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SCFA: mechanisms and functional importance in the gut. — Source link

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Short Chain Fatty Acids – mechanisms and functional importance in the gut.

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Abstract

In recent years, the importance of the gut microbiota in human health has been revealed and many publications have highlighted its role as a key component of human physiology. Owing to the use of modern sequencing approaches, the characterization of the microbiome in healthy individuals and in disease has demonstrated a disturbance of the microbiota, or dysbiosis, associated with pathological conditions. Microbiota establishes a symbiotic crosstalk with their host: commensal microbes benefit from the nutrient-rich environment provided by the gut and the microbiota produces hundreds of proteins and metabolites that modulate key functions of the host, including nutrient processing, maintenance of energy homeostasis and immune system development. Many bacteria-derived metabolites originate from dietary sources. Among them, an important role has been attributed to the metabolites derived from the bacterial fermentation of dietary fibers, namely the short chain fatty acids (SCFAs) linking host nutrition to intestinal

homeostasis maintenance. SCFAs are an important fuel for intestinal epithelial cells (IEC) and regulate IEC functions through different mechanisms to modulate their proliferation, differentiation as well as functions of subpopulations such as enteroendocrine cells, to impact gut motility and to strengthen the gut barrier functions as well as host metabolism. Recent findings show that SCFAs, and in particular butyrate, also have important intestinal and immuno-modulatory functions. In this review, we discuss the mechanisms and the impact of SCFAs on gut functions and host immunity and consequently on human health.

Introduction

Humans are colonized, at birth, by bacteria, archaea, fungi and viruses, which are collectively called the microbiota. Distinct microbiota inhabit all epithelial surfaces of the body: skin, oral cavity, respiratory, gastrointestinal and reproductive tracks [(1)]; with the largest and most diverse microbiota residing in the colon. The intestinal microbiota is composed of 100 trillions of bacteria which represent ~25 times as many genes as our own *Homo sapiens* genome. The diversity and complexity of the microbiota is influenced by the host genetic background, the diet and the environment. Reciprocally, this microbiota encodes thousands of genes absent in human genome that exert diverse functions often associated with beneficial physiological effects for its host [(2-4)]. From this close symbiotic relationship emerged the notion that humans and their microbiota form a composite organism, namely a holobiont [(5)]. Advances in next-generation sequencing and bioinformatics tools have shown that this relationship is far more complex than anticipated. Indeed, over the last decade, studies highlighted that perturbation of the microbiota, referred to as dysbiosis, and loss of bacterial diversity affect different host systems, particularly metabolic and immunes processes, that participate to host physiology and pathophysiologic conditions [(2)]. Moreover, growing lines of evidence suggest that the dialogue between microbiota and the host systems has a homeostatic role beyond the gut, and contributes directly to the global wellbeing of the host. In agreement with this, animal studies have demonstrated that microbiota is implicated in liver diseases, allergy, diabetes, airway hypersensitivity, autoimmune arthritis and even neurological disorders [(6-8)].

The human body has evolved to functionally interact with thousands of naturally occurring or microbiota-derived metabolites. Thus, the intestinal microbiome provides an extended repertoire of molecules and metabolites that influence the host health. Amongst those molecules, short chain fatty acids (SCFAs), derived from bacteria-dependent hydrolysis of fibers, have attracted considerable attention for their role in host health (figure 1A). Indeed, decreased abundance of SCFA-producing bacteria or decreased genomic potential for SCFA-production have been identified in many studies such type-1 diabetes, type-2 diabetes, liver cirrhosis, inflammatory bowel diseases (IBD) and atherosclerosis [(9-14)]. Here, we aim to provide an overview of bacterial SCFAs production in the gut, their impact on intestinal cells and host functions, and their different mechanisms of action.

SCFAs production and transport

Production of SCFAs

Complex dietary carbohydrates are metabolised by intestinal microbiota through an extensive set of enzymes, mostly absent in mammals and belonging to the large family of carbohydrate-active enzymes (CAZyme) (reviewed in [15]). The degradation of dietary fibers by gut microbiota, produces organic acids, gases and large amount of SCFAs. Acetate (C2), propionate (C3) and butyrate (C4) are the main SCFAs produced (60:20:20 mmol/kg in human colon). SCFAs can reach a combined concentration of 50–150 mM mainly in the colon where the microbial biomass is the highest [16-19]. Substrates for bacterial fermentation include non-digestible carbohydrates derived from dietary fibers such as polysaccharide plant cell walls, resistant starch, soluble oligosaccharide and endogenously products, such as mucin [20]. Aside bacterial fermentation, SCFAs can also be found in plant oil and animal fats. Butter contains 3 to 4% of butyrate in the form of tributyrin [21]. However, when fermentable fibers supply decreases, some bacterial species can switch to amino acids and protein fermentation as an alternative energy source, also contributing to SCFAs and branched chain fatty acids (BCFA) production [22, 23]. The BCFAs, *i.e.* isovalerate, 2-methylbutyrate and isobutyrate, are present at lower concentrations than SCFAs and originate only from proteins breakdown. Acetate is a net fermentation product for most gut bacteria while butyrate and propionate are produced by more specific bacterial species. Butyrate is produced from acetate, lactate, amino acids and various carbohydrates via the glycolysis from two different pathways, the butyryl-CoA:acetate CoA-transferase or the phosphotransbutyrykase and butyrate kinase pathway. Using FISH probes and PCR, Flint and colleagues have shown that specific families belonging to the Clostridiales order (Firmicutes) have the capabilities to produce butyrate: Lachnospiraceae (*Coprococcus, Eubacterium, Anaerostipes, Roseburia),* Ruminococcaceae (*Faecalibacterium, Subdoligranulum),*Erysipelotrichaceae (*Holdemanella*) [24-26]. The butyrate-producing capability of Clostridiales has been confirmed using *in vitro* culture in other genera such as *Clostridium, Butyrivibrio, Lachnoclostridium, Marvinbryantia, Oscillibacter, Flavonifractor, Erysipelatoclostridium, Anaerotruncus, Dorea and Blautia, Ruminiclostridium* [27, 28]. Propionate is produced in the gut from various substrate, including amino acids, carbohydrates, lactate and 1,2-propanediol. Hence, most hexoses and pentoses enter the succinate pathway and result in succinate production, a precursor of propionate. The succinate pathway is present in Bacteroidetes and some Firmicutes, such as the Negativicutes (*Veillonella,*

Phascolarctobacterium). Some other Firmicutes, belonging to the Negativicutes (*Megasphera*), the Lachnospiraceae (*Coprococcus*) and the Ruminocaccaceae, use the acrylate pathway, in which lactate is the substrate to produce propionate. The propanediol pathways is present in Proteobacteria and Lachnospiraceae species and use deoxyhexose sugars (*e.g.* fucose) as substrates. The commensal bacterium *Akkermansia muciniphila,* member of the Verrucomicrobia phylum also produces propionate from this later pathway [(29)]. Some bacteria, notably in the Lachnospiraceae family, can produce both propionate and butyrate but from different substrates, *e.g. Roseburia inulivorans* [(30)].

In vitro experiments have shown that Bacteroides growth is reduced relatively to Firmicutes and Actinobacteria because SCFAs negatively impact Bacteroides at mild acid pH [(31)]. Thus, SCFAs production by Firmicutes and Bacteroides may to be regulated by pH variations, with more Firmicutes fermentation in proximal colon (pH≈5.6) and conditions favoring Bacteroides fermentation in the distal colon with a more neutral pH (pH≈6.3) [(32)]. This selective gradient is limiting the propionate production and promoting butyrate formation in the more proximal part of the colon [(24)]. Intestinal pH is not the only factor that impact microbiota composition and consequently SCFAs production. Indeed, intestinal gases production (*e.g.* oxygen and hydrogen) and diet composition and intake (*e.g.* type of fibers and iron) have been reported to influence the microbiota composition and the gut SCFAs concentration [(33, 34)].

SCFAs transport

In the host, SCFAs have distinct roles depending of their absorption and local physiologic concentrations [(35, 36)]. Acetate, propionate and butyrate are weak acids with a pKa of 4.8 for butyrate. In physiological conditions the colonic pH range from 5.5 to 6.7, thus most of SCFAs are in the ionized form and require transporters for absorption [(37, 38)]. SCFA transporters are expressed at different level: in the small intestine: MCT1 (*SLC16A1*), SMCT2 (SLC5A12) and SLC16A7 and in the colon: MCT1 (*SLC16A1*), SMCT2 (*SLC5A12*), SMCT1 (*SL5CA8*) and SLC26A3[(20, 39)]. The transporters MCT1, SMCT1, SLC26A3 show affinities for all three major SCFAs while the other ones are more selective, *e.g.* SMCT2 only transports butyrate. Butyrate is mainly absorbed via MCT-1 that is expressed both at apical and basolateral membrane of colonic epithelial cells [(39, 40)]. From approximately 20mM in gut lumen, butyrate concentration on portal vein reaches a range of 5-10µM. The liver significantly uptakes butyrate as there is almost no splanchnic release [(41, 42)]. Butyrate venous concentration ranges from 0.5µM to 3.3µM [(32)]. Similarly, a larger amount of propionate is found in portal vein, around 32µM but

there is only a very small release from the liver. Venous concentration of propionate ranges from 3.8 to 5.4µM. In contrast, acetate is weakly absorbed by epithelial cells and the liver. The portal vein concentration of acetate is 98µM-143µM. Hence, liver efficiently metabolizes the butyrate and propionate released by the gut epithelium and avoids any acute increase even in the case of artificial enema [32, 41].

Cellular uptake of SCFAs in their anionic form are through H^+ or Na^+ coupled transporters. Thus, butyrate transport directly participates in electrolyte absorption with increases of Na^+ and Cl^- absorption and release of bicarbonate (HCO_3^-) in the lumen [39, 43, 44]. Interestingly, electrolytes absorption is region specific due to the different transporter expression levels in each gut regions [45]. Transport of butyrate is electroneutral though SMCT2 (Na^+), resulting in the transport of one Na^+ for each butyrate anion absorbed [46]. On the contrary, SMCT1 transport is electrogenic as two Na^+ are transported with one butyrate anion. This results in electrolytes and water absorption [47, 48]. MCT1 is a proton coupled transporter and has no direct role in ion transport. However, MCT1 indirectly regulates bicarbonate secretion through Na^+/H^+ and Cl^-/HCO_3^- exchangers. Interestingly, butyrate modulates the expression of many transporters including MCT1 and SMCT1, therefore potentially increasing electrolyte exchanges as well as its own transport. Butyrate blocks Cl^- secretion by inhibiting NKCC1 expression and increases expression of the Na^+/H^+ transporter NHE3 through HDAC inhibition and a SP1/3 dependent pathway [49-52].

Mechanisms

SCFAs receptors

The human genome encodes for six potential G protein-coupled receptors (GPCR) sensitive to SCFAs: GPR41 (FFAR3), GPR42, GPR43 (FFAR2), GPR109a (HCAR2), GPR164 (OR51E1) and OR51E2. GPR41 and GPR109a are exclusively Gαi/o coupled receptors whereas GPR43 can be coupled to either Gαβγq and $G\alpha_{i/o}$ and OR51E2 is αs coupled [53]. GPR42 was recently identified as a functional GPCR modulating Ca^{2+} channel flux, but only the Gβγ pathway downstream this receptor was explored [54]. The GPR41, GPR43 and GPR109a are expressed in numerous organs including the small and large intestine by various cell types: immune cells, adipose tissues, heart, skeletal muscle or neurons [20]. GPR43 (FFAR2), GPR41 (FFAR3) recognize acetate, butyrate and propionate with affinities that differ between species, whereas only butyrate activates GPR109a (Figure 1B) [55-58]. Schematically, GPR41 activation by

propionate and butyrate and GPR109a activation by butyrate lead to the inhibition of cAMP (cyclic adenosine monophosphate) accumulation and protein kinase A (PKA) and MAP kinases (ERK et p38) activation. On the other hand, GPR43 is activated by the three main SCFAs with approximately the same affinities. GPR43 engagement stimulates the phospholipase-Cβ, which releases intracellular Ca^{2+} and activates the protein kinase C (PKC) in addition to cAMP accumulation inhibition and PKA and ERK activation [59]. The highly polymorphic GPR42 is activated by propionate and modulates Ca^{2+} by a yet unknown mechanism that could be similar to GPR43 due to the very high homology between these two receptors. In humans, GPR42 is expressed in the colon and in sympathetic ganglia [54]. Butyrate is the ligand of GPR164 (OR51E1) expressed all along the gastrointestinal tract and specifically by enteroendocrine cells [60, 61]. The olfactory receptor OR51E2 (Olfr78 in mouse) is activated by propionate and acetate and result in cAMP and Ca^{2+} increase. Olfr78 is expressed at the gut mucosal level by PYY-positive colonic enteroendocrine cells [62]. It is also detected in various tissues, including kidney, blood vessels, lung and specific nerves in the heart and gut [63].

Transcriptional regulations and post-translational modifications

SCFAs have a broad impact in the host: metabolism, differentiation, proliferation mainly due to their impact on gene regulation. Indeed, several studies revealed that butyrate regulates the expression of 5 to 20% of human genes [64-66]. Within the cells, butyrate and propionate exhibit strong inhibition capacity of lysine/histone deacetylase (K/HDAC) activity, with butyrate being more potent than propionate [67, 68]. Moreover, butyrate is metabolized into acetyl-CoA which stimulates histone acetyltransferase (HAT) by further enhancing histone acetylation (Figure 1B) [66, 69]. By their HDAC inhibitor and HAT stimulatory properties, SCFAs promote post-translational modification of histones through increasing their acetylation. Histone hyperacetylation leads to an increased accessibility of transcription factors to the promoter regions of targeted genes owing the modulation of their transcription. HDAC inhibition by butyrate does not only up-regulate gene transcription, repression of several genes such as *LHR*, *XIAP* or *IDO-1* has been reported [27, 70]. In colonic cell line, 75% of the upregulated genes are dependent of the ATP citrate lyase (ACL) activity and 25% are independent at 0.5mM concentration but the proportion is reversed at high concentration (5mM). This suggests that the gene regulation mechanisms are different, depending on the butyrate concentration. It has been shown that butyrate does not only tune histone acetylation level but also acetylation of other proteins, “K/HDAC” inhibitor, including transcription factors such as SP1 and FOXP3

[71, 72]. SCFAs derived from the gut microbiota also promote crotonylation through their histone acetylase properties [73]. Histone crotonylation is abundant in the small and large bowel epithelium as well as in the brain. Crotonyl-CoA modification of histones is linked to the cell cycle regulation. Moreover, several studies have shown that butyrate also modifies DNA and protein methylation and phosphorylation levels [74-76].

Novel role of butyrate as ligand of transcription factors

Beside the extensive described effects of SCFAs on host physiology through GPRs and post-translational modifications, a novel role emerged for butyrate as ligand of two transcription factors, expanding our knowledge on microbial-host crosstalk. By exploring the mechanisms involved in the microbial modulation of Angiopoietin-like protein 4 (ANGPTL4), Alex and co-workers demonstrated that SCFAs induce ANGPTL4 transcription and secretion through a novel role as selective modulator of peroxisome proliferator-activated receptor γ (PPARγ) in colonic cell lines [77]. In this study, Alex and co-workers showed that butyrate promotes, similarly to PPARγ ligands, the interactions between PPARγ and multiple coactivators and binds into PPARγ binding pocket with a conformation similar to the known PPARγ agonists, the decanoic acid [77]. The evidence suggests, for the first time, an original function of butyrate as ligand for a transcription factor. This original mechanism was also reported for another nuclear transcription factor, the aryl hydrocarbon receptor (AhR) in human colonic cell lines [78]. This latter study described a ligand-dependent activation of human AhR by butyrate in synergy with its role as HDAC inhibitor. By using selective ligand antagonists and structural modelling, it emerges that butyrate activates human AhR by binding into its ligand binding pocket similarly to the AhR ligand FICZ [78]. Together these reports provide an expanded view of the possible mechanisms for butyrate to modulate human transcription factors activity that might apply to other transcription factors (Figure 1B).

Functional impact of SCFA on the host.

SCFA, regulators of the gut metabolism, proliferation and differentiation

SCFAs are efficiently taken up from the gut lumen by the intestinal epithelial cells (IEC) with different fates (Figure 1B). Butyrate is the primary energy source of IECs, being oxidized via the β-oxidation in the mitochondria. This catabolic process represents from 73% to 75% of oxygen consumption by human colonocytes, by which part of butyrate is converted into ketone bodies [79-81]. The main substrates of colonocytes are by order of preference, butyrate > ketone

bodies > amino acid > glucose. By using a high level of oxygen, the colonocytes metabolism maintains epithelial hypoxia with an oxygen partial pressure [pO_2] of less than < 1% oxygen (7.6 mmHg), thus favoring anaerobic commensals [82]. The capacity to produce ketone bodies and oxidize butyrate is a crucial difference between the small and large bowel. Epithelial cell butyrate oxidative capacity has been determined *in vitro* to be between 1 to 5 mM of butyrate, therefore when a greater concentration is available, SCFAs can affect cells functions such as K/HDAC inhibition [69, 83]. Moreover, butyrate absorption increases the pyruvate dehydrogenase kinases (PDK) which negatively regulates the pyruvate dehydrogenase (PDH) complex. The PDH decarboxylates pyruvate to produce acetyl-CoA and NADH, both necessary to the tricarboxylic acid (TCA) [84]. This dual action pushes the colonocyte metabolism from glycolysis to β-oxidation. After transport into the cells, butyrate enhances oxidative phosphorylation, which consumes oxygen [83]. Similarly, it has been demonstrated that fatty acid oxidation is reduced in germ-free mice compared to conventional mice [85]. Butyrate is not the only fatty acid metabolized. Acetate is a substrate for cholesterol and fatty acids synthesis and is metabolized in muscles. Propionate is a precursor for the synthesis of glucose in the liver [20, 25, 85]. Acetate and butyrate are also major substrates for lipogenesis in rat colonocytes [86].

Through the production of SCFAs, gut microbiota actively communicates with host cells and strongly modulates a variety of cellular mechanisms. Two of the main functions influenced by SCFAs and thus gut microbiota are cell proliferation and differentiation. Indeed, the proliferative activity of crypt epithelial cells as well as the migration of mature epithelial cells along the crypt-villus axis are greatly attenuated in antibiotic-treated and germ-free mice [87]. At physiological state, butyrate favors cells differentiation and inhibits proliferation. First evidences on IECs were demonstrated on cell lines [88, 89]. In these studies, long term incubation of intestinal cancerous cell lines with SCFAs resulted in differentiated phenotypes coupled to decreased cell proliferation. High concentration of butyrate is associated with inhibition of stem cells and proliferative cells in the crypts, through a HDAC inhibition-dependent binding of FOXO3 to promoters of key genes in the cell cycle [90]. Butyrate concentration near the crypt base is estimated to be 50–800 µM dose equivalent [85, 91, 92]. These studies indicate that butyrate concentration is low in the deep crypts and increasing in a gradient along the lumen-to-crypt axis. Butyrate metabolization by differentiated cells on the epithelium plateau may result in a protective depletion in the crypts that is protective for stem cells proliferation. Hence, the crypt

structure is suggested to be an adaptive mechanism protecting the gut epithelial stem cells of butyrate high concentration found in the lumen [90].

Interestingly, butyrate has a dualistic role in epithelial cellular metabolisms: it supports healthy cells as primary energy source for IECs and represses cancerous cells expansion. This is known as the "butyrate paradox" or "Warburg effect" (66). This is explained by a metabolic shift occurring in cancerous cells using preferentially glucose as energy source. The inhibition of cell proliferation is generally characterized by an increase in reactive oxygen species (ROS) production, DNA damages and cell cycle arrest, suggesting that SCFAs initiate apoptosis signaling in cancer cells [93-96]. Indeed, through the activation of the pro-apoptotic protein BAX, the upregulation of apoptosis-inducing factor-mitochondria associated 1 isoform 6 (AIFM1) and the reduction of mitochondrial membrane potential, SCFAs stimulate the cytochrome c release which drives caspase 3 activation [93]. Coherently, the induction of the CDK inhibitors p21 and p27 and the downregulation of heat-shock cognate 71 kDa protein isoform and survival is observed, leading to growth arrest [97, 98].

Another mechanism for propionate to inhibit cell proliferation is suggested to involve its role as GPCR agonist. In human monocyte and lymphoblast cancer cell lines, Bindels and colleagues observe that the effect on cell proliferation is dependent on GPR43 activation [99]. GPR43 displays a dual coupling through G_i and G_q protein families. While PLC blockage does not influence cell proliferation, increase in cAMP, mediated by the inhibition of G_i subunit, slightly reduces the propionate anti-proliferative effect, suggesting a mechanism dependent on cAMP levels [99].

Considering the important metabolic shift occurring in cancer cells, the production and availability of a large variety of metabolites are modified among which acetyl-CoA. Acetyl-CoA is crucial in several metabolic pathways and a fundamental cofactor for HATs. Consequently, different cell metabolites are produced, such as a large amount of lactate, which in turn could stimulate the growth of commensal bacteria and partially explain the anti-tumorigenic effect of some probiotics [100].

Regulation of gut endocrine functions, importance on host physiology

Among intestinal epithelial cells, enteroendocrine cells (EECs) play an important role in host physiology by secreting hormones that regulate food intake, insulin secretion and gut functions

in response to a variety of stimuli [101]. Among these stimuli, fiber-rich diets or infusion with SCFAs have been associated with increased circulating levels of gut hormones [102, 103]. Supporting these results, expression of butyrate receptors GPR43, GPR41 and GPR109a have been reported in enteroendocrine cells [104-106]. Acute stimulation of EECs by SCFAs is shown to trigger hormones secretion such as GLP-1 and PYY. The mechanism involves GPR43 activation leading to increased intracellular calcium, corresponding to the activation of a G_q coupled receptor [107]. Several studies have confirmed the role of GPR43 in the EEC response to SCFAs using additional knockout models or agonists[108-110]. In particular EECs, the L-cells, GPR41 is also involved in the GLP-1 secretory response as suggested by the results in GPR41 knockout animals or GPR41 agonists [106, 107]. However, GPR41 stimulation also inhibits GIP secretion from glucose insulinotropic polypeptide (GIP) producing EECs [111]. This inhibition of GIP-producing cells could correspond to the activation of Gi/o pathways which are mainly resulting in inhibitory responses. The exact role of GPR41 in GLP-1 secretion remains to be fully understood. The possibility of GPR41 hetero-dimerization with GPR43 has been recently highlighted and could explain a role of GPR41 in GLP-1 stimulatory activity[112]. Additionally, species differences are described in response to the different SCFAs. If propionate and acetate are strong stimuli for PYY and GLP-1 secretion in rodents at low concentrations, much higher concentrations are required to induce secretion in human [110, 113]. These divergences can be explained both by the variation of SCFAs affinities to the receptor families as well as the different receptors expression levels. Indeed, GPR41 is expressed in fewer EECs in humans compared to rodents [106, 114]. The role of other SCFAs receptors GPR109a, GPR42, OR51E1 and OR51E2, is still to be deciphered but some studies show that they are also enriched in some EECs subpopulations [62, 115].

In addition to the SCFA-dependent acute stimulation of gut hormone secretion, it emerged that SCFAs also tune EECs identity and consequently long-term hormonal production. Indeed, animals fed with fiber-rich diets have, in addition to a higher circulating gut hormone levels, an elevated number of enteroendocrine cells [102]. Supporting this result, an increase differentiation of epithelial cells into L-cells by SCFAs have been reported, with a higher GLP-1, PYY and serotonin production [103, 116-120]. GPR43 and GPR41 play important but different roles in the differentiation of enteroendocrine cells. GPR43 stimulation increased the number of the PYY-producing cells and *PYY* expression but not the number of GLP-1-positive cells which is dependent on GPR41 [116, 117].

Moreover, receptor-independent pathways are also involved in the expression regulation of gut hormone genes. Indeed, butyrate HDAC inhibitory activity highly increased *PYY* expression in human L-cells with a much stronger effect than GPR43 stimulation [116]. Modulation of *PYY* gene expression is associated with increased production and secretion both in basal and stimulated conditions and could explain the long-term effects of SCFAs on circulating gut hormone levels seen with fiber-enriched diets. Butyrate also impacts EECs responses to external stimuli by regulating the expression of receptors sensing exogenous molecules deriving from the microbiota. In particular, butyrate increases Toll-like receptors (TLR) expression in L-cells leading to an amplified stimulation by TLR ligands and a consequent higher NF-κB activation and butyrate-dependent *PYY* expression [121].

Due to their important functions on host, gut hormones link SCFAs and the modulation of other gut functions such as electrolyte absorption. Indeed, PYY is strongly associated with the modulation of electrolyte and water absorption functions due to the expression of NPY receptors on epithelial cells and neuronal cells [122, 123]. As SCFAs stimulate PYY release, they impact electrolyte absorption [124]. Similarly, serotonin is also important in water and electrolyte absorption. SCFAs also increase serotonin production, and blockade of serotonin receptors decreases butyrate-dependent electrolyte absorption [119, 125]. These results indicate that regulation of electrolytes absorption by SCFAs is mediated by multiple pathways including gut hormone modulations.

SCFAs have also been associated with tuning of intestinal transit [125]. Acute effect of SCFAs on gut motility is hormone dependent with an important role of PYY [126, 127]. Moreover, germ-free animals have decreased gut motility which is partially restored by SCFAs infusion in the colonic lumen, with butyrate having the highest effect [128]. The gut motility dysfunction in germ-free mouse could be partially explained by the highly dysregulated gut endocrine functions. However, no difference could be found in non-producing serotonin mouse model using TPH1 knockout mice [128]. This suggests that serotonin might not play an important role in the SCFAs-dependent regulation of gut motility and effects previously described could be minor compared to other pathways [125]. Interestingly, SCFAs, and mostly butyrate, have a direct effect on gut motility through regulation of enteric neurons [126]. Indeed, some enteric neurons express GPR41 and can therefore respond to SCFAs [106]. Additionally, HDAC inhibition by butyrate increases gut motility on the long term by increasing the number of

acetylcholine and substance P positive neurons, highlighting the importance of distinct mechanisms triggering similar effects [129].

Butyrate and other SCFAs are therefore important regulators of EECs functions, both by acutely stimulating gut hormone secretion, and modulating their production. Indeed, SCFAs increase EECs subpopulation cell numbers and regulate genes expression. Different mechanisms including receptor activation and HDAC inhibition are involved in these functions, highlighting the important and diverse roles of SCFAs as signaling molecules. Modulations of gut hormones participate in the many roles of SCFAs on host physiology including gut homeostasis.

Barrier function and Immune responses

In the last decade, SCFAs have attracted considerable attention for their impact on host immune responses and barrier functions. SCFAs play one of their major roles by maintaining an environment favourable for commensal bacteria and controlling pathogens growth. By stabilising the transcription factor HIF, butyrate increases O_2 consumption by IECs favouring the physiologic hypoxia in the colon [130]. Maintenance of the colonic anaerobic environment is key to favour the anaerobes commensal component of the gut microbiota and control the pathogens level such as *Salmonella* in a virtuous cycle [131-133]. However, enteric pathogens such as *Salmonella enterica* serovar Typhimurium are highly adapted to the colonic environment and utilize the gut microbiota-derived butyrate to compete with resident bacteria [134]. Besides effect on the O_2 level in the intestinal tract, butyrate promotes the epithelial barrier functions by reducing the epithelial permeability via HIF [130]. Moreover, butyrate reduces epithelial permeability by the regulation of IL-10 receptor, occludin, zonulin and claudins, reinforcing the tight junctions and the trans-epithelial resistance *in vitro* [135, 136]. Another important mechanism involved in the epithelial barrier function is the modulation of the mucus layer thickness protecting the mucosa. In the colon, MUC2 is the predominant mucin glycoprotein produced by the goblet cells. Treatment with butyrate increases *MUC2* production both *in vitro* and in human colonic biopsies [32, 137]. SCFAs enhance the epithelial barrier functions by modulating the antimicrobial peptides (AMP) secretion by the gut epithelium. Butyrate increases the level of colonic LL-37 *in vitro* and an *in vivo* [138, 139]. Activation of GPR43 by butyrate induce RegIIIγ and β-defensins expression by the activation of the mTOR pathway and STAT3 phosphorylation in mouse IECs [140]. The modulations of β-defensins in

epithelial cells relies on the inhibition of HDAC3 [141]. Interestingly, SCFAs and butyrate in particular, promote AMPs targeting both gram positive and negative bacteria.

It is now clear that gut microbiota plays an important role in intestinal homeostasis by controlling the human immune response notably by the production of SCFAs. Indeed, SCFAs have a global anti-inflammatory effect by up-regulating both anti-inflammatory and down-regulating pro-inflammatory cytokines by different mechanisms and consequently promoting mucosal homeostasis [142]. This anti-inflammatory effect can be mediated by IECs as binding of SCFAs to GPR43 and GPR109a induces Ca^{2+} efflux and membrane hyperpolarization which activate the inflammasome activating protein NLRP3 thereby induce the release of IL-18 with a protective effect on DSS colitis mouse model [143]. *In vitro* experiments demonstrate that the increase of protein acetylation by butyrate decreases IL-8 production in IECs [144]. Moreover, butyrate, and to a lesser extent propionate, upregulate the production of TGFβ1 in IECs, a cytokine promoting anti-inflammatory regulatory T cells (Treg) [145, 146]. Our group have shown that butyrate acts independently of the main GPCRs, *via* its HDAC inhibition property and the SP1 transcription factor present on the human TGFβ1 promoter [28]. Moreover, in mice, fiber supplementation promotes vitamin A metabolism in small intestine epithelial cells by increasing *RALDH-1*. The production of retinoic acid by epithelial cells, the active metabolite of vitamin A, is crucial for the tolerogenic imprinting of dendritic cells [147].

The impact of SCFAs go beyond the epithelial cells, with similar mechanisms reported in macrophages and dendritic cells (DCs). In mice, macrophages stimulation with butyrate, imprints through HDAC3 inhibition, a metabolic reprogramming and elevates antimicrobial peptides. Hence, upon stimulation, AMPs belonging to the S100 family, ficolin and lysozyme are increased [148]. Here again, butyrate has a stronger antimicrobial effect than propionate and no protective impact is detected with acetate. Butyrate treatment of DCs derived from human donors, decreases their capacity to present antigens and increases IL-10 production leading to a tolerogenic phenotype [149]. Upon LPS treatment, butyrate induces the IL-23 production by DCs thus promoting the differentiation of naive T lymphocytes into pro-inflammatory Th17 [150]. Another study shows that DCs treated with butyrate induce the differentiation of naive T lymphocytes into anti-inflammatory Tr1 producers of IL-10 [151]. By regulating the transcriptional activity, butyrate decreases the inflammatory response of macrophages exposed to inflammatory microbial molecules such as LPS and induces their polarisation through a M2 anti-inflammatory phenotype [152, 153]. Similarly, butyrate-dependent activation of GPR109a

411 increases the tolerogenic response of colonic macrophages and DCs reducing colonic
412 inflammation and promoting homeostasis [154]. Furthermore, it has been shown that butyrate
413 pre-treatment down-regulates nitric oxide (NO), IL-6, and IL-12 in mice independently of
414 TLRs and GPCRs pathways. Neutrophils migration is increased upon treatment with SCFAs,
415 in a GPR43-dependent mechanism [155].

416 Regulatory T cells (Treg) are critical for limiting intestinal inflammation and have thus been
417 subject of considerable attention to improve diseases such as inflammatory bowel disease
418 (IBD). Many studies showed that Tregs depend on microbiota-derived signals for proper
419 development and function [145, 146, 156, 157]. Recently several groups identify SCFAs as key
420 metabolites for promoting differentiation of naive T lymphocytes into Treg cells in the intestine
421 [71, 145, 146, 152, 154, 158, 159]. By interacting directly with naive T cells, butyrate and propionate
422 increase the acetylation of the promoter of the transcription factor Foxp3 essential for the
423 differentiation of Tregs, leading to an increase of Foxp3 expression [71, 152, 158]. Another group
424 suggested that propionate might induce the same changes via GPR43 [71, 159]. Moreover,
425 butyrate-dependent activation of GPR109a increases the tolerogenic response of colonic
426 macrophages and DCs, promoting Treg and IL-10 producing T cells[154]. Interestingly, SCFAs
427 increase the TGFβ1 production by IECs via its HDAC inhibition property thus promoting the
428 Tregs differentiation in the gut [28, 145, 146]. Altogether, these studies highlight that the molecular
429 mechanisms induced by SCFAs to control Treg-development are complex and involve many
430 cells types involved in the tolerogenic environment such as myeloid cells and IECs.

431 Impact of SCFAs on other lymphocyte populations such as B cells has not been as extensively
432 studied than their Treg counterparts. Acetate supplementation in mice increases intestinal IgA
433 in a GPR43 dependent mechanism [160]. Dietary fibres and SCFAs enhance antibody response
434 to bacteria by supporting B cell differentiation into plasma B cells via the increase of histone
435 acetylation and of B cell metabolism [161, 162]. Mechanistically, it is through the downregulation
436 of B cell AID and Blimp1, dependent on their HDAC inhibitory activity that SCFAs inhibited
437 class-switch DNA recombination, somatic hypermutation and plasma cell differentiation.
438 Interestingly, SCFAs also modulate the fate of B-cell producing autoantibodies and reduce
439 autoimmunity in lupus-prone mice [162].

Conclusion:

The past decade of biological research through a combination of translation-focused animal models and studies in humans has highlighted the overarching roles that the gut microbiota plays in human health. It has become clear that dysbiotic microbiota is associated with a wide range of pathologies such as obesity, diabetes, cardiovascular diseases, autoimmune diseases and neuronal disorders. Despite the lack of evidence in human, causality has been demonstrated in rodent models. Factors such as antibiotics use, modern sanitation, quality of diet and environmental factors linked with the lifestyle changes that occurred in the last century in developed societies are suggested to contribute to a decrease in the diversity of the human microbiome [163].

Diet and nutritional status are important determinants in human health. Numerous studies have shown that diet modulates the composition and functions of the microbiota in humans and animal models [164-166]. These interventional studies showed that microbiota composition is dynamic, can shift rapidly to dietary changes and that this shift is individual dependent and depends on the microbiota diversity of the donor. Thus, the role of diet in shaping microbiota is changing our view of the strategies to take to improve the systemic health. Indeed, it is though that nutritional interventions could manipulate the microbial ecology and consequently modulate human physiology with beneficial health outcomes. However, what constitutes an optimal health-promoting microbiota and how individuals with distinct microbiota can achieve such level of diversity are still open questions.

As discussed in this review, the gut microbial metabolites, SCFAs, are well known to exert a wide beneficial impact to the host [167, 168]. Hence, fiber-induced increase of SCFA-producing bacteria has been proposed to play an important role in the prevention and treatment of many diseases. Supporting this idea, clinical studies reported that prebiotics and dietary fibers increased the relative abundance of these beneficial SCFAs-producing bacteria and butyrate fermentation, leading to the improvement of type 2 diabetes and ulcerative colitis [169, 170]. However, the microbiota produces a vast number of metabolites that modulate host responses, sometimes in synergy with SCFAs [121]. Many studies support the benefits of increasing both the amount and the variety of dietary fibers ingested but it is difficult to establish whether it is a direct role of SCFAs or the increased bacterial diversity that impact host homeostasis. As the microbiota is a complex ecosystem, much work remains to be done to investigate fully the functions of SCFAs alone or with other beneficial metabolites in physiology and pathophysiology.

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Conflict of interest:

The authors declare that there is no conflict of interest regarding the publication of this paper.

Figure legend:

Figure 1: A. Functional impact of SCFA on the host. B. Mechanisms: 1: GPCR-dependent signaling, 2. Histone and transcription factor acetylation by SCFAs, 3. Role of butyrate as ligand of transcription factors.

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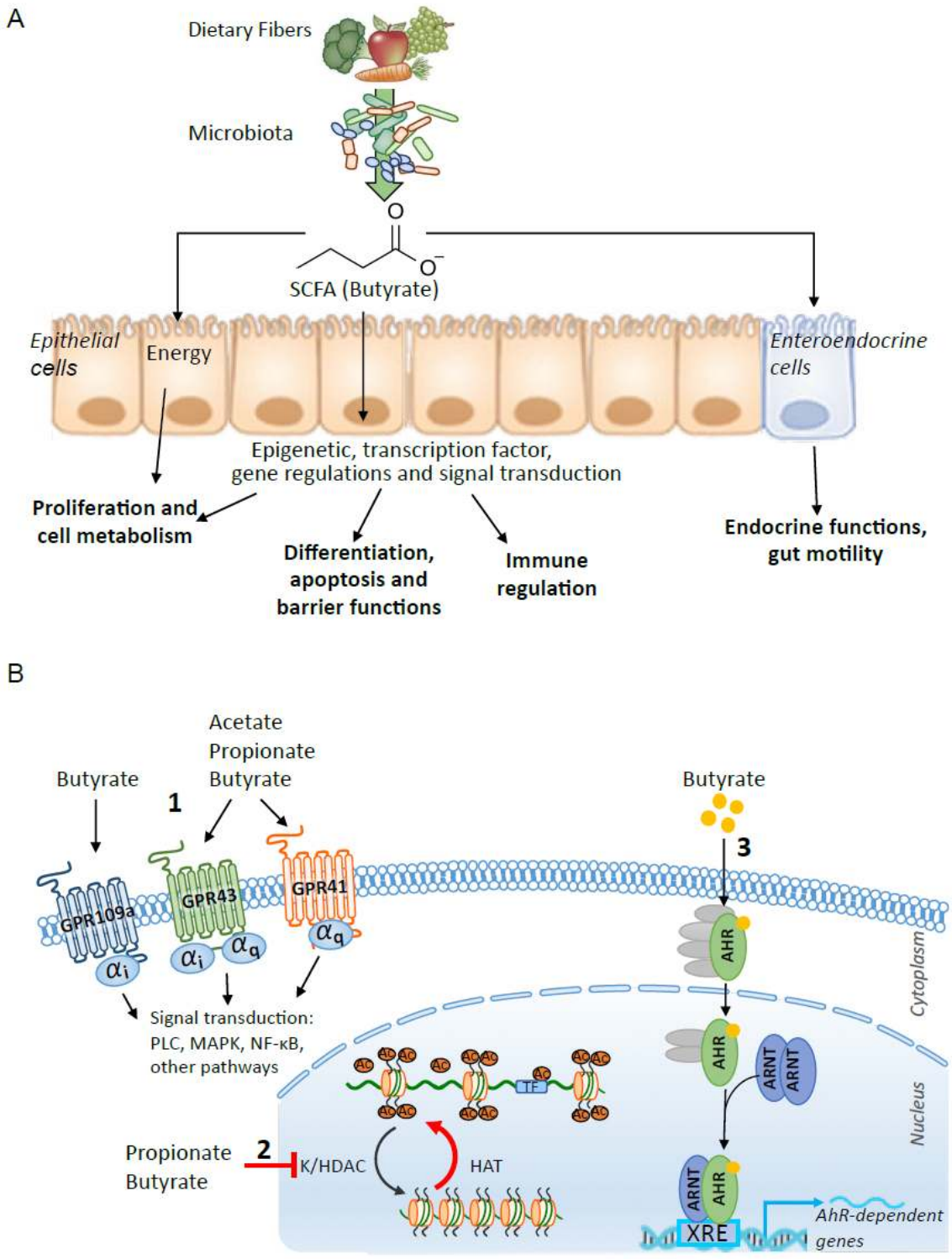
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