J Atheroscler Thromb, 2017; 24: 660-672.
http://doi.org/10.5551/jat.RV17006

Review

Are Short Chain Fatty Acids in Gut Microbiota Defensive Players for Inflammation and Atherosclerosis?

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Intestinal flora (microbiota) have recently attracted attention among lipid and carbohydrate metabolism researchers. Microbiota metabolize resistant starches and dietary fibers through fermentation and decomposition, and provide short chain fatty acids (SCFAs) to the host. The major SCFAs acetates, propionate and butyrate, have different production ratios and physiological activities. Several receptors for SCFAs have been identified as the G-protein coupled receptor 41/free fatty acid receptor 3 (GPR41/FFAR3), GPR43/FFAR2, GPR109A, and olfactory receptor 78, which are present in intestinal epithelial cells, immune cells, and adipocytes, despite their expression levels differing between tissues and cell types. Many studies have indicated that SCFAs exhibit a wide range of functions from immune regulation to metabolism in a variety of tissues and organs, and therefore have both a direct and indirect influence on our bodies. This review will focus on SCFAs, especially butyrate, and their effects on various inflammatory mechanisms including atherosclerosis. In the future, SCFAs may provide new insights into understanding the pathophysiology of chronic inflammation, metabolic disorders, and atherosclerosis, and we can expect the development of novel therapeutic strategies for these diseases.

Key words: Short chain fatty acids, Butyrate, Microbiota, Inflammation, Atherosclerosis

Introduction

There are up to 100 trillion (1×10^{14}) microbes in the human intestinal tract[1], including bacteria, fungi, and viruses. Collectively, these are called the intestinal microbiota. The microbiota and eukaryotic species in our intestines activate countless numbers and amounts of enzymes, and in doing so they play a fundamental role in the control of physiological functions[2]. The interaction between microbiota and the host influences immunological homeostasis, and changes in this interaction are associated with various inflammatory diseases[3].

Fatty acids with a carbon number between 2 and 6 are considered short chain fatty acids (SCFAs) and have the following names: C2: acetic, C3: propionic, C4: butyric, C5: valeric, and C6: caproic acid. The main constitutive materials in animals are acetate, propionate, and butyrate[4]. In humans, SCFAs are produced from dietary fibers and resistant starches that cannot be decomposed by digestive enzymes through fermentation by the microbiota in the cecum and colon[4, 5].

Atherosclerosis is associated with lipid accumulation and inflammation in the arterial wall. Aggravation of inflammation in the arterial wall induces instability of atheromatous plaques and formation of occlusive thrombosis, leading to atherosclerotic cardiovascular disease (ASCVD) events, including acute coronary syndrome and stroke. Recent studies suggest that gut microbiota and the metagenome are associated with the inflammatory condition known as atherogenesis[6, 7]. This brief review will focus on SCFAs, especially butyrate, and their effects on various inflammatory mechanisms including atherosclerosis. As for the connections between gut microbiota, immunity, and atherosclerosis, recent excellent reviews should also be consulted[8-11].

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Received: April 4, 2017
Accepted for publication: April 7, 2017

Table 1. SCFAs (Acetate, Propionate, Butyrate) production by microbiota in the Gut

SCFAs	Pathways/Reactions	Producers	References
Acetate	from pyruvate via acetyl-CoA	Most of the enteric bacteria	Louis P, *et al*., Nat Rev Microbiol. 2014, 12: 661-72.[12]
		(Representative of species bacteria) *Akkermansia muciniphilia*,	Rey FE, *et al*,. J Biol Chem. 2010, 285: 22082-90.[13]
		Bateroides spp., *Bifidobacterium* spp., *Prevotella* spp.,	
		Ruminococcus spp.	
	Wood-Ljungdahl pathway	*Blautia hydrogentrophica*, *Chrostridium* spp., *Streptococcus* spp.	
Propionate	succinate pathway	*Bacteroides* spp., *Phascolarctobacterium succinatutens*,	Louis P, *et al*., Nat Rev Microbiol. 2014, 12: 661-72.[12]
		Dalister spp., *Veilonella* spp.	Scott KP, *et al*., J Bacteriol. 2006, 88: 4340-9.[14]
	acrylate pathway	*Megasphaera elsdenii*, *Coprpcoccus catus*	
	propanediol pathway	*Salmonella* spp., *Roseburia inulinivorans*, *Ruminocossus obeum*	
Butyrate	phosphotransbutyrylase/ butyrate kinase route	*Coprococcus comes*, *Coprococcus eutactus*,	Duncan SH, *et al*., Appl Environ Microbiol. 2002, 68: 5186-90.[15]
			Louis P, *et al*., Nat Rev Microbiol. 2014, 12: 661-72.[12]
	butyryl-CoA: acetate CoA-transferase route	*Anaerostripes* spp. (A, L), *Coprococcus catus* (A), *Eubacterium rectale* (A),	
		Eubacterium hallii (A, L), *Faecalibacterium prausnitzii* (A), *Roseburia* spp. (A)	

Citationed from Koh A, *et al*, Cell 165, 1332-45[8]
spp., species; (A), acetate is the substrate for producing butyrate; (L), lactate is the substrate for producing butyrate

Intestinal Bacterial Flora and Short Chain Fatty Acids

In accordance with systematic taxonomy, microbiota are organized by phylum, class, order, family, genus, and species. It has been difficult to culture microbiota, since most are obligate anaerobes, and thus many of their specific roles have remained unknown. However, recent developments in genetic analysis, including 16S ribosomal RNA sequencing and metagenomics analysis, have begun to reveal their function[1]. The predominant microbiota bacteria that produce SCFAs are classified as *Ruminococcaceae* (*cluster IV*) and *Eubacterium* (*cluster XIVa*) in the order *Clostridia*, class *Clostridia*, and phylum *Firmicutes*. The predominant producers of SCFAs are shown in **Table 1**[8, 12-15].

SCFAs account for 2-10% of the total energy consumption in humans, are the main energy source for large intestinal epithelial cells, and affect the production of mucins (mucus). In addition, SCFAs physiologically influence blood flow to the colon mucous membrane, the absorption of fluids and electrolytes, the autonomic nervous system, and the secretion of gut hormones[8]. A considerable part of the beneficial effect of prebiotics (usually non-digestive fiber compounds) is thought to be due to SCFAs produced by intestinal microbes[16]. Research has shown that the concentration of SCFAs is 70-140 mmol/L in the proximal colon and 20-70 mmol/L in the distal colon[17]. In general, acetate is thought to be more prevalent, followed by proprionate and butyrate; however, assessing the ratios of SCFAs is extremely difficult since their production depends on the various types of fermentation substrates. Animal experiments have shown that the total amount of SCFAs may be approximately 400-600 mmol/day when 60 g/day of undigested carbohydrates reach the colon[18]. In humans, however, since most studies on SCFAs have been conducted using fecal samples through absorp-

tion, the abovementioned estimates of intestinal concentrations may not reflect actual conditions, and many issues are yet to be elucidated. **Table 2** shows the concentrations of SCFAs in the feces of adult humans that have been previously reported[19-33].

Nearly all SCFAs absorbed by the colon are thought to pass through the portal vein from the colon capillaries and reach the liver, though the concentrations of SCFAs in the human portal vein are broad-ranging. **Table 3** shows the results of the several studies that have reported on the concentrations of SCFAs in the portal vein[4, 34-36].

The concentrations of SCFAs in healthy adult human peripheral blood are estimated as 100 to 150 μmoL/L for acetate, 4 to 5 μmoL/L for propionate, and 1 to 3 μmoL/L for butyrate, indicating that these concentrations in peripheral blood are vastly lower than in the intestinal tract[4]. In experiments using rats, a correlation has been found between the cecum content and portal or aortic serum concentrations of total SCFAs after feeding with highly fermentable fiber diets (pectin, guar gum, and fructo-oligosaccharides)[37]. Oral administration of tributyrin (a prodrug of butyrate) increased plasma butyrate concentrations in the portal vein to 2.4 mmol/L at 1 h and 0.7 mmol/L at 2.5 h[38].

Short Chain Fatty Acids and Their Receptors

Several receptors for SCFAs have been found to be G-protein coupled receptors (GPR). Among these, GPR41 and 43 have been renamed as free fatty acid receptor (FFAR) 3 and FFAR2, respectively. GPR41/FFAR3 is distributed throughout the entire body with a high degree of expression in the intestinal tract, immune cells, and fatty tissues[39, 40], and is thought to be associated with adiposity and energy homeostasis[41].

GPR43/FFAR2 is expressed in intestinal tract epithelial cells and immune system cells, which suggests that it is related to cell chemotaxis and activation[8, 39]. Interestingly, GPR43/FFAR2 is also expressed within adipocytes in white adipose tissue, and experiments using GPR43/FFAR2 -/- mice have shown that GPR43/FFAR2 signaling with SCFAs may be effective in lipolysis control in adipocytes[42].

GPR109A/hydroxycarboxylic acid receptor (HCA) 2, which is known to be a niacin receptor, has been identified as a receptor to butyrate as well as beta-hydroxybutyric acid, a ketone body[43, 44]. Its expression sites are intestinal tract epithelial cells, immune cells, and adipocytes[8]. GPR109A/HCA2 participates in homeostasis of regular T cells (Treg) in the colon and fat metabolism in adipose tissues[45, 46]. In addition, GPR109A/HCA2 signaling accelerates inflammation in hypertrophic adipose tissues[46]. Recently, olfactory receptor (Olfr) 78, which is a member of the GPR family, has been reported to be a novel SCFA receptor[47, 48]. Olfr78 is thought to be associated with regulation of hormone secretion and blood pressure[47, 48]. **Table 4** shows the characteristics and physiological functions of SCFA receptors[8, 47, 48].

Short Chain Fatty Acids and T Cells

SCFAs regulate T cell polarization and induction[49]. Propionate (at concentrations of 2.0 to 5.0 mmol/L) inhibits the proliferation of lymphocytes stimulated by mitogens[50]. Propionate (250 to 500 μmol/L) suppresses the Th1-type immune response in stimulated human peripheral blood mononuclear cells[51]. Butyrate (1.0 mmol/L) inhibits the proliferation of T lymphocytes, and more than 2.0 mmol/L of butyrate induces apoptosis in activated T lymphocytes, but not primary macrophages[52]. Trompette *et al*. have reported that there are differences in the intestinal *Firmicutes*/*Bacteroides* ratio (F/B ratio) and microbiota composition between high- and low-fiber diets in mice, and that a high-fiber diet increases blood concentrations of SCFAs (approximately 1.0 to 2.0 mmol/L) and attenuates allergic inflammation of the lungs[53]. The authors suggested that propionate is involved in bone marrow hematopoiesis and in the enhanced generation of macrophage and dendritic cell (DC) precursors and subsequent seeding of the lungs by DCs with high phagocytic capacity, but with an impaired ability to activate Th2 effector cells in the lung[53]. In addition, they suggested that these effects are induced via GPR41/FFAR3 but not GPR43/FFAR2[53].

Compounds acting as histone deacetylase (HDAC) inhibitors may be an effective treatment for inflammatory bowel disease and other pro-inflammatory cytokine-related diseases[54]. As SCFAs are widely known to have HDAC inhibitory activity, they may be involved in the expression of cytokines in T cells and the induction of Treg cells via inhibition of HDAC[55]. SCFAs (acetate 5-20 mmol/L, propionate 0.5-1.0 mmol/L) promote naïve $CD4^+$ T cell polarization into Th1 and Th17 effector cells producing interleukin (IL)-17, interferon-γ, and/or IL-10[56]. This effect is independent of GPR41 and GPR43, but directly dependent on the HDAC inhibitor activity and subsequent enhancement of mTOR-S6 kinase activity[56]. More than 1 mmol/L of butyrate induces Fas-mediated apoptosis of T cells by inhibiting HDAC 1 activity to induce Fas promoter hyperacetylation and Fas upregulation in T cells[57].

When butyrate is supplied into the colons of T cell-dependent colitis mouse models, the number of

Table 2. Fecal concentration of individual SCFAs (Acetate, Propionate, Butyrate) by human adults

	Subjects (*n*); age	Reported measure	Acetate	Propionate	Butyrate	Total SCFAs	Unit	References
Healthy subjects	10; 21-34 years	Mean (SD)	218 (99)	72 (37)	58.7 (54.5)	378 (188)	μmol/g dry weight	Whelan K, *et al.*, J Nutr. 2005, 135: 1896-902.[20]
	20; 20-40 years	Mean (SEM)	320.3 (24.9)	97.3 (10.5)	93.8 (9.13)	511.4 (41.9)	μmol/g dry weight	Boler BM, *et al.*, Br J Nutr. 2011, 106: 1864-71.[21]
	13; 23-58 years	Median (IQR)	52.2	23.2 (13.6-37.3)	36.8 (5-128)	119.3 (64.5-197.0)	μmol/g wet weight	Lewis SJ, *et al.*, Gut. 1997, 41: 245-51.[22]
	60; 18-24 years	Mean (SEM)	198.4 (14.2)	55.2 (4.7)	50.5 (4.9)	304.1	μmol/g dry weight	Lecerf JM, *et al.*, Br J Nutr. 2012, 108: 1847-58.[23]
	27; 18-55 years	Mean (SEM)	35.8 (2.4)	11.4 (1.2)	10.0 (1.1)	61.1 (4.4)	μmol/g	Reimer RA, *et al.*, J Hum Nutr Diet. 2012, 25: 373-7.[24]
	12; 18-65 years	Mean (SD)	48	13.98	13.31	80.91	μmol/g	Fernando WM, *et al.*, Benef Microbes. 2010, 1: 197-207.[25]
	46; 31-66 years	Mean (95%CI)	44.7 females (39.7, 50.3)	12.3 females (10.7, 14.0)	11.7 females (9.8, 14.0)	69.5 females (61.3, 78.7)	μmol/g	McOrist AL, *et al.*, J Nutr. 2011, 141: 883-9.[26]
			58.6 males (49.8, 69.0)	16.1 males (13.4, 19.5)	15.4 males (12.1, 19.6)	90.5 males (76.3, 108)		
	36	Median (IQR)	43.7 (34.0-52.2)	13.1 (9.2-18.5)	8.8 (5.2-11.5)	91.8 (73.1-107.5)	μmol/g	Nemoto H, *et al.*, Dig Dis Sci. 2012, 57: 2955-64.[27]
	20; 22-55 years	Mean (SEM)	42.13 (3.8)	11.5 (1.2)	11.28 (1.4)	67.3 (6.2)	μmol/g	Tiihonen K, *et al.*, Br J Nutr. 2010, 103: 1070-8.[28]
	8; 31-59 years	Mean (SD)	NR	NR	NR	92.7 (33.9)	μmol/g	McOrist AL, *et al.*, Br J Nutr, 2008, 100: 138-46.[29]
	20; 23-28 years	Mean (SEM)	NR	NR	NR	78.5 (6.4)	μmol/g	Hylla S, *et al.*, Am J Clin Nutr, 1998, 67: 136-42.[30]
	30	Mean (SD)	50.5 (12.6)	13.6 (5.2)	14.1 (7.6)	84.6 (22.9)	mmol/L	Schwiertz A, *et al.*, Obesity. 2010, 18:190-5.[31]
Obese subjects	20; 22-55 years	Mean (SEM)	47.2 (3.8)	13.6 (1.3)	14.7 (1.5)	78.8 (6.2)	μmol/g	Tiihonen K, *et al.*, Br J Nutr. 2010, 103:1070-8.[28]
	91	Mean (SD)	58.5 (19.1)	17.6 (7.6)	18.3 (9.7)	102 (33.5)	mmol/L	Brinkworth GD, *et al.*, Br J Nutr. 2009; 101: 1493-502.[32]
	32; 20-65 years	Mean (SEM)	NR	NR	NR	34 (6)	mmol/24h	Benassi-Evans B, *et al.*, Mutat Res, 2010; 703: 130-6.[33]
	35 over weight	Mean (SD)	56.0 (18.2)	18.3 (7.9)	18.5 (10.1)	98.7 (33.9)	mmol/L	Schwiertz A, *et al.*, Obesity. 2010, 18: 190-5.[31]
	33 obese		59.8 (18.3)	19.3 (8.7)	18.1 (10.0)	103.9 (34.3)		

Citationed from Verbeke KA, *et al.*, Nutr Res Rev. 28:42-66[19]
Mean, the mean value; SD, standard deviation; SEM, standard error of the mean; IQR, interquartile range; 95%CI, 95% confidence limit; NR, not reported.

Table 3. Portal concentrations of individual SCFAs (Acetate, Propionate, Butyrate) by human

Subjects	(*n*); age	Treatment	Reported measure	Acetate	Propionate	Butyrate	Unit	References
Died suddenly	6; 16-89 years	Not fasting	Mean	258	88	29	μmol/L	Cummings JH, *et al.*, Gut 1987 28, 1221-7.[4]
Surgical patients	5; -	Fasting	Mean	114	32	9	μmol/L	Dankert J, *et al.*, Clin Chim Acta. 1981 110: 301-7.[34]
Surgical patients	28; 23-74 years	Fasting	Mean	128	34	18	μmol/L	Peters SG, *et al.*, Gut. 1992 33: 1249-52.[35]
Surgical patients	10; -	Fasting 10 g Lactulose was injected into the caecum at surgery (Peak value)	Mean	241	39	27	μmol/L	Peters SG, *et al.*, Gut. 1992 33: 1249-52.[35]
Surgical patients	6; -	Fasting 6.7 g Lactulose was injected into the caecum at surgery (Peak value)	Mean	166	31	22	μmol/L	Peters SG, *et al.*, Gut. 1992 33: 1249-52.[35]
Surgical patients	7; 54-73 years	Fasting	Mean	236	18	26	μmol/L	van der Beek CM, *et al.*, J Nutr. 2015 145: 2019-24.[36]
Surgical patients	7; 54-73 years	Fasting butyrate: 100 mmol/L; 60 mL enema at surgery (Peak value)	Mean	NR	NR	92	μmol/L	van der Beek CM, *et al.*, J Nutr. 2015 145: 2019-24.[36]

Mean, the mean value; NR, not reported

Treg cells increases in the colonic lamina propria, and bowel inflammation is attenuated[58]. The ingestion of propionate increases Treg cells in intestinal mucosa in germ-free mice via GPR43/FFAR2[59]. By inhibition of HDAC, butyrate and to a lesser extent propionate, but not acetate, promotes transcription of the *FoxP3* gene, which is the transcription factor for Treg differentiation, and increases the expression of the *FoxP3* gene[55]. On the other hand, 1 mmol/L of butyrate causes the induction of Th17 cells and also exacerbates inflammation by the production of IL-23 in stimulated dendritic cells (DCs)[60].

Thus, SCFAs, especially butyrate and propionate, play a complicated role in Treg differentiation and intestinal tract immune regulation[9, 53, 61, 62].

Short Chain Fatty Acids and Neutrophils, Monocytes, and Macrophages

The chemotaxis of neutrophils is activated by inflammatory mediators [tumor necrosis factor (TNF)-α, IL-17, etc.] and chemokines [chemokine (C-X-C motif) (CXCL) 1, 8, etc.]. SCFAs (optimal concentrations for migration are 0.1-3.0 mmol/L) affect the chemotaxis and the viability of neutrophils[63]. SCFAs (4.0-12 mmol/L propionate, 0.4-3.2 mmol/L butyrate) inhibit TNF-α production by neutrophils in the presence of lipopolysaccharide (LPS)[64]. The suppression of nuclear factor-kappa B (NF-κB) activity and the inhibition of HDAC are thought to be the underlying mechanisms[64, 65]. On the other hand, neutrophils increase the production of IL-8, IL-6, and IL-1β at high concentrations (20 mmol/L) of SCFAs while lower concentrations (0.02-2.0 mmol/L) do not induce cytokine secretion. However, lower concentrations of SCFAs enhance TLR2-induced production of IL-8 and TNF-α production[66]. SCFAs suppress large intestine inflammation in dextran sulfate sodium-induced colitis mice by the induction of apoptosis of neutrophils via GPR43/FFAR2[67] and via HDAC inhibition[68]. In addition, SCFAs produce and release reactive oxygen species (ROS) as well as nitric

Table 4. The characteristics and physiological functions of SCFA receptors

	G protein	Ligand	EC50	Expression	Physiological function
GPR41/ FFAR3	Gi/o	C1-C5 Propionate(C3), Butyrate(C4) C3＞C4＞C2	12-274 μmol/L for C3	Colonic, colonic LP cells (mast cells), spleen, lymphnodes, bonemarrow, adipocytes, peripheral mononuclear cells, peripheral nervous system, etc.	Increased energy expenditure, leptin expression, decreased food intake, hematopoiesis of DCs from bonemarrow, increased Treg cells, etc.
GPR43/ FFAR2	Gi/o, Gq11	C1-5 Acetate (C2), Propionate (C3) C2 ≒ C3＞C4	259-537 μmol/L	Colonic, colonic LP cells (mast cells, neutrophils, eosinophils, and Tregs), polymorphonuclear cells, adipocytes, skeltal muscle, etc.	Anti-lipolysis, increased insulin sensitivity, preadipocyte differentiation, expansion and differentiation of Tregs, protection against IBD, etc.
GPR109A/ HCA2	Gi/o, Gβγ	Niacin β-D-OHB＞ Butyrate (C4)	0.7 mmol/L (mouse), 1.6 mmol/L (human) for C4	Apical membrane of colonic epithelium, macrophages, monocytes, DCs, neutrophils, adipocytes (white and brown), etc.	Anti-lipolysis, triglyceride lowering, protection against colitis, increased of Treg generation, increased IL-10 producing T cells, etc.
Olfr78	NR	Propionate(C3)＞ acetate (C2)	920 μmol/L for C3 2.35 mmol/L for C2	Nurons, enteroendocrine cells, colon (epithelial enteroendocrine cells), renal afferent arteriole, juxtaglomerular cells, smooth muscle cells (blood vessels)	Regulation of hormone secretion (GLP-1, PYY), blood pressure regulation (renin-angiotensin-aldosterone pathway)

Citationed from koh A, *et al*., Cell. 165: 1332-45[8)]
Citationed from Fleischer J, *et al*., Cell Tissue Res. 361: 697-710[47)], Pluznick J., Gut Microbes. 5: 202-7[48)]
NR, not reported; EC50, half maximal (50%) effective concentration; GPR, G-protein coupled receptor; FFAR, free fatty acid receptor; HCA, hydroxycarboxylic acid receptor; Olfr, olfactory receptor; LP, lamina propria;
Treg, regulatory T cell; DC, dendritic cell; GLP-1, glucagon-like peptide; PYY, peptide YY; IBD, inflammatory bowel disease

oxide (NO) involving neutrophil bacteria phagocytosis[69)]. Thus, SCFAs have both suppressing and promoting functions in neutrophils.

SCFAs also affect immunoregulation in monocytes and macrophages. In experiments using human monocytes, SCFAs (0.2-20 mmol/L) reduce the production of TNF-α and monocyte chemotactic protein-1 (MCP-1) under LPS stimulation and increase the production of prostaglandin E2 (PGE2)[70)]. The suppression of NF-κB activity and inhibition of HDAC are thought to be the underlying mechanisms, as in the case of neutrophils. Butyrate increases the production of PGE2 by upregulating the expression of phospholipase A2 (PLA2) and cyclooxygenase-2 (COX2) in Kupffer cells (at butyrate concentrations of 0.5-10 mmol/L)[71)], in human peripheral blood mononuclear cells (1.0-2.0 mmol/L)[72)], and in a mouse macrophage cell line (0.2-1.0 mmol/L)[73)] (**Fig. 1 (A)**). In a human macrophage cell line, butyrate (1.0 mmol/L) increases the production and release of ROS when under LPS stimulation and increases caspase-1 expression and IL-1β production[74)].

Short Chain Fatty Acids and Atherosclerosis

Hazen *et al*. have revealed that gut microbial-derived metabolites trimethylamine (TMA) and trimethylamine N-oxide (TMAO) are proatherogenic in mice and humans[6, 11, 75)]. They have also shown that plasma TMAO concentrations can predict an enhanced risk of major adverse cardiac events in two independent cohorts[76)].

Recently, Aguilar *et al*. demonstrated that a butyrate-supplemented chow diet suppresses atherosclerotic lesions in apoE knockout mice[77, 78)]. They also observed that butyrate reduces chemotaxis protein-1 (CCL2/MCP-1), vascular cell adhesion molecule-1, and matrix metalloproteinase-2 production in the lesion site, resulting in a lower migration of macrophages and increased collagen deposition and plaque stability[77)], and that peritoneal macrophages from butyrate-treated mice present lower ROS and NO release[78)].

On the other hand, Kasahara *et al*. demonstrated that the lack of microbiota in apoE-deficient mice

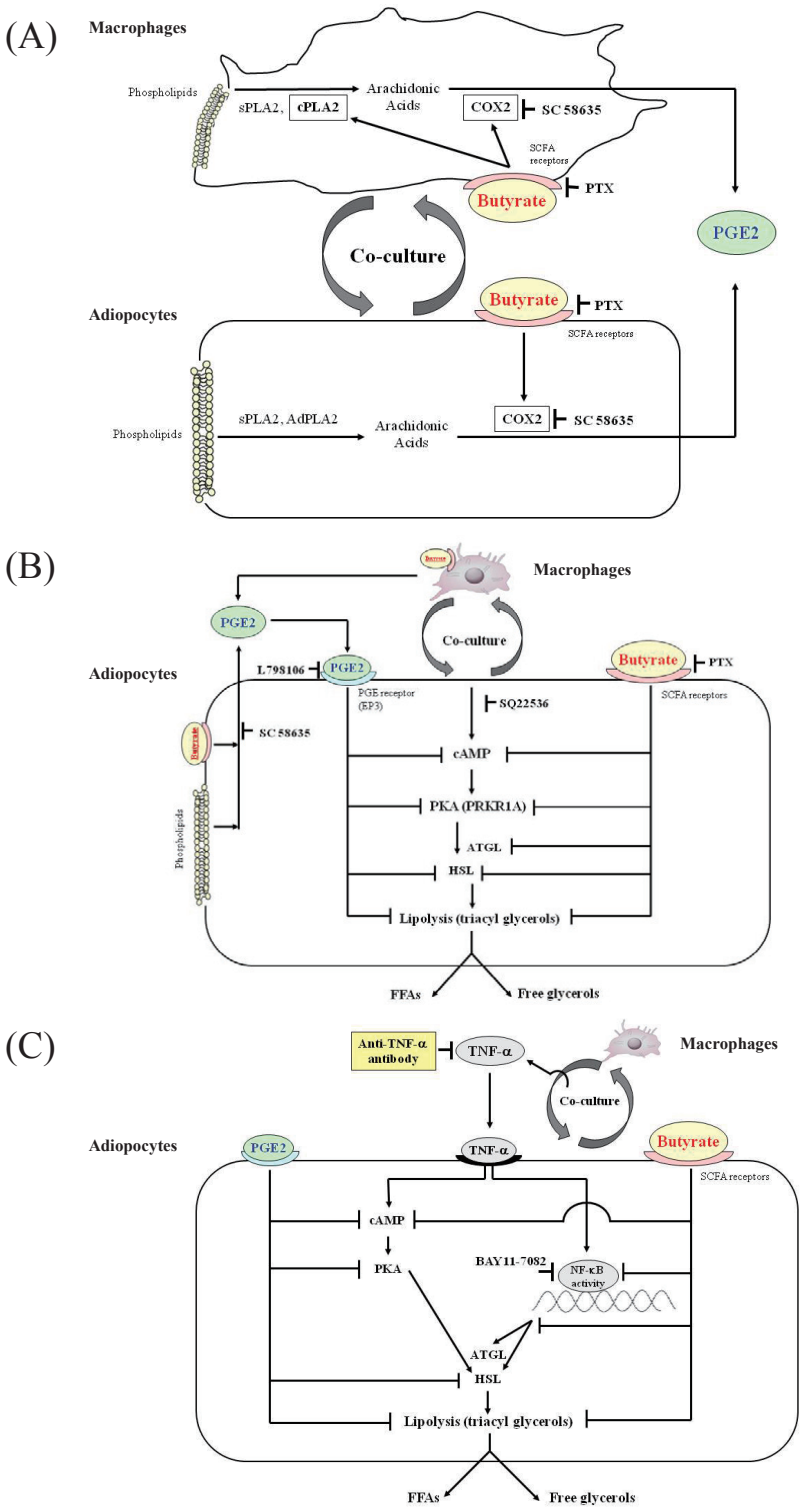

Fig. 1. Hypothetical pathways based on the results of the suppressive effect of butyrate depends on the prostaglandin E2 (PGE2)-mediated pathway

Cited from the reference 73

(A) The effect of butyrate on PGE2 production in the interaction between co-cultured macrophages and adipocytes. Co-culture elevates calcium-dependent cytosolic phospholipase A2 (cPLA2) activity in macrophages, secretory PLA2 (sPLA2) activity in adipocytes and macrophages, and the expression of adipose-specific PLA2 (AdPLA2) protein and mRNA in adipocytes. Butyrate elevates cPLA2 activity to a greater degree in macrophages. Co-culture elevates cyclooxygenase-2 (COX2) expression in both cells, and butyrate further enhances COX2 expression in both cells. Butyrate increases PGE2 production more than co-culture alone.

(B) The effects of butyrate and PGE2 on lipolysis in co-cultured adipocytes. Co-culture increases cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) levels in adipocytes and increases the release of free fatty acids (FFAs) and free glycerol into the medium (lipolysis). Butyrate suppresses cAMP and PKA levels, and exogenous PGE2 via prostaglandin E receptor 3 (EP3) has a lesser effect than butyrate. These suppressive effects may reduce the activities of lipases, including adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), thus resulting in inhibition of lipolysis.

(C) The effects of butyrate and exogenous PGE2 on cAMP- and nuclear factor-kappa B (NF-κB)–mediated lipolysis in tumor necrosis factor-α (TNF-α)–stimulated 3T3-L1 adipocytes. Co-culture increases TNF-α production. TNF-α increases cAMP, leading to increased lipolysis. Anti–TNF-α antibody, butyrate, or exogenous PGE2 decrease cAMP levels and reduce lipolysis in TNF-α–stimulated 3T3-L1 cells. The GPR109A-mediated pathway may be the predominant pathway regulating the effect of butyrate on lipolysis in TNF-α–stimulated 3T3-L1 cells.

PTX (pertussis toxin), an inhibitor blocking G-protein coupled receptor (GPR) 41- and/or GPR109A-mediated signaling; SC 58635, COX2 selective inhibitor; L798106, EP3 selective antagonist; SQ22536, adenylyl cyclase selective inhibitor; BAY11-7082, NF-κB–selective inhibitor

causes a significant reduction in atherosclerotic lesion formation in spite of a significant increase in plasma and hepatic cholesterol concentrations, and suggested that this might be associated with the attenuation of LPS-mediated inflammatory responses[79]. They also found that gut microbiota can regulate cholesterol homeostasis via bile acid metabolism under hypercholesterolemia[79]. Ryan demonstrated shifts in the composition of the gut microbiome in apoE-deficient mice fed high fat/cholesterol in conjunction with

plant sterol esters or oat β-glucan, and increased concentrations of cecum acetate or butyrate, and found that these feedings attenuated the microbial production of TMA and reduced serum cholesterol concentrations[80].

In accordance with experimental data, recent clinical work has shown that coronary artery disease is linked with an alternation of gut microbiota, and that gut microbiota may be a diagnostic marker of morbidity from coronary artery disease[81, 82]. Emoto *et al.* reported that the incidence of coronary artery disease is related to a decreased prevalence of the phylum *Bacteroidetes* and increased F/B ratio in the intestinal tract[81].

Therefore, it is possible that the use of SCFAs, prebiotics, or probiotics (live microbiota) to improve the gut microbiota environment can allow SCFAs to prevent metabolic disorders and prevent ASCVD.

Short Chain Fatty Acids and Visceral Adipose Tissues

Adipose tissues not only store energy through the accumulation of triglycerides, but secrete various adipokines that affect metabolism throughout the body. In hypertrophic adipose tissues, the invasion of macrophages activates the secretion of free fatty acids (FFAs), TNF-α, IL-6, MCP-1, plasminogen activator inhibitor-1, and other pro-inflammatory cytokines and chemokines, which leads to metabolic abnormalities, including insulin resistance, resulting in the promotion of atherogenesis[83, 84]. These interactions between adipocyte-derived FFAs and macrophage-derived adipokines represent a vicious cycle[85].

Using a co-culture system with adipocytes and macrophages, we found increased production of TNF-α, IL-6, MCP-1, and the release of free glycerol and FFAs into the medium. Butyrate (0.1-1.0 mmol/L) significantly reduces these effects[86]. Butyrate inhibits the phosphorylation of mitogen-activated protein kinases and NF-κB activity in co-cultured macrophages and suppresses lipolysis caused by the suppression of adipose triglyceride lipase expression and hormone-sensitive lipase phosphorylation in adipocytes[86]. In these co-culture conditions, butyrate increases the production of PGE2, and approximately 40% of the suppressive effect of butyrate on lipolysis depends on the PGE2-mediated pathway[73] (**Fig. 1 (A-C)**).

Intraperitoneal administration of butyrate (1.0 g/kg) in db/db mice suppresses obesity-induced inflammation and the expression of IL-1, IL-6, and TNF-α mRNA in epididymal, subcutaneous adipose tissues by inhibiting the NOD-like receptor 3 inflammation signaling pathway[87]. In experiments using explants of human omental and subcutaneous adipose tissues, propionate (3 mmol/L) suppresses the production of resistin, which is an adipocyte-derived adipokine and pro-inflammatory cytokine[88].

In hypertrophic visceral adipose tissues, a decrease of the CD4 (+) Treg and an increase of the $CD153^{+}PD\text{-}1^{+}CD44^{hi}CD4^{+}$ T cell population ratio have been observed. This change in T cell populations may induce macrophages into adipose tissues as well as decreases in the M2 macrophage population ratio, and an increase in the M1 macrophage population ratio[89-91]. However, it has not been clarified whether SCFAs are directly associated with changes in the M1/M2 macrophage ratio or in T cell differentiation and population in adipose tissues. This issue therefore requires further study. Finally, mast cells may be involved in the inflammation of visceral adipose tissues prior to macrophage invasion[92]. Nevertheless, the effect of SCFAs on mast cells remains unknown.

Short Chain Fatty Acids and Metabolic Abnormality

Several reports have shown that obesity is associated with changes in the relative abundance of the two dominant bacterial phyla, *Firmicutes* and *Bacteroides*. Increased F/B ratios are observed in the guts of obese adults[81, 93]. Recent cohort studies in Chinese and European women have shown that regardless of the difference in race and eating habits, type 2 diabetes patients are characterized by a moderate degree of gut microbial decrease in the abundance of some universal butyrate-producing bacteria[94, 95].

Dietary fibers promote metabolic benefits on body weight and glucose control, and several studies have demonstrated the impact of an SCFA-enriched diet, establishing a direct causal relationship between fiber fermentation and improved metabolism in humans[8, 96, 97]. Lin *et al.* have shown that butyrate, propionate, and acetate protect against diet-induced obesity and insulin resistance and that butyrate and propionate, but not acetate, induce gut hormones and reduce food intake[98]. De Vadder *et al.* have demonstrated that propionate and butyrate activate intestinal gluconeogenesis via complementary mechanisms[99]. Increased production of acetate by an altered gut microbiota in rodents leads to activation of the parasympathetic nervous system, which promotes increased glucose-stimulated insulin secretion, increased ghrelin secretion, hyperphagia, obesity, and related sequelae[100]. Oral supplementation of SCFAs may thus improve impaired glucose metabolism in humans[101].

Dietary acetic acid reduces serum cholesterol and

triglycerides (TG) in rats fed a cholesterol-rich diet[102]. Propionate inhibits fatty acid synthesis and to a lesser extent cholesterol synthesis, while butyrate is a potent activator of both synthetic pathways in rat isolated liver cells[103]. A high propionate diet reduces hepatic gene and protein expression of lipogenic enzymes leading to reduced hepatic TG concentrations in high-fat fed mice[104]. Although it has been reported that a high-cholesterol diet does not alter gut microbiota composition in mice[105], further examination is needed to reveal the association between SCFAs and lipid metabolism. Interestingly, Tarini *et al*. demonstrated in healthy adult humans that supplementation with the fermentable dietary fiber inulin significantly increased postprandial serum acetate, propionate, and butyrate concentrations at 4-6 h, and significantly decreased postprandial serum FFAs concentrations at 4 h. They also showed that inulin significantly increased plasma glucagon-like peptide-1 concentrations at 30 min, and reduced ghrelin at 4.5 h and 6 h[106]. Thus, compositional changes of microbiota might influence adiposity and glucose and lipid metabolism by regulating food intake.

Conclusion and Perspectives

SCFAs may suppress inflammation by reducing migration and proliferation of immune cells, reducing many types of cytokines, and inducing apoptosis. Thus, SCFAs are thought to have anti-inflammatory effects. However, marked changes of SCFAs concentrations in blood or various tissues are thought to cause disorders related to immunological and metabolic imbalances. Thus, gut bacteria exert both beneficial and harmful effects[107]. Therefore, it may be important to estimate the appropriate concentrations of SCFAs to maintain a normal metabolism and immune system for the prevention and treatment of diseases using diet and SCFAs. Recently, Bergeron *et al*. demonstrated that a lower carbohydrate diet (39-40% energy) high in resistant starches was associated with higher plasma TMAO levels in spite of reduced postprandial insulin and glucose responses, while there was no difference in TMAO affected by resistant starches when carbohydrate intake was high (51-53% energy)[108]. It may be necessary to develop food patterns or medications to reduce plasma TMAO concentrations and maintain appropriate concentrations of SCFAs.

Although there have been only a small number of studies thus far, in the future SCFAs may provide new insights into the pathophysiology of inflammatory diseases, and we can expect the development of novel therapeutic strategies against chronic inflammation, metabolic disorders, and atherosclerosis.

Conflict of Interest

All authors declare that they have no conflict of interest.

Sources of Funding

Some studies in this review were supported by a Grant-in-Aid for Scientific Research (C), JSPS KAKENHI grant number 26350166.

Author Contributions

All authors contributed equally to the preparation of the manuscript and approved the final manuscript.

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