDACs have also been shown to deacetylate more than 50 transcription factors and nonhistone targets, greatly extending their regulatory functions [98–100].

Butyrate is often described as an inhibitor of histone deacetylase (HDAC) activity (Box 1) based on a report describing the accumulation of acetylated histones in butyrate-treated cancer cells [44]. Due to the Warburg effect, cancer cells undergo a metabolic shift to primarily aerobic glycolysis. Under these conditions, butyrate and propionate accumulate, resulting in decreased HDAC activity and altered histone acetylation. However, if cancer cells are grown in low glucose concentrations then oxidative metabolism is increased, butyrate or propionate are oxidized to acetyl-CoA, and histone acetylation is not affected [45]. Thus, the epigenetic effects of butyrate exposure are strongly dependent on glucose metabolism and need to be considered when extrapolating results obtained in intestinal cancer cell lines to normal intestinal cells *in vivo* [45]. Indeed, many of the described effects of SCFAs on the epithelial barrier and integrity were performed *in vitro* using cancer or immortal intestinal cell lines (reviewed in [11,46]).

A role for SCFAs in histone modification of tissues in the body was definitively shown by the dietary supplementation of germ-free (GF) mice with microbially produced acetate, propionate, and butyrate. These SCFAs increased the acetylation of histone H4 and H3 in a tissue-specific fashion [47]. Moreover, this recapitulated much of the changes observed in chromatin states and gene expression due to microbial colonization, although the magnitude of the effects was less than in conventionally colonized mice [47]. Acetyl-CoA, an essential cofactor for histone acetyltransferase

(HAT) enzymes, can be converted from acetate or formed from the oxidation of propionate or butyrate. An increase in the intracellular pool of acetyl-CoA can increase HAT activity. Additionally, butyrate is reported to inhibit histone deacetylases [48] potentially by binding to the Zn^{2+} in the catalytic site [49].

Butyrate-producing *Clostridium* promotes Treg Development in the Colon

Tregs expressing Foxp3 play an important role in regulating inflammation in the mucosa and were shown to be induced by colonization of the intestine by microbiota. GF mice have substantially lower numbers of Tregs in the colon mucosa, but this could be reversed by oral administration of the spore-forming component of the colonic bacteria, and in particular the spore-forming clusters IV and XIVa of the genus *Clostridium* [50,51]. The mechanism was linked to increased secretion of latent TGF-β by epithelial cells exposed to butyrate as well as upregulation of membrane metalloproteases involved in maturation of TGF-β. Treg induction was also shown to be independent of mucosal lymphoid tissue, suggesting that Treg differentiation from naïve T cells occurs in the lamina propria of the colonic mucosa [33]. Later, Furusawa *et al*. [51] showed that administration of 0.1 mM butyrate to peripheral naïve $CD4^+$ T cells promoted histone H3 acetylation of the *Foxp3* gene promoter and intronic enhancers conserved in the noncoding region. These results were consistent with the earlier finding that *in vivo* administration of the HDAC inhibitor trichostatin A (TSA) leads to expansion of $Foxp3^+$ Tregs, increased suppressive activity of Tregs, and attenuation of colitis in mice [52]. More recently, Smith *et al*. showed that exposure of colonic Tregs to propionate induces a GPCR43-dependent decrease in expression of HDAC6 and HDAC9 [53]. Although Treg cells express multiple HDACs, HDAC9 is particularly important in regulating Foxp3-dependent suppression [52]. Moreover, butyrate exposure increases acetylation of several lysine residues in the forkhead domain of Foxp3 transcription factor that appear to be directly involved in optimal Treg function [54].

SCFAs Promote Intestinal Homeostasis

Butyrate and niacin signaling through receptor GPCR109a has been shown to confer anti-inflammatory properties in colonic macrophages and dendritic cells (DCs) [41], which are important for intestinal homeostasis [55]. In another study, using human monocyte-derived DCs, both butyrate and propionate strongly downregulated cytokine and chemokine gene expression in both immature and mature human monocyte-derived DCs, whereas only minor effects were seen with acetate [56]. In some studies, the anti-inflammatory effects of butyrate and propionate on macrophages and DCs were shown to be independent of GPCR receptors and or phenotypically reproduced by a pharmacological HDAC inhibitor [54,57].

Recently, butyrate – but not propionate or acetate – was shown to inhibit proliferation of stem/progenitor cells in the intestinal crypt [58]. It was proposed that the crypt structure, gradient diffusion, and efficient metabolism of butyrate by mature enterocytes would limit its access to stem and progenitor cells near the bottom of the crypt (Figure 2). This may explain why colonocytes express high levels of butyrate-metabolizing enzymes compared to propionate-metabolizing enzymes. The transcription factor involved in inhibition of cell proliferation was identified as FoxO3, which regulates several key cell-cycle genes [58]. It is not known precisely how butyrate affects FoxO3, but the acetylation of several lysine residues of FoxO3 are known to affect its regulatory activity (Figure 2). It is further suggested that butyrate induces rapid differentiation processes, possibly linking intestinal crypt morphological development to stem cell protection [58,59].

Butyrate further affects intestinal barrier function by physiologic hypoxia restoration through hypoxia-inducible factor (HIF) depleting local O_2 concentrations, as well as a direct increase in the

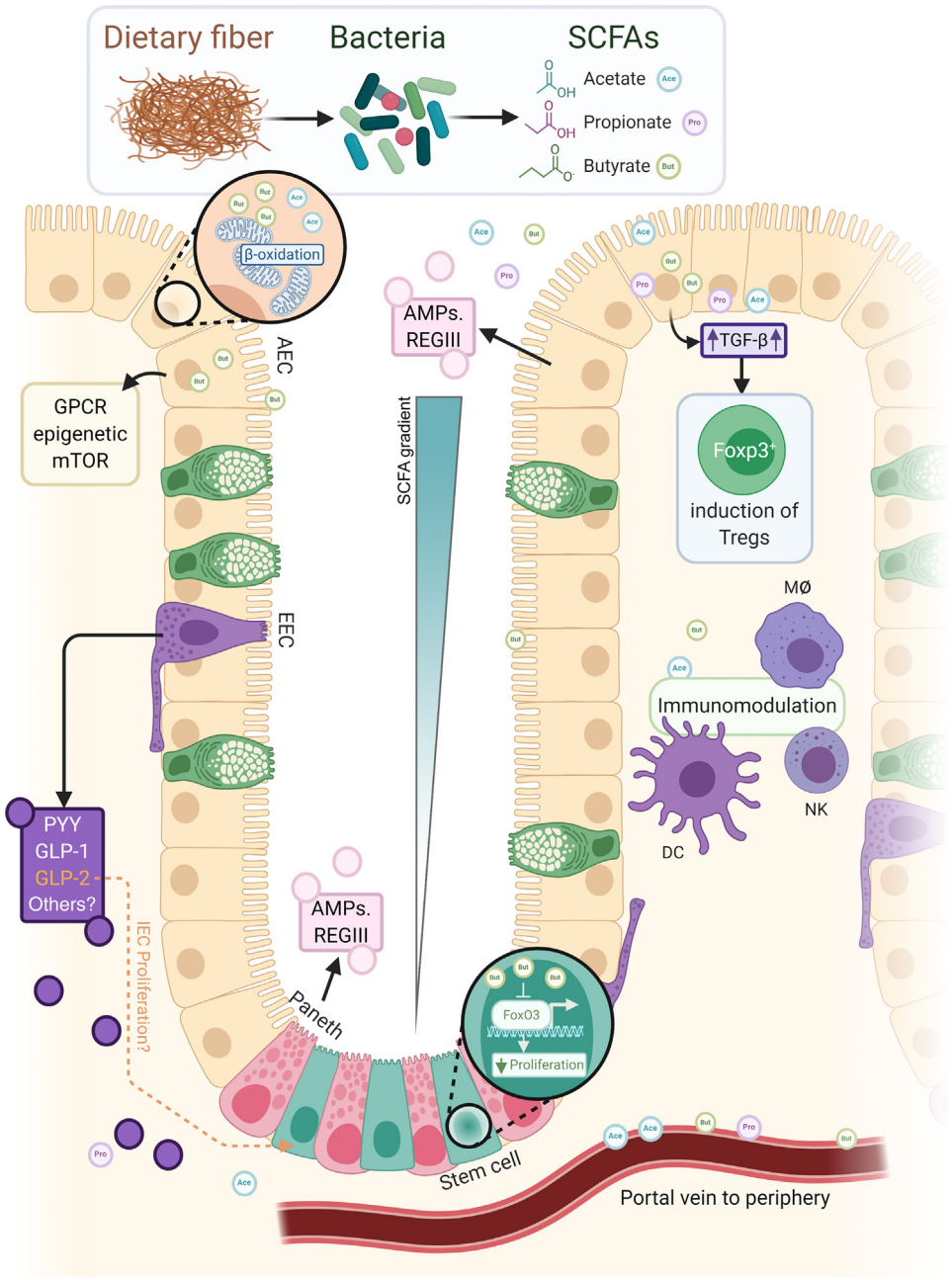

Figure 2. Interactions of Short-chain Fatty Acids (SCFAs) with the Colonic Epithelium by Binding to G-protein-coupled Receptors (GPCRs), mTor-signaling, or Epigenetic Mechanisms. Butyrate can be metabolized by mature enterocytes via anaerobic β-oxidation providing an energy supply. This has been proposed as an evolutionary adaptation to reduce the concentration of butyrate in the intestinal crypt where it can inhibit proliferation of stem/progenitor cells [61]. Inhibition of stem cell replication is mediated through FoxO3, a transcription factor involved in inhibition of cell proliferation, which is regulated by acetylation [61]. Butyrate induces expression of antimicrobial peptides (AMPs) and TGF-β in enterocytes [50,53]. Activated TGF-β promotes the development of inducible IL-10-producing T regulatory cells (Tregs) in the colon, which are important in controlling proliferation of effector T cells and suppressing inflammatory responses.

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tight-junction-related proteins occludin and zonula occludens, and also downregulation of claudins 1 and 2 [60].

Several studies have reported that SCFAs increase the production of antimicrobial peptides (AMPs) by epithelial cells from pigs, rabbits, and humans [61,62]. In a clinical trial, cathelicidin (LL-37) was induced in human rectal epithelium of an intervention group given an enema containing sodium butyrate (80 mM), twice daily for 3 days compared to placebo [61]. Recently, Zhao *et al*. [3] identified that SCFAs regulate intestinal expression of antimicrobial REGIIIβ/γ and β-defensins through a GPCR43-dependent mechanism. Previous studies have also linked IL-22 production with activation of STAT3, indicating a relationship between antimicrobial regulation of the host microbiome through nutrient-sensing pathways [3,63], like the SCFA axis, thereby also altering the composition of the microbiological environment [3].

SCFAs Beyond the Gut

As mentioned previously, SCFAs transported across the intestine can enter the circulation and directly affect metabolism or the function of peripheral tissues. Although the liver clears a major part of SCFAs from the portal circulation, acetate reaches peripheral concentrations of 19–160 μmol/l compared with propionate (1–13 μmol/l) and butyrate (1–12 μmol/l) [65]. Recent evidence suggests that SCFAs are likely to play an important role in the aetiology of several diseases, linking systemic effects with human diet and microbiota. The effects of butyrate on abundance of Tregs in the colon mucosa [63,64] and intestinal homeostasis are considered to be beneficial in several chronic diseases, including IBD [66]. Some major areas of therapeutic interest are outlined in more detail in the following text, and current knowledge of the effects of SCFAs on other organs and tissues, besides the gut, and also the developing fetus *in utero*, are summarized in Figure 3.

Type 1 Diabetes Mellitus

Genetic susceptibility plays a role in type 1 diabetes mellitus (T1D) but, as with other autoimmune and allergic diseases, there is compelling evidence of a role for environmental factors in the etiology of the disease [67]. Feeding specialized diets in the form of acetylated or butylated resistant starches, which release large amounts of the acetate or butyrate in the colon after bacterial fermentation, prevented onset of diabetes in nonobese diabetic (NOD) mice [68]. These results suggest that high-fiber diets and the microbiota might function cooperatively to reduce the risk of T1D in susceptible individuals. Interestingly, a combined acetate- and butyrate-yielding diet provided complete protection, which suggested that these SCFAs contribute to protection through distinct mechanisms [68]. The acetate-yielding diet alone altered the composition of B cell subsets in the spleen, whereas both diets decreased the frequency of autoreactive T cells in lymphoid tissues and reduced the expression of CD86 in IL-12-producing mature marginal-zone B cells, a subset implicated in the pathology of autoimmune disease [69]. In agreement with previous studies (discussed previously for the periphery) the butyrate-yielding diet increased the number and function of Tregs. The protection from diabetes provided by SCFAs probably also involves the engagement of metabolite-sensing GPCRs, such as GPCR43, on enteroendocrine-producing cells and pancreatic beta cells which play important roles in glucose tolerance [70].

Asthma

Recently, acetate administered in the drinking water to pregnant mice only during gestation was shown to protect the offspring *in utero* from induced allergic airway disease [71]. The mechanism

Butyrate induces anti-inflammatory properties in colonic macrophages and dendritic cells (DCs) via GPCR109a [41,56,57]. In enteroendocrine cells, butyrate induces the release of appetite-regulating hormone PYY and glucagon-like peptide 1 (GLP-1) which can enter blood vessels and decrease blood glucose levels by stimulating the production of insulin [31,32]. Additionally, release of GLP2 induces crypt cell proliferation and inhibition of apoptosis, resulting in an increase in villous height and in the expansion of the absorptive mucosal surface in the small and large intestine [64].

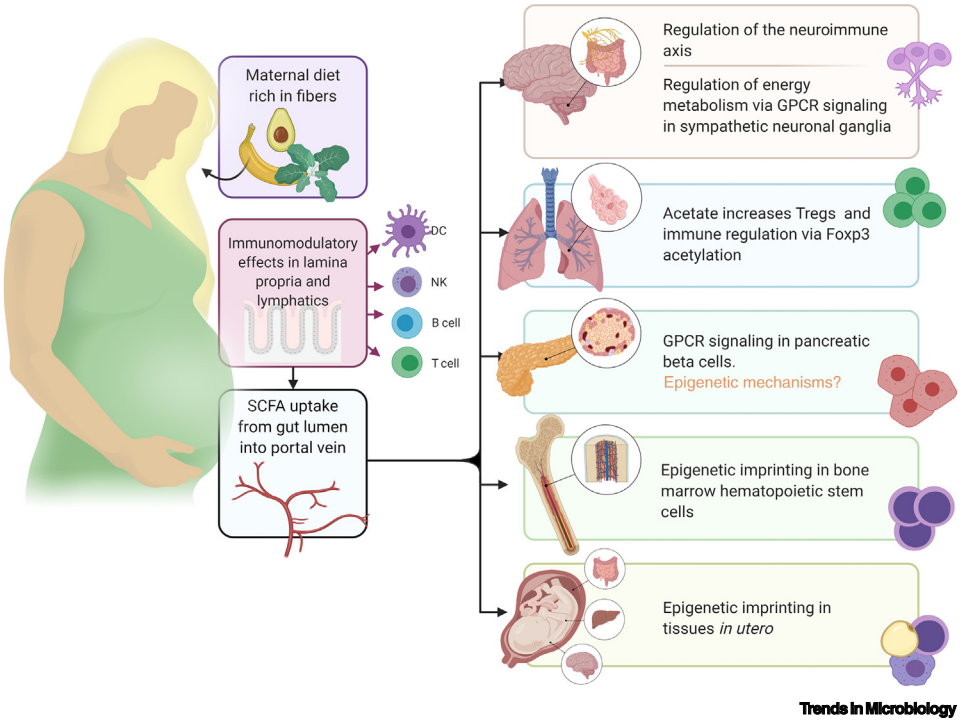

Figure 3. Effects of Short-chain Fatty Acids (SCFAs) on Organs and Tissues beyond the Gut, Including the Developing Fetus. Diets rich in nondigestible fiber increase microbial fermentation and the production of SCFAs in the colon, which promotes mucosal homeostasis through direct effects on intestinal epithelial cells and immune cells in the lamina propria (Figure 2). Hormones and transported SCFAs enter the blood vessels and, finally, the portal vein. Via the vascular system, SCFAs can influence energy homeostasis, endocrine and immune functions in different issues via G-protein-coupled receptor (GPCR)-dependent signaling, and epigenetic mechanisms, impacting on health and resilience to disease. SCFAs can also be found in the brain and cerebrospinal fluid (CSF) where they might influence growth and differentiation of neurones and synapses and inflammatory responses of brain glial cells.

of protection was linked to increased acetylation at the Foxp3 promoter and higher numbers of Tregs in the lung for at least 16 weeks after birth. Addition of acetate to the drinking water strongly decreased expression of three genes in the fetal lung (*Nppa*, *Ankrd1*, and *Pln*). This was most likely mediated by acetylation of Foxp3 in the lung epithelial cells, which was bound in higher amounts to the *Nppa* promoter of fetuses from pregnant mice supplemented with acetate than in the control mice. Acetate still protected against allergic airway disease in GPCR43$^{-/-}$ mice, but a role for GPCR41, which also binds acetate, cannot be ruled out [72].

There is also indirect evidence that the effect of acetate on immune regulation in the lung can be translated to humans. SCFAs were measured in a cohort of pregnant women, and data were collected on visits to the general practitioner for infant cough or wheeze in the first year. Strikingly higher levels of serum acetate, but not propionate or butyrate, were significantly associated with fewer respiratory problems in the infants [71].

Influence of SCFAs on the Blood–Brain Barrier and Neuroimmunoendocrine Function

There is growing interest in the role of the microbiota in the interconnected gut–brain axis, and here, SCFAs have already been implicated in the regulation of neuroimmune and neuroendocrine function [73]. The intestinal mucosa is innervated with a network of neurones regulating digestive processes that are influenced by secreted immune effector molecules [74]. The inner submucosal

plexus, which is closer to the intestinal lumen, mainly innervates the mucosa and the muscularis mucosae and plays a sensory role, for example, in regulating blood flow in the intestine and epithelial functions. Thus, SCFAs transported across the gut could have direct roles on the activity of neurones, such as the vagal afferent nerves which play a key role in satiety, stress response, and mood (reviewed in [73]). Recently, oral supplementation of mice with acetate, propionate, and butyrate in the drinking water for 1 week gave protection from the subsequent effects of chronic psychosocial stress [75].

SCFAs entering into blood vessels can also be transported across the blood–brain barrier (BBB) into brain and CSF [76,77]. This might directly influence levels of neurotrophic factors that regulate growth and differentiation of neurones and synapses in the brain. Although the mechanisms remain unclear, SCFAs have already been shown to modulate learning and memory in a range of brain disorders [78–80] and to regulate neuropeptides that favor appetite suppression [14].

In addition, SCFAs play a role in the permeability of the BBB. The BBB of GF mice is more permeable to small molecules than is the case with conventional mice, and their recolonization with a complex microbiota or SCFA-producing bacteria restores the BBB integrity [81].

Antiviral Immunity

Another aspect of immunity which appears to be regulated by microbial SCFAs concerns the priming and function of natural killer (NK) cells in the tissues. Ganal *et al*. [82] compared nonmucosal-associated mononuclear phagocytes in GF and conventionally housed mice and revealed increased acetylation at the transcription start sites of proinflammatory genes such as interleukin 6 (*IL-6*) and interferon-beta-1 (*Ifnb1*) in conventional specific-pathogen-free (SPF) mice. These epigenomic modifications were associated with increased expression of IL-6 and IFN-1 in mononuclear phagocytes in response to microbial ligands and viral infection. Increased expression of these cytokines led to enhanced priming of NK cells residing at nonmucosal sites and more effective antiviral immunity [82].

Metabolic Disease, Obesity, and Type 2 Diabetes Mellitus

As mentioned previously, SCFA metabolism by the host can increase energy harvest from the diet – which might contribute to obesity depending on the total caloric intake. By contrast, several studies in rodents have shown that SCFAs can protect against obesity by increasing energy expenditure and appetite control (reviewed in [65]). Propionate can influence control of body weight via sympathetic nervous system activity, as mentioned previously [38]. Additionally, SCFAs decrease expression of PPARγ, resulting in increased oxidative metabolism in the liver and adipose tissue as well as reduced body fat accumulation, hepatic steatosis, and increased insulin sensitivity [83]. Results from a recent randomized clinical trial in type 2 diabetic patients indicated that SCFAs may be a promising strategy to treat pancreatic dysfunction in early-stage type I and type 2 diabetes in humans [84].

Based on studies in rodents, SCFAs are considered to have a beneficial rather than detrimental effect on host metabolism. However, there are conflicting results on the beneficial effects of SCFAs on glucose homeostasis in humans, and further well controlled long-term intervention studies are needed to confirm the beneficial role of SCFAs in metabolic disease [65].

New-generation Probiotics for Increasing SCFA Production

Numerous studies on the fecal microbiota of IBD patients report a decreased abundance of the *Clostridium* clusters IV and XIVa, which includes butyrate-producers such as *Faecalibacterium prausnitzii* and *Roseburia intestinalis* [85]. Given the relatively high abundance of *F. prausnitzii* in the fecal microbiota of healthy adults and the role of butyrate in intestinal homeostasis (discussed

previously) there is much interest in using this human commensal as one of a new generation of probiotics for prevention and treatment of inflammatory diseases linked to the gut [86]. Moreover, the beneficial effects of *F. prausnitzii* may not be limited to butyrate production [86–88]. Similarly, administration of butyrate-producing *Clostridium tyrobutyricum* was shown to attenuate colitis in mice [89]. Additionally, *Clostridium butyricum* was demonstrated to have antidiabetic effects in mice [90] and neuroprotective effects in a mouse model of vascular dementia [80]. In the future we can expect more reports of interventions using prebiotics and probiotics to modulate gut microbiota to increase SCFA production and treat disease.

Concluding Remarks and Future Perspectives

Increasing evidence supports the notion that reduced intake of NDPs and production SCFAs by microbial fermentation in the colon lies behind the increasing incidence of diseases which have been steadily increasing in high-income countries over the past 50 years. However, the key to fully exploiting the opportunities for using SCFAs in disease prevention and treatment requires a better understanding of the mechanisms by which SCFAs exert their effects in the gut and other tissues and organs in the body. Furthermore, most research to date has focused on butyrate but, unlike acetate and propionate, it is typically present in undetectable or very low concentrations in the body. SCFAs appear to influence health through three principal mechanisms: (i) altering levels of HAT and HDAC activity, (ii) signaling by specific fatty acid-sensing GPCRs, and (iii) anti-inflammatory mechanisms in the periphery and tissues due to the first two mechanisms.

Unraveling the precise mechanisms by which SCFAs promote intestinal homeostasis, and protect from or ameliorate diseases, is further complicated by potentially broad effects of SCFAs on GPCR signaling, as well as epigenomic modifications. Moreover, we currently know little about the effects of SCFAs on the HAT- and HDAC-mediated post-translational modification of transcription factors, and how this alters their function, activity, and stability (see Outstanding Questions). The development of new tissue-specific GPCR41 and/or GPCR43 knockout mice will help to elucidate the effects of SCFAs in the body. Studies in organoids or primary cells more closely mimicking the conditions *in vivo* can also give further insights into the activities of SCFAs, especially in combination with selective agonists and antagonists of SCFA-sensing receptors [91]. In the future, SCFA receptor-selective pharmacological drugs might open up new possibilities for therapeutics, including applications in neurodegenerative diseases and behavior, by reinforcing BBB integrity, modulating neurotransmission, and influencing levels of neurotrophic factors.

Outstanding Questions

Can we discover selective agonists or antagonists, and produce cell-type-specific knockouts of SCFA GPCRs, to elucidate the precise mechanisms by which microbially produced SCFAs impact on host physiology?

Can we devise studies on organoids, with sustained exposure to SCFAs at concentrations found in the body, to translate findings to humans and uncover the precise mechanisms?

What are the effects of circulating acetate and propionate on animal and human physiology?

How do SCFAs influence the acetylation and activity of transcription factors?

What are the effects of SCFAs on different HAT and HDAC activities in different organs and tissues?

Can we devise strategies to effectively increase SCFAs in the colon, and produce SCFA-receptor-selective pharmacological drugs, in order to explore the possibilities for disease prevention and treatment?

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Declaration of Interests

There are no interests to declare.

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