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

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15 **Abstract**

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

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

34

35 **Introduction**

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

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

65 SCFAs production and transport

66 *Production of SCFAs*

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

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

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

115 *SCFAs transport*

116 In the host, SCFAs have distinct roles depending of their absorption and local physiologic
117 concentrations^(35, 36). Acetate, propionate and butyrate are weak acids with a pKa of 4.8 for
118 butyrate. In physiological conditions the colonic pH range from 5.5 to 6.7, thus most of SCFAs
119 are in the ionized form and require transporters for absorption^(37, 38). SCFA transporters are
120 expressed at different level: in the small intestine: MCT1 (*SLC16A1*), SMCT2 (*SLC5A12*) and
121 *SLC16A7* and in the colon: MCT1 (*SLC16A1*), SMCT2 (*SLC5A12*), SMCT1 (*SL5CA8*) and
122 *SLC26A3*^(20, 39). The transporters MCT1, SMCT1, *SLC26A3* show affinities for all three major
123 SCFAs while the other ones are more selective, *e.g.* SMCT2 only transports butyrate. Butyrate
124 is mainly absorbed via MCT-1 that is expressed both at apical and basolateral membrane of
125 colonic epithelial cells^(39, 40). From approximately 20mM in gut lumen, butyrate concentration
126 on portal vein reaches a range of 5-10 μ M. The liver significantly uptakes butyrate as there is
127 almost no splanchnic release^(41, 42). Butyrate venous concentration ranges from 0.5 μ M to
128 3.3 μ M⁽³²⁾. Similarly, a larger amount of propionate is found in portal vein, around 32 μ M but

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

134 Cellular uptake of SCFAs in their anionic form are through H⁺ or Na⁺ coupled transporters.
135 Thus, butyrate transport directly participates in electrolyte absorption with increases of Na⁺
136 and Cl⁻ absorption and release of bicarbonate (HCO₃⁻) in the lumen ^(39, 43, 44). Interestingly,
137 electrolytes absorption is region specific due to the different transporter expression levels in
138 each gut regions ⁽⁴⁵⁾. Transport of butyrate is electroneutral though SMCT2 (Na⁺), resulting in
139 the transport of one Na⁺ for each butyrate anion absorbed ⁽⁴⁶⁾. On the contrary, SMCT1
140 transport is electrogenic as two Na⁺ are transported with one butyrate anion. This results in
141 electrolytes and water absorption ^(47, 48). MCT1 is a proton coupled transporter and has no direct
142 role in ion transport. However, MCT1 indirectly regulates bicarbonate secretion through
143 Na⁺/H⁺ and Cl⁻/HCO₃⁻ exchangers. Interestingly, butyrate modulates the expression of many
144 transporters including MCT1 and SMCT1, therefore potentially increasing electrolyte
145 exchanges as well as its own transport. Butyrate blocks Cl⁻ secretion by inhibiting NKCC1
146 expression and increases expression of the Na⁺/H⁺ transporter NHE3 through HDAC inhibition
147 and a SP1/3 dependent pathway ⁽⁴⁹⁻⁵²⁾.

148 **Mechanisms**

149 *SCFAs receptors*

150 The human genome encodes for six potential G protein-coupled receptors (GPCR) sensitive to
151 SCFAs: GPR41 (FFAR3), GPR42, GPR43 (FFAR2), GPR109a (HCAR2), GPR164 (OR51E1)
152 and OR51E2. GPR41 and GPR109a are exclusively G α i/o coupled receptors whereas GPR43
153 can be coupled to either G α β γ q and G α i/o and OR51E2 is α s coupled ⁽⁵³⁾. GPR42 was recently
154 identified as a functional GPCR modulating Ca²⁺ channel flux, but only the G β γ pathway
155 downstream this receptor was explored ⁽⁵⁴⁾. The GPR41, GPR43 and GPR109a are expressed
156 in numerous organs including the small and large intestine by various cell types: immune cells,
157 adipose tissues, heart, skeletal muscle or neurons ⁽²⁰⁾. GPR43 (FFAR2), GPR41 (FFAR3)
158 recognize acetate, butyrate and propionate with affinities that differ between species, whereas
159 only butyrate activates GPR109a (Figure 1B) ⁽⁵⁵⁻⁵⁸⁾. Schematically, GPR41 activation by

160 propionate and butyrate and GPR109a activation by butyrate lead to the inhibition of cAMP
161 (cyclic adenosine monophosphate) accumulation and protein kinase A (PKA) and MAP kinases
162 (ERK et p38) activation. On the other hand, GPR43 is activated by the three main SCFAs with
163 approximately the same affinities. GPR43 engagement stimulates the phospholipase-C β , which
164 releases intracellular Ca²⁺ and activates the protein kinase C (PKC) in addition to cAMP
165 accumulation inhibition and PKA and ERK activation ⁽⁵⁹⁾. The highly polymorphic GPR42 is
166 activated by propionate and modulates Ca²⁺ by a yet unknown mechanism that could be similar
167 to GPR43 due to the very high homology between these two receptors. In humans, GPR42 is
168 expressed in the colon and in sympathetic ganglia ⁽⁵⁴⁾. Butyrate is the ligand of GPR164
169 (OR51E1) expressed all along the gastrointestinal tract and specifically by enteroendocrine
170 cells ^(60, 61). The olfactory receptor OR51E2 (Olf78 in mouse) is activated by propionate and
171 acetate and result in cAMP and Ca²⁺ increase. Olf78 is expressed at the gut mucosal level by
172 PYY-positive colonic enteroendocrine cells ⁽⁶²⁾. It is also detected in various tissues, including
173 kidney, blood vessels, lung and specific nerves in the heart and gut ⁽⁶³⁾.

174 *Transcriptional regulations and post-translational modifications*

175 SCFAs have a broad impact in the host: metabolism, differentiation, proliferation mainly due
176 to their impact on gene regulation. Indeed, several studies revealed that butyrate regulates the
177 expression of 5 to 20% of human genes ⁽⁶⁴⁻⁶⁶⁾. Within the cells, butyrate and propionate exhibit
178 strong inhibition capacity of lysine/histone deacetylase (K/HDAC) activity, with butyrate being
179 more potent than propionate ^(67, 68). Moreover, butyrate is metabolized into acetyl-CoA which
180 stimulates histone acetyltransferase (HAT) by further enhancing histone acetylation (Figure
181 1B) ^(66, 69). By their HDAC inhibitor and HAT stimulatory properties, SCFAs promote post-
182 translational modification of histones through increasing their acetylation. Histone
183 hyperacetylation leads to an increased accessibility of transcription factors to the promoter
184 regions of targeted genes owing the modulation of their transcription. HDAC inhibition by
185 butyrate does not only up-regulate gene transcription, repression of several genes such as *LHR*,
186 *XIAP* or *IDO-1* has been reported ^(27, 70). In colonic cell line, 75% of the upregulated genes are
187 dependent of the ATP citrate lyase (ACL) activity and 25% are independent at 0.5mM
188 concentration but the proportion is reversed at high concentration (5mM). This suggests that
189 the gene regulation mechanisms are different, depending on the butyrate concentration. It has
190 been shown that butyrate does not only tune histone acetylation level but also acetylation of
191 other proteins, “K/HDAC” inhibitor, including transcription factors such as SP1 and FOXP3

192 ^(71, 72). SCFAs derived from the gut microbiota also promote crotonylation through their histone
193 acetylase properties ⁽⁷³⁾. Histone crotonylation is abundant in the small and large bowel
194 epithelium as well as in the brain. Crotonyl-CoA modification of histones is linked to the cell
195 cycle regulation. Moreover, several studies have shown that butyrate also modifies DNA and
196 protein methylation and phosphorylation levels ⁽⁷⁴⁻⁷⁶⁾.

197 *Novel role of butyrate as ligand of transcription factors*

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

216 **Functional impact of SCFA on the host.**

217 *SCFA, regulators of the gut metabolism, proliferation and differentiation*

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

223 bodies > amino acid > glucose. By using a high level of oxygen, the colonocytes metabolism
224 maintains epithelial hypoxia with an oxygen partial pressure [pO₂] of less than < 1% oxygen
225 (7.6 mmHg), thus favoring anaerobic commensals⁽⁸²⁾. The capacity to produce ketone bodies
226 and oxidize butyrate is a crucial difference between the small and large bowel. Epithelial cell
227 butyrate oxidative capacity has been determined *in vitro* to be between 1 to 5 mM of butyrate,
228 therefore when a greater concentration is available, SCFAs can affect cells functions such as
229 K/HDAC inhibition^(69, 83). Moreover, butyrate absorption increases the pyruvate
230 dehydrogenase kinases (PDK) which negatively regulates the pyruvate dehydrogenase (PDH)
231 complex. The PDH decarboxylates pyruvate to produce acetyl-CoA and NADH, both
232 necessary to the tricarboxylic acid (TCA)⁽⁸⁴⁾. This dual action pushes the colonocyte
233 metabolism from glycolysis to β-oxidation. After transport into the cells, butyrate enhances
234 oxidative phosphorylation, which consumes oxygen⁽⁸³⁾. Similarly, it has been demonstrated
235 that fatty acid oxidation is reduced in germ-free mice compared to conventional mice⁽⁸⁵⁾.
236 Butyrate is not the only fatty acid metabolized. Acetate is a substrate for cholesterol and fatty
237 acids synthesis and is metabolized in muscles. Propionate is a precursor for the synthesis of
238 glucose in the liver^(20, 25, 85). Acetate and butyrate are also major substrates for lipogenesis in
239 rat colonocytes⁽⁸⁶⁾.

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

255 structure is suggested to be an adaptive mechanism protecting the gut epithelial stem cells of
256 butyrate high concentration found in the lumen ⁽⁹⁰⁾.

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

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

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

282 *Regulation of gut endocrine functions, importance on host physiology*

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

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

308 In addition to the SCFA-dependent acute stimulation of gut hormone secretion, it emerged that
309 SCFAs also tune EECs identity and consequently long-term hormonal production. Indeed,
310 animals fed with fiber-rich diets have, in addition to a higher circulating gut hormone levels,
311 an elevated number of enteroendocrine cells ⁽¹⁰²⁾. Supporting this result, an increase
312 differentiation of epithelial cells into L-cells by SCFAs have been reported, with a higher GLP-
313 1, PYY and serotonin production ^(103, 116-120). GPR43 and GPR41 play important but different
314 roles in the differentiation of enteroendocrine cells. GPR43 stimulation increased the number
315 of the PYY-producing cells and *PYY* expression but not the number of GLP-1-positive cells
316 which is dependent on GPR41 ^(116, 117).

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

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

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

348 acetylcholine and substance P positive neurons, highlighting the importance of distinct
349 mechanisms triggering similar effects ⁽¹²⁹⁾.

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

357 *Barrier function and Immune responses*

358 In the last decade, SCFAs have attracted considerable attention for their impact on host immune
359 responses and barrier functions. SCFAs play one of their major roles by maintaining an
360 environment favourable for commensal bacteria and controlling pathogens growth. By
361 stabilising the transcription factor HIF, butyrate increases O₂ consumption by IECs favouring
362 the physiologic hypoxia in the colon ⁽¹³⁰⁾. Maintenance of the colonic anaerobic environment
363 is key to favour the anaerobes commensal component of the gut microbiota and control the
364 pathogens level such as *Salmonella* in a virtuous cycle ⁽¹³¹⁻¹³³⁾. However, enteric pathogens
365 such as *Salmonella enterica* serovar Typhimurium are highly adapted to the colonic
366 environment and utilize the gut microbiota-derived butyrate to compete with resident bacteria
367 ⁽¹³⁴⁾. Besides effect on the O₂ level in the intestinal tract, butyrate promotes the epithelial barrier
368 functions by reducing the epithelial permeability via HIF ⁽¹³⁰⁾. Moreover, butyrate reduces
369 epithelial permeability by the regulation of IL-10 receptor, occludin, zonulin and claudins,
370 reinforcing the tight junctions and the trans-epithelial resistance *in vitro* ^(135, 136). Another
371 important mechanism involved in the epithelial barrier function is the modulation of the mucus
372 layer thickness protecting the mucosa. In the colon, MUC2 is the predominant mucin
373 glycoprotein produced by the goblet cells. Treatment with butyrate increases MUC2 production
374 both *in vitro* and in human colonic biopsies ^(32, 137). SCFAs enhance the epithelial barrier
375 functions by modulating the antimicrobial peptides (AMP) secretion by the gut epithelium.
376 Butyrate increases the level of colonic LL-37 *in vitro* and an *in vivo* ^(138, 139). Activation of
377 GPR43 by butyrate induce RegIII γ and β -defensins expression by the activation of the mTOR
378 pathway and STAT3 phosphorylation in mouse IECs ⁽¹⁴⁰⁾. The modulations of β -defensins in

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

381 It is now clear that gut microbiota plays an important role in intestinal homeostasis by
382 controlling the human immune response notably by the production of SCFAs. Indeed, SCFAs
383 have a global anti-inflammatory effect by up-regulating both anti-inflammatory and down-
384 regulating pro-inflammatory cytokines by different mechanisms and consequently promoting
385 mucosal homeostasis⁽¹⁴²⁾. This anti-inflammatory effect can be mediated by IECs as binding
386 of SCFAs to GPR43 and GPR109a induces Ca²⁺ efflux and membrane hyperpolarization which
387 activate the inflammasome activating protein NLRP3 thereby induce the release of IL-18 with
388 a protective effect on DSS colitis mouse model⁽¹⁴³⁾. *In vitro* experiments demonstrate that the
389 increase of protein acetylation by butyrate decreases IL-8 production in IECs⁽¹⁴⁴⁾. Moreover,
390 butyrate, and to a lesser extent propionate, upregulate the production of TGFβ1 in IECs, a
391 cytokine promoting anti-inflammatory regulatory T cells (Treg)^(145, 146). Our group have shown
392 that butyrate acts independently of the main GPCRs, *via* its HDAC inhibition property and the
393 SP1 transcription factor present on the human TGFβ1 promoter⁽²⁸⁾. Moreover, in mice, fiber
394 supplementation promotes vitamin A metabolism in small intestine epithelial cells by
395 increasing *RALDH-1*. The production of retinoic acid by epithelial cells, the active metabolite
396 of vitamin A, is crucial for the tolerogenic imprinting of dendritic cells⁽¹⁴⁷⁾.

397 The impact of SCFAs go beyond the epithelial cells, with similar mechanisms reported in
398 macrophages and dendritic cells (DCs). In mice, macrophages stimulation with butyrate,
399 imprints through HDAC3 inhibition, a metabolic reprogramming and elevates antimicrobial
400 peptides. Hence, upon stimulation, AMPs belonging to the S100 family, ficolin and lysozyme
401 are increased⁽¹⁴⁸⁾. Here again, butyrate has a stronger antimicrobial effect than propionate and
402 no protective impact is detected with acetate. Butyrate treatment of DCs derived from human
403 donors, decreases their capacity to present antigens and increases IL-10 production leading to
404 a tolerogenic phenotype⁽¹⁴⁹⁾. Upon LPS treatment, butyrate induces the IL-23 production by
405 DCs thus promoting the differentiation of naive T lymphocytes into pro-inflammatory Th17
406⁽¹⁵⁰⁾. Another study shows that DCs treated with butyrate induce the differentiation of naive T
407 lymphocytes into anti-inflammatory Tr1 producers of IL-10⁽¹⁵¹⁾. By regulating the
408 transcriptional activity, butyrate decreases the inflammatory response of macrophages exposed
409 to inflammatory microbial molecules such as LPS and induces their polarisation through a M2
410 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 TGF β 1 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⁾.

440 **Conclusion:**

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

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

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

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481

482 **Conflict of interest:**

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

484

485 **Figure legend:**

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

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