Review article: short chain fatty acids as potential therapeutic agents in human gastrointestinal and inflammatory disorders

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Summary
Background: Butyrate, propionate and acetate are short chain fatty acids (SCFA), important for maintaining a healthy colon and are considered as protective in colorectal carcinogenesis. However, they may also regulate immune responses and the composition of the intestinal microbiota. Consequently, their importance in a variety of chronic inflammatory diseases is emerging.

Aims: To review the physiology and metabolism of SCFA in humans, cellular and molecular mechanisms by which SCFA may act in health and disease, and approaches for therapeutic delivery of SCFA.

Methods: A PubMed literature search was conducted for clinical and pre-clinical studies using search terms: 'dietary fibre', short-chain fatty acids', 'acetate', 'propionate', 'butyrate', 'inflammation', 'immune', 'gastrointestinal', 'metabolism'.

Results: A wide range of pre-clinical evidence supports roles for SCFA as modulators of not only colonic function, but also multiple inflammatory and metabolic processes. SCFA are implicated in many autoimmune, allergic and metabolic diseases. However, translating effects of SCFA from animal studies to human disease is limited by physiological and dietary differences and by the challenge of delivering sufficient amounts of SCFA to the target sites that include the colon and the systemic circulation. Development of novel targeted approaches for colonic delivery, combined with postbiotic supplementation, may represent desirable strategies to achieve adequate targeted SCFA delivery.

Conclusions: There is a large array of potential disease-modulating effects of SCFA. Adequate targeted delivery to the sites of action is the main limitation of such application. The ongoing development and evaluation of novel delivery techniques offer potential for translating promise to therapeutic benefit.
1 | INTRODUCTION

Short chain fatty acids (SCFA) comprise of 1-6 carbon based anions produced during bacterial fermentation, of which, acetate (C2), propionate (C3) and butyrate (C4) are most abundantly produced. SCFA are produced naturally within the colon by fermentation of carbohydrates, both dietary and endogenous, and protein that are accessible to the microbiota. Fermentation is a process that has also been exploited by humans for thousands of years in the production of foods and beverages, which consequently, contain SCFA.

Epidemiological evidence suggests there is an association between dietary fibre consumption and reduced risk of developing cardiovascular disease, diabetes and colon cancer.1-3 SCFA may play a role in conferring such effects by directly affecting cell activity and function. A strong body of research, mostly in animal models demonstrates that SCFA affect outcomes of health and disease. However, this is yet to be conclusively translated in a clinical setting. The aims of this review are: (1) to provide an overview of the putative mechanisms by which SCFA can modulate human health and disease; (2) to highlight the spectrum of diseases that have been linked to SCFA; and (3) to discuss aspects of SCFA physiology and metabolism in the context of utilising SCFA for therapeutic use.

2 | METHODS

A literature review was conducted using PubMed, Medline, Scopus and Google Scholar databases using combinations of search terms: ‘short-chain fatty acids’, ‘acetate’, ‘propionate’, ‘butyrate’, ‘dietary fibre’, ‘inflammation’, ‘immune’, ‘metabolism’, ‘fermentation’, ‘gastrointestinal’, ‘colon’, ‘Inflammatory bowel disease’, ‘colitis’, ‘autoimmune’, ‘allergy’. Both clinical and pre-clinical studies were included for the purposes of this explanatory review. Papers that were not in English or did not have a full-text were excluded from the review.

3 | SOURCES OF SCFA

Short chain fatty acids can be either ingested in the diet or produced via metabolic processes within body tissues during fasting conditions, after consumption of alcohol, and within the bowel lumen.4-5 By far the major source of SCFA in the colon is bacterial fermentation of carbohydrates, which can be endogenous or dietary in origin. The essential role of the microbiota in generating SCFA has been highlighted in studies of germ-free mice, in which very low levels of SCFA are produced compared to those in conventional mice, reflecting the relatively small amount of SCFA obtained directly from dietary sources and endogenous, non-microbial production.6 Additionally, protein can also be utilised as a substrate for SCFA production by the gut microbiota during dissimilatory amino acid metabolism, producing branched-chain fatty acids such as isobutyrate and iso-valerate.7 Dietary carbohydrates that reach the colonic lumen, usually referred to as dietary fibre, are the quantitatively most important substrate for saccharolytic bacteria in the colon and the consumption of fermentable fibres increases colonic production of SCFA.8

3.1 | SCFA production in vivo by fermentation of dietary fibres

There are many definitions of “dietary fibre”. Many countries, including Australia, Japan and those within the EU accept dietary fibre as carbohydrates with a degree of polymerisation greater than 2 that fail to be hydrolysed or absorbed in the small intestine, as outlined by Codex Alimentarius Alinorm.9 This definition is yet to be fully accepted in the USA, whereby dietary fibre is defined as non-digestible carbohydrates and lignin that are intrinsic and intact in plants. Irrespective of variations of definition, fibre is a crude term that represents a diverse group of non-digestible carbohydrates with varying structural and functional properties that include ability to hold water (solubility), to be metabolised to SCFA by intestinal microbiota (fermentation), to selectively stimulate the growth of health-promoting bacteria (prebiotic effect) and to bind and modulate other events such as the inhibition of emulsifier-induced bacterial translocation (post-biotic effect).10,11 Therefore, it is important to consider that not all subtypes of dietary fibre contribute to the SCFA pool and not all putative fibre-associated modulation of colonic health occurs via SCFA. For example, non-fermentable plant fibres such as cellulose cannot be utilised by the human gut microbiota, but increase faecal bulking, which stimulates peristalsis and hence reduces transit time through the colon.12 This effect presumably underlies its value for treating constipation and contributes to reducing the tumour load in rodent models of colorectal cancer.13,14

Fermentable fibres vary in the rapidity by which they are fermented15 and in the SCFA profiles resulting. Factors that modulate or influence these characteristics are outlined in Table 1. The delivery of SCFA to different regions of the colon also varies, and this has been of interest due to the rapid uptake of SCFA by epithelium and the subsequent local effects of such uptake. For example, the preventive effect of SCFA on colorectal carcinogenesis via the adenoma-carcinoma sequence in a rat model was clearly related to promotion of distal colonic fermentation rather than fermentation per se.14 Most data available on regional SCFA production profiles have been generated from animal studies and have been extrapolated to humans, where observations are largely restricted to studies of faeces and blood. Interpretation of the findings in faeces, for example, is limited as such measurements are unlikely to capture the dynamic processes of fermentation and absorption that occur more proximally along the colon. Information about concentrations of SCFA along the colon have relied on data from sudden death victims, which indicated that SCFA concentrations are highest in the proximal colon, means values being 69, 25 and 25 mmol/L for acetate, propionate and butyrate, respectively.35 These high concentrations of SCFA are reflected by the low luminal pH in the ascending colon, which contrasts higher pH and lower SCFA concentrations in the faeces.36,37 The use of an ingestible pH-sensing device that
1. The type of fibre: Slowly fermentable fibres may shift SCFA production from more rapidly fermented fibres from only the proximal colon towards the distal colon. Consumption of wheat bran with a relatively rapidly fermented resistant starch increased SCFA concentrations in the distal colon of pigs and rats compared to those fed only resistant starch. Healthy humans were able to increase faecal concentrations of SCFA, especially butyrate, by addition of wheat bran to resistant starch that failed to alter faecal SCFA alone. This observation is considered strong evidence that fermentation had been shifted distally as in the animal studies.

2. Gut transit time: Changing the rate of transit along the colon will alter the time for fermentation and, hypothetically alter SCFA absorption in the proximal colon. For example, faecal butyrate concentrations increase with more rapid colonic transit without a change in dietary fibre intake in humans. Drugs that slow or hasten colonic transit, such as loperamide and cisapride, respectively, alter SCFA production. However, SCFA themselves have been reported to alter transit time in rodent models by acting on colonic smooth muscle tissue, releasing polypeptide YY (PYY). This may occur via direct engagement of GPR43 (FFAR2) on colonic L-enteroendocrine cells. A rise in post-prandial PYY has been reported to alter transit time in rodent models by acting on colonic smooth muscle tissue, releasing polypeptide YY (PYY).

There are two major factors that influence the regional production of SCFA:

### TABLE 1 Factors that influence SCFA production via fermentation

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<th>Characteristic</th>
<th>Factor</th>
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| Amount of fermentation      | Size and structure of the fibre | • Speed and height of breath hydrogen rise following ingestion of short to long-chain fibres is proportional to chain length.  
• Oligosaccharides are utilised in hours within in vitro fermentation systems, whilst long-chain fibres are slowly fermented over days.  
• RS, resistant starch; NSP, non-starch polysaccharide. |
| Structure and function of the microbiota | Type of fibre | • Pectin preferentially produces acetate, guar gum produces propionate and RS produces butyrate.  
• Metabolic cross-feeding, for example, between B. adolescentis and E. hallii, B. adolescentis and Roseburia sp., causing conversion between acetate and butyrate, and lactate and butyrate respectively.  
• Butyrate-producing capacity of microbiota may decline with age and antibiotic use.  
• Colonic pH of 5.5 stimulates butyrogenic fermentation, whilst acetate is promoted at pH 6.5.  
| Non-dietary, non-microbial | Profile SCFA produced | • Roseburia sp., causing conversion between acetate and butyrate, and lactate and butyrate respectively.  
• Abundance of SCFA producing species: Bifidobacterium adolescentis, Eubacterium rectale, Eubacterium hallii, Faecalibacterium prausnitzii and Ruminococcus bromii.  
• Hydrogen-metabolising bacteria prevent accumulation of hydrogen that inhibits fermentation.  
• Inter-individual differences in faecal SCFA output in response to high RS and NSP diets highlight inherent variation in the functional capacity of microbiota amongst humans.  
• Indirect effects on microbiota: ageing, antibiotics, mucus production and colonic disease states.  
• pH—fermentation occurs ideally between 5.5 and 7.6.  
| Colonic pH                  |                               | 1. The type of fibre: Slowly fermentable fibres may shift SCFA production from more rapidly fermented fibres from only the proximal colon towards the distal colon. Consumption of wheat bran with a relatively rapidly fermented resistant starch increased SCFA concentrations in the distal colon of pigs and rats compared to those fed only resistant starch. Healthy humans were able to increase faecal concentrations of SCFA, especially butyrate, by addition of wheat bran to resistant starch that failed to alter faecal SCFA alone. This observation is considered strong evidence that fermentation had been shifted distally as in the animal studies.

2. Gut transit time: Changing the rate of transit along the colon will alter the time for fermentation and, hypothetically alter SCFA absorption in the proximal colon. For example, faecal butyrate concentrations increase with more rapid colonic transit without a change in dietary fibre intake in humans. Drugs that slow or hasten colonic transit, such as loperamide and cisapride, respectively, alter SCFA production. However, SCFA themselves have been reported to alter transit time in rodent models by acting on colonic smooth muscle tissue, releasing polypeptide YY (PYY).

3.2 | Endogenous metabolic processes that generate SCFA

There are a few pathways for genesis of SCFA endogenously. First, fat oxidation, predominantly during starvation-associated ketosis leads to the production of acetate, propionate and butyrate. Long-chain fatty acids and pyruvate may be converted to acetyl-CoA, which is then broken down to acetate through the actions of acetyl-CoA hydrolase. Animal studies have found that acetyl-CoA hydrolase is expressed in all tissues, with highest activity found in liver and muscle.Secondly, acetate may also be produced in the liver on exposure to ethanol through the actions of alcohol dehydrogenase and aldehyde dehydrogenase. Plasma acetate flux has been reported to increase 2.5 times in healthy males who consumed 24 g of radio-labelled ethanol, resulting in a rapid increase to serum concentrations. Serum acetate concentrations in healthy males and females may rise to approximately 500 μmol/L, 75 min after a 0.5 g/kg dose...
of alcohol. Thus, propionate may be produced endogenously from branched-chain amino acids and odd-chain fatty acids via propionyl-CoA. The contribution of metabolically derived to plasma levels of SCFA varies according to the setting. In healthy humans, fatty acid oxidation and ketosis may contribute to a 60 µmol/L increase in plasma acetate levels in those fasting for three consecutive days, but this was not seen in those who underwent a 12-h overnight fast. The contribution of metabolic processes to plasma SCFA levels is not thought to be significant in those living a normal lifestyle. For propionate and butyrate, utilisation in the tissues readily accounts for any metabolic contribution. Thus, plasma concentrations are generally <10 µmol/L and butyrate <5 µmol/L. Variation in plasma SCFA levels observed in healthy volunteers over the course of a day is most likely due to changes in the rate of colonic fermentation according to its exposure to dietary carbohydrates. However, there are two exceptions. First, diabetic individuals have elevated fasting plasma SCFA levels close to double that of healthy controls, a reflection of increased metabolic production from fatty acid oxidation and ketosis due to poor glycaemic control. Secondly, the plasma acetate response to ethanol can be considerable as outlined above.

### 3.3 SCFA intake via food

The fermentation process has been utilised for millennia by humans in the production of various fermented foods and drinks. Unlike that carried out by the gut microbiota, this process of fermentation must be tightly controlled to ensure the final product is free from pathogenic bacteria and potentially toxic metabolites. Due to the odorous properties of SCFA, their concentrations are routinely quantified during quality control processes within the food and beverage industry. However, limited published information exists regarding SCFA quantities in foods.

Within the dairy industry, predominately lactic acid producing bacteria are selected for starter cultures responsible for fermentation. Commonly selected cultures in commercial products include *Bifidobacterium lactis*, *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Lactobacillus bulgaris*. These bacteria utilise the Embden-Meyerhoff-Parnas pathway, fermenting hydrolysed sugars to pyruvate and lactate. These products may be further broken down to acetic acid during glycolysis in carbon restrictive environments. Acetic acid producing bacteria from the *Acetobacter* and *Glucobacter* genera are found in starter cultures used to create traditional fermented products such as vinegar and kombucha. Consequently, these products will or are likely to have higher levels of acetic acid than other fermented foods. The addition of the fungus, *Penicillium roqueforti* to starter cultures of soft cheeses further enhances SCFA production from lipolysis, resulting in production of other SCFAs such as propionate and butyrate. This occurs predominately during the ripening process of blue cheeses.

Bacterial starter cultures chosen for fermentation are highly sensitive to environmental factors. Oxygen levels, temperature and pH must be tightly controlled to create optimal conditions. Hydrolysed lipids are a key substrate for SCFA production and thus, the concentration at which they are present in the fermentation mixture will determine the capacity of SCFA production. Addition of lipase to starter cultures is utilised in cheese manufacturing in order to improve SCFA production for enhanced flavour. Furthermore, fibres such as inulin may also improve viability of bacterial cultures and has been reported to increase SCFA production in yoghurt.

### 4 PHARMACOKINETICS OF SCFA

Within the colon, approximately 60% of SCFA diffuse from the lumen through the epithelium to the lamina propria with the remaining portion taken up directly by epithelial cells. Epithelial uptake can be by diffusion of unionised SCFA, or receptor-mediated via by both hydrogen- (MCT-1) and sodium-coupled (SMCT-1) transporters, the latter of which has a higher affinity for SCFA. Receptor-mediated uptake is a dynamic process that is sensitive to both physiological and microbial factors in the colonic environment. While expression of these transporters on the apical membrane of colonic epithelial cells is constitutive, it is also regulated by factors in the local environment. Butyrate has been shown to induce translocation modulation of MCT-1 on colonic epithelial cells within 30 min of exposure, resulting in increased abundance on the apical membrane. This effect is also seen in mice fed pectin. Bile acids may competitively inhibit both SMCT-1 and MCT-1, although less potently in the latter. Furthermore, microbiota may have both positive and negative effects on butyrate uptake. For example, enteropathogenic *Escherichia coli* induces endocytosis of MCT-1, whilst this phenomenon is attenuated in the presence of *Lactobacilli acidophilus*, stimulating MCT-1 function.

Short chain fatty acids delivered to the lamina propria enters the blood vessels and are taken via the portal vein to the liver where the vast majority is metabolised on first pass; only acetate is detectable at physiologically significant concentrations in the peripheral blood. It has been estimated that 36% of colonic-derived acetate becomes systemically available, in contrast to only 9% of propionate and 2% of butyrate. In healthy subjects, fasting levels of acetate in peripheral blood have been reported to rise from a mean of 54 to 169 µmol/L 6-8 h after consumption of a 24-h high resistant starch diet, although this rise of >100 µmol/L has not been achieved through the use of inulin, guar gum or XOS fibre supplements. Circulating concentrations of SCFA levels are likely to be tightly controlled by energy levels within the body, as SCFA are known substrates for the production of glucose, fatty acids and ketone bodies, outlined in Figure 1. Intravenously delivered acetate is rapidly metabolised, creating a temporary increase in plasma levels that is cleared within an hour. In studies using radiolabelled SCFA, endogenous turnover of acetate is much lower in humans than in smaller animals such as dogs and rats, reflecting differences in the production and utilisation of acetate within cells and tissues of these animals. Such endogenous variation highlights a major limitation to...
extrapolation of peripheral effects of SCFA in animal models of human disease to the human situation.4

4.1 Molecular and metabolic interactions of SCFA

SCFA can modulate cellular activity both extra- and intracellularly.

1. Extracellular activity via SCFA-specific G-protein coupled receptors (GPRs): GPR41 (also known as free fatty acid receptor 3 [FFAR3]), GPR43 (FFAR2) and GPR109a (PUMA-G) are activated by ionised SCFA and signal downstream to the nucleus.79,80 SCFA-specific GPRs are expressed at a functional level on the surface of a wide range of cells that include gut epithelial cells, adipocytes, enteroendocrine L-cells, innate immune cells, and neurons of the automatic and somatic sensory ganglia.81,82 In transfected cell lines, human GPR43 may be activated by acetate and propionate at an effective half-maximal concentration (EC50) of approximately 50-200 μmol/L, depending on the expression system and cell line used.79,83 Propionate can activate GPR41 with an EC50 of 10 μmol/L.83 Given that these concentrations may be found in the circulation, it is plausible that GPR43 and 41 are biologically active systemically. GPR109a is selective for butyrate but is relatively less potent, requiring a concentration closer to 1 mmol/L, a concentration only found in the lumen of the terminal ileum and along the length of the colon.35,84

2. Intracellular inhibition of histone deacetylases (HDACs): These enzymes are involved in epigenetic regulation of gene expression.85 While HDACs are a family of enzymes constitutively expressed within all cells, SCFA appear to inhibit only type 1 and type 2 HDAC.86 The mechanism of action for such inhibition has yet to be fully elucidated, but it has been proposed that a pair of butyrate molecules could sit in the hydrophobic binding cleft of the active site.87 Butyrate is the most potent HDAC inhibitor (HDACi), with half-maximal inhibitory concentration (IC50) of 10-100 μmol/L in human cell lines.85 Propionate and, to a lesser extent acetate, also have inhibitory properties at a concentrations of 100-1000 μmol/L.88

3. Intracellular energy supply for colonic epithelium: Beta-oxidation of butyrate specifically is the preferred energy source for colonic epithelial cells (see below).

4. Substrates for the Krebs cycle: The main site of SCFA metabolism via the Kreb’s cycle is within hepatocytes of the liver, where propionate is a major substrate for gluconeogenesis, and butyrate for lipogenesis.31 Furthermore, cholesterol can be synthesized from acetate and butyrate. Alterations in cellular energy levels as a direct consequence of these metabolic changes will also influence cell behaviour.89

5. Induction of apoptosis: At higher concentrations, butyrate and other SCFA induce apoptosis of cells. Extracellular concentrations that have this action in vitro are in the range of 2-10 mmol/L; a concentration of 8 mmol/L induced apoptosis in normal colonic crypt cells.70,91 Only surface colonic epithelial cells will be exposed to such concentrations under physiological conditions, and such an action probably plays an important role in epithelial cell kinetics. This effect, however, may hypothetically occur in crypt cells when very high luminal concentrations are achieved; this might at least partly underlie the increased colonic epithelial permeability, heightened translocation of Salmonella and increased mucus secretion induced by high doses of rapidly fermentable substrates in rat and humans (to a lesser extent).92-94 Furthermore, high doses of dietary uncooked potato starch that achieved high SCFA luminal concentrations were associated with induced epithelial injury and promoted carcinogenesis.92,95,96

FIGURE 1 Systemic metabolism of SCFA. (1) SCFA produced in the colon drains into the portal vein; (2) portal venous SCFA undergo first-pass metabolism by the liver; (3) within the liver SCFA may enter a number of metabolic pathways depending on the metabolic state. Acetate and butyrate may be converted to the central metabolite, acetyl-CoA. Consequently, these SCFA can be utilized to form lipids and ketone bodies. SCFA may also enter the citric acid cycle and become utilized for glucose production via gluconeogenesis. (3) Acetate may also pass through into the peripheral circulation and can be detected in peripheral blood.
Given the range of molecular and metabolic interactions characterised for SCFA, it remains unclear how or why SCFA show preference for HDAC inhibition or GPR activation within different cell types, before being metabolised. The kinetics between GPR and SCFA transporters on the surface of cells, and what potential factors regulate interaction with cognate SCFA also remains unknown. Variation in SCFA concentration at different sites of the body, in SCFA transporter expression (for example, expression of SMCT-1 is almost exclusively limited to brush border of colonic epithelial cells, while MCT-1 is expressed by almost all tissues) and in the concentration required to elicit an effect via GPR/HDAC, results in a scenario where SCFA-mediated effects are extremely tissue specific. Regardless, the aforementioned mechanisms have been heavily implicated in health and disease, both within the colon and at peripheral sites.

5 | SCFA IN HEALTH AND DISEASE

5.1 | Maintenance of the colonic epithelium

It is well-established that SCFA play a critical role in colonic health, particularly in maintenance of the epithelial lining. Their plentiful presence is critical for the turnover and differentiation of colonic epithelial cells. Very low luminal SCFA exposure to the colonic epithelium is associated with mucosal hypoplasia, reduced epithelial proliferation and differentiation of the epithelium, and mucosal atrophy in experimental animals and humans when luminal substrates are reduced via parenteral nutrition, elemental diet or diversion of the faecal stream. In animal models, consumption of a diet high in resistant starch induces changes in metabolic gene expression towards glucose and fatty acid metabolism within the colonic epithelial cells, likely through increased uptake of SCFA.

There is also evidence that high concentrations might have toxic effects on the epithelium in rats (as discussed above), just as SCFA do on cell lines in vitro. Thus, colonic epithelium starved or swamped with SCFA have impaired structure and function. However, whether differences in SCFA delivery to the epithelium within the physiological range have specific modulating effects on epithelial homeostasis, such as affecting epithelial turnover and differentiation or barrier function, has not been convincingly demonstrated in humans and animals. This is despite multiple actions of butyrate particularly in many cellular processes. Some of these included modulation of specific enzyme systems such as urokinase, reduction in paracellular permeability or promotion of epithelial cell restitution after injury in the Caco-2 model of colonic epithelium, stimulation of mucus production in another cell line, which in turn prevented epithelial adherence of pathogenic bacteria, HDAC1-mediated promotion of expression of structural proteins responsible for maintaining tight junctions, and increased epithelial biosynthesis of retinoic acid, an important mediator of immune homeostasis in the gut.

All of these phenomena are consistent with a positive effect of butyrate in epithelial turnover and barrier function.

5.2 | Anti-tumour effects

Butyrate has been extensively studied for its anti-tumour effects in both human colonic carcinoma cell lines and in animal models. Its topical delivery to the colonic epithelium reduced early tumourigenic effects (aberrant crypt formation) in the rat azoxymethane model. To date, however, the development of butyrate as a chemotherapeutic agent has not progressed beyond phase I studies. A major limitation has been delivering butyrate to the correct location at sufficient concentrations. It needs to be delivered topically. If the rectum is used as the portal of entry, it needs to be given frequently, since twice daily enemas failed to specifically reduce aberrant crypt formation in rats, but five times daily did.

The use of oral preparations of butyrate itself failed to deliver it to the distal colon. Fibre combinations can enhance distal colonic delivery, but such combinations have not shown consistent benefits in human intervention studies. Modified forms of resistant starch composed of butyrate molecules esterified to the sugar backbone can increase butyrate delivery to the distal colon and have anti-tumourigenic effects in rats, but the effects of this strategy in humans have not yet been clearly reported.

The complexity of butyrate’s effects is compounded by confusion over the “butyrate paradox” by which this SCFA has seemingly both pro-tumour and anti-tumour properties. The effects of butyrate have been shown to be altered by the degree of differentiation of the cell. Migration of intestinal epithelial crypt cells to the surface epithelium is increased in the presence of SCFA, therefore promoting epithelial cell proliferation. It has been suggested that butyrate may confer its anti-tumour effect via inhibition of HDACs that control gene expression within the cell. Indeed, the butyrate paradox is likely to be a result of the complex role of SCFA in regulating cellular metabolism and epigenetics, seen in the Warburg effect. Cells in a cancerous state primarily undergo anaerobic glycolysis. This causes butyrate to accumulate within the cell to a concentration whereby it may inhibit HDAC, resulting in increased expression of apoptotic genes. Conversely, healthy cells metabolise butyrate whilst undergoing oxidative phosphorylation. Within this metabolic pathway it is converted to acetyl-CoA, a co-factor that promotes expression of cell proliferation genes. This may drive polyp formation in vivo. In the adenomatous polyposis coli (APC) (MHS2)/− mouse model of colorectal cancer, butyrate acted as an oncometabolite driving polyp formation, when administered as a colonic enema at concentrations between 50 μM and 0.5 mM. However, engagement of butyrate-specific GPR109a on colonic epithelial cells may also confer protection from cancer by upregulating tumour suppressor genes Slc5a8, Msh2 and Msh3.

5.3 | Anti-inflammatory effects on the colonic epithelium

Short chain fatty acids, particularly butyrate, have effects on colonic epithelial cells that are regarded as anti-inflammatory. Studies in vitro have highlighted that SCFA can control expression of
inflammatory cytokines such as IL-8, IL-17, IL-1β, IL-6, IL-12 and TNF-α by colonic epithelial cells. This may be through activation of the MEK-ERK and p38 MAPK intracellular signalling pathways by SCFA engaging GPR41 and GPR43 on the surface of colonic epithelial cells. Murine models have implicated both a pro- and anti-inflammatory role for SCFA dependent effects on the colonic epithelium, ultimately reflecting the complex nature of homeostasis within the gut. This may also involve SCFA-dependent activation of the NLRP3 inflammasome via GPR43 and GPR109a, promoting production of IL-18. Indirect effects of butyrate on inflammation via the maintenance of a healthy colonic epithelial barrier as outlined above are also of likely importance.

5.4 Effects of SCFA on immune cells

As illustrated in Figure 2, SCFA may directly affect immune cells, not only via long-recognised direct inhibitory effects on cell division, but also via modulation of cell signalling, epigenetic regulation and metabolism, the molecular mechanisms of which have now also been described. These could occur during the innate response by affecting macrophages and granulocytes, as well as antigen presentation by dendritic cells, and also during adaptive immune responses by affecting T and B cell function.

5.5 Effects on innate immune cells

Butyrate regulates many subsets of innate cells of the mucosal immune system via inhibition of NF-κB nuclear translocation and HDAC. First, CD11b+CD11c+ intestinal macrophages down regulate production of inflammatory IL-6 and IL-12 in response to butyrate. Secondly, polarisation of macrophages to the alternative M2 phenotype in the presence of IL-4 may be enhanced, attenuating DSS-induced inflammation in a murine colitis model. Thirdly, butyrate down-regulates genes involved in activation of lamina propria-residing CD103+ dendritic cells, thus giving them a tolerance-promoting phenotype. In addition, engagement of GPR109a by butyrate on this dendritic cell subset appears to control the activity of the tolerogenic retinoic acid-producing enzyme, RALDH. Indeed, co-culture of CD4+ helper (Th) cells with SCFA-treated dendritic cells drives the conversion of T cells towards a Foxp3+ regulatory T (Treg) cell phenotype. Conversely, SCFA-treated GPR109-/- dendritic cells fail to induce Treg formation and, instead promote the inflammatory Th17 phenotype.

Neutrophils in vitro chemotactically respond to acetate at concentrations typically found in human peripheral blood. However, it is not known if this could modulate neutrophil function in the periphery, as the presence of such a concentration gradient has not been characterised in vivo. Indeed, neutrophils exposed to SCFA appear to downregulate expression of the inflammatory receptors C5aR and CXCR2 via a GPR43-dependent mechanism. Furthermore, butyrate decreases neutrophilic expression of the inflammatory mediators TNF-α and nitric oxide. It is important to consider that, in the context of infection, SCFA could potentially exert unfavourable effects on the host by inhibiting pro-inflammatory activity. Addition of SCFA to the site of subcutaneous infection in mice was found to impair phagocytosis by neutrophils recruited to the site of infection and was also associated with increases in bacterial load. During colonic Citrobacter rodentium infection in mice, however, provision of acetate enhances IL-6-dependent recruitment of neutrophils to sites of infection. Taken together, these observations highlight that SCFA may modulate neutrophilic activity. The extent to which this is pro- or anti-inflammatory is likely to be dependent on the immunological milieu within the site of action.

5.6 Effects regulatory T cells

Direct effects of SCFA on Treg cells have been of interest as deficiencies in these cells have been implicated in both mucosal and systemic immune disease. Indeed, transfer into germ-free mice of a group of SCFA-producing strains of Clostridia isolated from the human intestinal microbiota promoted the generation of protective colonic Treg cells. Mice given 150 mmol/L SCFA in the drinking water have increased colonic T cell expression of Foxp3, the lineage specification factor of Treg cells. The mechanism through which this occurs remains unclear. Initial murine studies reported that this Foxp3 induction was dependent on engagement of GPR43 by SCFA, both individually and in combination. However, as T and B cells do not express functional levels of this receptor, SCFA-mediated HDAC inhibition has been implicated. Within Treg cells, the HDAC9 isoform has been proposed to directly contribute to increased transcription of Foxp3. As a consequence, increased acetylation of signalling protein S6 Kinase (S6K) within the mTOR pathway may also increase cellular metabolism, contributing to enhanced suppressive function.

Although SCFA increase expression of the colonic homing receptor, GPR15 on Treg cells, they also appear to boost peripheral Treg numbers in murine models. This effect is yet to be studied in humans, but the ratio of Treg:Th17 cells increases in human peripheral blood mononuclear cells incubated in vitro with SCFA. Numerous studies have highlighted similar anti-inflammatory changes to peripheral blood mononuclear cells. As reported concentrations of SCFA used in these in vitro studies are upwards of 50-fold greater than physiological concentration in the peripheral blood, it is unlikely that such effects could be validly translated to the same situation in vivo. SCFA may also confer anti-inflammatory effects on immune cells in the dermis, a site where SCFA-producing commensal bacteria are present. Topical application of butyrate induced the formation of Foxp3+ Treg cells in the skin of mice and was also associated with reduced swelling in a model of contact hypersensitivity.

5.7 Effects on B cells and antibody production

Increased appreciation for effects of SCFAs on B cells is also emerging. Increased delivery of acetate to the colon via acetylated-starches resulted in a tolerogenic B cell phenotype in non-obese diabetic (NOD) mice; with splenic B cells from these mice found to
FIGURE 2 Immune-modulating effects of SCFA. A, Mechanisms of SCFA action within cells. SCFA can engage G-protein coupled receptors on the surface of cells altering intracellular signalling pathways. SCFA may also pass into the nucleus, altering gene expression and mTOR signalling by inhibiting activity of histone deacetylases. Within the cell, SCFA may also be used as an energy source, altering cellular metabolism.

B, The spectrum of immune cell targets with reported changes to phenotype.
have reduced expression of MHCII and co-stimulatory molecules CD80 and CD86. SCFA may both indirectly and directly enhance IgA and IgG antibody responses. GPR43 engagement by SCFA on dendritic cells upregulates retinoic acid production, which in turn has been found to induce IgA production by B cells. SCFA have also been reported to directly activate the mTOR pathway in B cells, leading to, increased glucose uptake, glycolytic activity and terminal differentiation into antibody-secreting plasma cells. Such observations highlight an important role for SCFA in regulating B cell function via metabolic changes within the cell.

5.8 SCFA in immune disorders

Short chain fatty acids have now been implicated in a wide spectrum of inflammatory diseases shown in Table 2. Although the majority of studies show the association of SCFA with protection from development of disease, the opposite may occur. Thus, provision of SCFA in the drinking water caused levels of SCFA to accumulate in the renal tissue, resulting in severe T-cell mediated inflammation. Furthermore, the potency of each SCFA varies across disease models and has resulted in a varying array of proposed mechanisms put forward. For example, the protective effect of SCFA in asthma models has been reported to occur via propionate-mediated effects on dendritic cell development and subsequent antigen-presenting function in the house-dust mite model, but also via acetate-mediated promotion of protective Treg cells in utero in the allergic airways disease model. Collectively, these studies lack supportive data in humans, in whom many studies have been unable to provide an adequate dose of fermentable fibre or have failed to examine the putative mechanisms put forward by animal studies. There is a need for increased collaboration between immunologists, nutritionists and dietitians to ensure studies are correctly designed to translate into clinical findings.

5.9 Metabolic effects

Short chain fatty acids can be used for de novo synthesis of glucose and lipids, providing a rationale for studying a potential role for SCFA in modulating energy homeostasis and metabolism. Multiple metabolic effects of SCFA have been reported and these include the following:

1. Control of obesity: Epidemiological evidence suggests a protective role for fibre in the development of obesity. If causal, SCFA may be involved. SCFA activation of GPR43 in white adipose tissue decreases fatty acid uptake, supressing fat accumulation. Furthermore, GPR43 activation on brown adipocytes promotes mitochondrial biogenesis, increasing the capacity of the cell to utilize energy for thermogenesis. Although these effects are difficult to observe in vivo, a distal colonic infusion of acetate was shown to increase fasting fat oxidation in overweight men. Control of energy intake may also be mediated by SCFA. GPR41 activation by propionate stimulates production of the satiety hormone leptin by adipocytes. In contrast, enhanced SCFA delivery might promote obesity. Comparative studies of faecal microbiota have highlighted that obese and overweight individuals have different bacterial profiles and faecal concentrations of SCFA, particularly propionate, are increased in the obese. However, as no assessment of the diets of the subjects studied was made, the results cannot be interpreted in terms of enhanced capacity to harvest energy in the form of increased SCFA production, as was deduced from microbiota studies in lean and obese mice.

2. Control of glucose homeostasis: A role for SCFA in maintaining glucose homeostasis has also been documented; consumption of an arabinoxylan-rich diet increases plasma SCFA and improve glucose tolerance in type II diabetics. Mechanistically, SCFA can directly stimulate GPR43 on colonic enteroendocrine cells to produce glucagon-like peptide (GLP)-1, improving glucose homeostasis during feeding. Propionate has also been reported to elicit a similar effect on ileal cells by activating a GLP-1 dependent neuronal network via GPR43, highlighting that direct consumption of SCFA may also improve glucose tolerance. Studies in Gpr43−/− mice have also implicated a role for direct stimulation of GPR43 in promoting pancreatic β-cell proliferation. However, SCFA were not observed to stimulate this pathway. Propionate may also signal via a GPR41-mediated gut-brain pathway to increase intestinal gluconeogenesis, shown to be associated with improved glucose homeostasis and decreased weight gain in mice. To confuse the issue, Perry et al have proposed that increased colonic acetate production could promote increased glucose stimulated β-cell insulin release via the parasympathetic nervous system, leading to insulin resistance.

3. Appetite regulation: GPR41 activation by propionate stimulates production of the satiety hormone, leptin, by adipocytes. Inulin challenge, purported to specifically increase colonic propionate production, was reported to be associated with reduced desire for high energy foods and led to decreased energy intake in healthy men. However, inulin has multiple effects, including intestinal distension with its potential for inducing abdominal symptoms and elevating production of other SCFA. Given that acetate can cross the blood-brain barrier, SCFA may act as signalling molecules in a bi-directional gut-brain pathway responsible for control of energy homeostasis. Indeed, acetate has also been reported to have a role in modulating central appetite regulation in humans. Murine studies have elucidated that this may be via inhibition of neuronal circuits within the brain that control release of the PYY in the gut. Moreover, GPR41 has been found to be expressed on sympathetic ganglia of humans. Taken together, these observations suggest that SCFA may regulate neuronal pathways that control feeding behaviours.

4. Cardiovascular effects: SCFA may also play a role in regulating blood pressure. Provision of acetate in the drinking water reduces cardiac hypertrophy, systolic and diastolic blood pressure
in deoxycorticosterone acetate-treated mice. Although the mechanism behind such observations are not fully elucidated, GPR41 is expressed on the surface of murine endothelial cells, potentially implicating a role for propionate in regulating blood pressure.

6 | UTILISING SCFA AS A THERAPEUTIC FOR INFLAMMATORY DISEASE

Human randomised control trials are needed to confirm the wide range of therapeutic and protective effects of SCFA reported in animal studies. However, these animal studies do not directly translate into human intervention studies owing to the huge quantities of dietary fibre used. Daily fibre intake by a mouse on a murine high fibre diet has been estimated to be equivalent to 274 g/day for a human, approximately 9.7 times that of the recommended intake of 30 g/day. Such doses in humans would neither be possible or tolerated. Therefore, alternative approaches are needed to obtain adequate levels of SCFA in the colon and peripheral tissues in humans. Several delivery methods can be applied as shown in Figure 3.

### 6.1 | Delivery by a pro-drug approach

Tributyrin, a butyrate prodrug, may increase serum butyrate to a median level of 52 μmol/L with an oral dose of 200 mg/kg given three times daily. However, clinical use of tributyrin has been limited by large intra-individual variation in serum butyrate and the impractical number of capsules required. Incorporation of tributyrin in microcapsules made from the 8-membered sugar ring molecule, γ-cyclodextrin appears to protect the compound from release in the upper gastrointestinal tract. This reduces the unpleasant sensory characteristics of tributyrin and promotes delivery to the small intestine and colon. There are as yet no reports of its application in a clinical trial.

### 6.2 | Fermentable fibre

Highly fermentable fibres, such as inulin and guar gum can produce a significant rise in plasma SCFA levels within 6 h when consumed as a 20-30 g supplement. Furthermore, longer term consumption in studies ranging from days to weeks, of a similar dose of resistant starch increases faecal levels of total SCFA and butyrate. These fibre doses are reported as tolerable within study cohorts but
gastrointestinal symptoms, are generally increased, making it unlikely that regular consumption of such doses would be tolerated amongst all members of the general public.

Combining these fibres with slowly fermentable wheat bran may promote the generation of SCFA along the entire length of the colon. Of particular interest has been high amylose maize starch (HAMS), a source of type 2 resistant starch that is commercially available and can provide high levels of resistant starch after cooking and freezing. Consumption of breads and baked goods containing HAMS increased faecal SCFA output in human subjects with hypertriglyceridaemia. Arabinoxylan oligosaccharides (AXOS), another type of highly fermentable fibre produced by enzymatic hydrolysis of arabinoxylan, can be produced in bread by the addition of xylanase into the dough prior to baking. Consumption of a 140 g serving of white bread containing 18 g of added AXOS increased peripheral serum levels of acetate, butyrate and total SCFA in young adults and was also observed to improve overnight glucose tolerance. A major limitation of utilising a dietary approach to delivering SCFA in the colon is the variable nature of SCFA production seen in response to consumption of fermentable fibre, as discussed earlier. Furthermore, encouraging the general public to consume an adequate dose of fermentable fibre that will achieve sufficient boosting of SCFA production has been challenging. This has highlighted a need for more targeted approaches that are compatible with a wider variety of gut microbiota profiles.

6.3 | Chemically modified resistant starch

Esterification of SCFAs to resistant starch protects them from release and absorption in the small intestine. Such chemical modification combines both postbiotic and dietary approaches, generating efficient and preferential delivery of each SCFA to the colon when compared to unmodified resistant starch. Thus, consumption of these starches produces a significant increase in SCFA levels in stoma digesta from ileostomy patients and faeces from healthy controls. Moreover, consumption of 40 g of butyrylated starch per day protected healthy controls from the development of colonic tumourigenic adducts induced by concurrent consumption of a 300 g/day high red meat diet. These starches confer significant protection against autoimmune T1 diabetes in NOD mice. Esterification of propionate to inulin has also been reported, with the synthetic process proposed as a more efficient alternative to using starch, with 10 g of propionated inulin estimated to deliver the equivalent amount of propionate to the colon as 90 g of non-starch polysaccharide. This dose has also been observed to increase post-prandial plasma PYY and GLP-1, with long-term ingestion reducing weight gain in obese individuals. These studies highlight therapeutic effects achieved through targeted and efficient delivery of SCFA to the colon, representing a potentially desirable approach for use in future randomised control trials.

There are potential short-comings of these approaches. Practical aspects such as taste and smell may be an issue, despite being reported as tolerable when consumed as a drink in healthy subjects. Whether they can be incorporated into food without imparting unpleasant taste needs also to be addressed. Their safety has received limited attention. As discussed above, SCFA may be toxic at very high luminal concentrations in the colonic lumen. The presence of SCFA in defined gut microbiota growth medium inhibits the growth of key Bacteroidetes species of the human gut microbiota. Furthermore, the lowering of the luminal pH is associated with inhibited metabolic activity of luminal bacteria, which potentially may have deleterious effects on other functions of the microbiota. However, little is known of what luminal concentrations of SCFA and of what pH profiles are achieved by these compounds. On the other hand, inhibition of fermentation will lead to more starch being available for fermentation in the distal colon, which could result in reduced protein fermentation. Clearly, more studies are needed to determine the physiological and microbiological effects of such modified carbohydrate use.

6.4 | Direct “postbiotic” delivery of SCFA

Direct delivery of SCFA in the gut without the need for fermentation has been explored as a “postbiotic” treatment for disease. There has been particular focus in IBDs and colitis, whereby impaired SCFA-epithelial interaction may contribute to disease pathology. A number of open-label and RCTs trialling postbiotic butyrate and SCFAs within IBD and colitis patients have been conducted, the results of which are summarised in Table 3. Treatment with SCFA enemas appeared to improve histological and endoscopy disease scores in an initial open label study in diversion colitis patients. This effect was less clear in randomised placebo-controlled clinical trials in larger cohorts of patients with active distal
<table>
<thead>
<tr>
<th>Disease</th>
<th>No.</th>
<th>Design</th>
<th>Treatment arms 1</th>
<th>Comparator</th>
<th>Dose duration</th>
<th>Results</th>
<th>Secondary end-points</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diversion colitis</td>
<td>4</td>
<td>Observation</td>
<td>SCFA (46:23:31) 130 mM (pH 7)</td>
<td>Saline (sequential)</td>
<td>60 mL b.d. 2-60 weeks</td>
<td>Improved endoscopic appearance + histological scores</td>
<td>Saline had no benefits</td>
<td>197</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>RCT</td>
<td>SCFA (46:23:31), 130 mM (pH 7)</td>
<td>Isotonic saline</td>
<td>60 mL b.d. 2 weeks</td>
<td>No differences in histological endoscopic scores</td>
<td></td>
<td>201</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>RCT</td>
<td>Butyrate 600 mM</td>
<td>Saline + 3 mM butyrate</td>
<td>30 mL b.d. 30 days</td>
<td>No differences in end-points vs placebo</td>
<td>Butyrate-mediated up-regulation of gene expression associated with mucosal repair</td>
<td>203</td>
</tr>
<tr>
<td>Distal ulcerative colitis—active</td>
<td>12</td>
<td>Observation</td>
<td>SCFA (80:30:40)</td>
<td>Isotonic saline</td>
<td>100 mL b.d.</td>
<td>Improved disease activity (1 remission, 9 improved) and histology scores</td>
<td></td>
<td>207</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>RCT</td>
<td>#1 SCFA (46:23:31) 130 mM (pH 5.5)</td>
<td>Isotonic saline</td>
<td>60 mL b.d. 8 weeks</td>
<td>No differences in disease activity score</td>
<td>No differences across groups for partial clinical response, or endoscopic and histological scores</td>
<td>199</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>RCT</td>
<td>#2 Butyrate 100 mM (pH 5.5)</td>
<td>Isotonic saline</td>
<td>100 mL b.d. 6 weeks</td>
<td>No difference in overall clinical score or proportion of responders</td>
<td>SCFA better than placebo for stool frequency (P &lt; 0.05), urgency (P = 0.02) and patient self-evaluation (P &lt; 0.05)</td>
<td>208</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>RCT</td>
<td>SCFA (54:20:27), 150 mM (pH not stated)</td>
<td>Isotonic saline</td>
<td>60 mL noce 6 weeks</td>
<td>No differences in disease response-butyrate 7/19 vs placebo 9/19 (P = 0.51)</td>
<td>No differences in complete response, or in disease activity, endoscopic or histological scores</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>91</td>
<td>RCT</td>
<td>Butyrate 80 mM (pH 7)</td>
<td>Saline + 0.8 mM butyrate</td>
<td>100 mL b.d. 6 weeks</td>
<td>No differences in change in disease activity scores (P = 0.23)</td>
<td>No differences in change in stool frequency, rectal bleeding, endoscopic appearance, histological indices (except mucin depletion less with SCFA; P = 0.03)</td>
<td>198</td>
</tr>
</tbody>
</table>

(Continues)
ulcerative colitis and diversion colitis, with SCFA enemas not improving clinical indices of disease when compared to placebo.\textsuperscript{198-201}

Although enemas improved clinical indices of disease when compared to pre-treatment, a higher than expected response to placebo occurred in many trials. Saline enemas, the usual placebo control, do alter rectal epithelial proliferative kinetics in healthy subjects\textsuperscript{202} and when infused twice daily into the distal colon of rats treated with azoxymethane, reduced the development of aberrant crypts compared with no-infusion controls.\textsuperscript{112} Furthermore, variation in concentration and frequency of the enemas provided in these studies have likely contributed to mixed results seen. For example, butyrate infusion into the distal colon of rats had no specific effect over saline infusions on aberrant crypt formation when given twice a day, but have a significant effect when delivered five times per day.\textsuperscript{112} Hence, positive biological effects of saline (control) enemas reduce the power of detecting specific effects and twice-daily use may well be insufficient as a therapy. In other words, clinical efficacy of enemas are likely to be impractical to achieve as an extended period of contact between SCFA and mucosa may be required for therapeutic effects. This has dampened enthusiasm for the enema approach, despite recent studies highlighting that they may reduce inflammation by inducing changes to expression of mucosal genes involved in cytokine expression and metabolism of reactive oxidative species.\textsuperscript{203,204}

Slow-release preparation of oral butyrate, utilising pH-dependent release mechanisms, is an alternative approach to colonic enema that had benefits in patients with diverticulosis and Crohn’s disease in pilot studies.\textsuperscript{205,206} However, this approach has not been further developed and requires testing in larger placebo-controlled RCTs.

TABLE 3 (Continued)

<table>
<thead>
<tr>
<th>Disease</th>
<th>No.</th>
<th>Design</th>
<th>Active</th>
<th>Comparator</th>
<th>Dose duration</th>
<th>Primary end-point</th>
<th>Secondary end-points</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal ulcerative colitis— refractory to oral 5-ASA + steroids</td>
<td>51</td>
<td>RCT</td>
<td>4 g 5-ASA + butyrate, 80 mM (pH not stated)</td>
<td>4 g 5-ASA + saline</td>
<td>80 mL b.d. 6 weeks</td>
<td>No difference in proportion in clinical remission/ improved: Butyrate: 6/12 vs control 1/13</td>
<td>Improvement with butyrate in stool frequency (P &lt; 0.01), urgency/tenesmus (P &lt; 0.05) and patient self-evaluation (P &lt; 0.01)</td>
<td>209</td>
</tr>
</tbody>
</table>

Oral butyrate

| Diverticulosis with previous diverticulitis | 73  | [52]\textsuperscript{a} | 2 × 150 mg butyrate (microencapsulated) | Placebo | 2 tablets o.d. 12 months | Greater number of episodes of diverticulitis with butyrate vs placebo: clinically diagnosed 2/30 vs 7/22 (P = 0.04); ultrasound diagnosed 6 vs 1 (P = 0.02) | Better subjective symptom improvement with butyrate than placebo (P = 0.014) | 205  |

Mild ileo-colonic Crohn’s disease | 13  | Observation | 2 g butyrate (enteric coated) |   | 2/day 8 weeks | Clinical remission in 7, response in 2 | Improved endoscopic and histological scores | 206  |

Mild active UC | 30  | [52]\textsuperscript{a} | 2.4 g 5ASA + 4 g butyrate (Eudragit-S-coated) | 2.4 g 5ASA | Daily 6 weeks | No differences in clinical remission/or response: Butyrate (n = 12) 7/5 vs control (n = 13) 5/5 | No differences in improvement in clinical activity, histological and endoscopic scores | 210  |

\textsuperscript{a}Number analysed per-protocol.

Food containing SCFA is another potential way of delivering SCFA to the systemic circulation. SCFA ingested orally in solution are rapidly absorbed into the circulation and have been reported to cause a transient increase in blood acetate levels.\textsuperscript{211} Furthermore, they are likely to be adsorbed by epithelial surfaces in the oesophagus, stomach and intestine. Consumption of vinegars, which are naturally high in acetic acid, is anecdotally believed to promote health benefits.\textsuperscript{212} In small human studies, vinegar consumption reduces plasma triglyceride levels.\textsuperscript{219} Long-term fasting that promotes ketogenesis and increases circulating SCFA is associated with reduced

6.5 | Promotion of endogenous SCFA

Utilising endogenous metabolic processes that produce SCFA to attain health benefits is an intriguing possibility. Low-level alcohol consumption, that creates a transient increase in plasma acetate levels, is associated with a lower risk of heart disease and diabetes.\textsuperscript{218} Pownall et al proposed that protective effects of alcohol may be mediated by acetate acting via GPR43 on adipocytes, inhibiting lipolysis and decreasing plasma triglyceride levels.\textsuperscript{219} Long-term fasting that promotes ketogenesis and increases circulating SCFA is associated with reduced
circulating inflammatory cytokines and improved glucose tolerance.\textsuperscript{220,221} The ketone body \(\beta\)-hydroxybutyrate, is structurally similar to that of butyrate and inhibits NLP3-inflammasome mediated inflammation in mouse models.\textsuperscript{222} Further studies are required to define whether increasing endogenous SCFA is causally associated with reducing disease before utilising such a strategy.

7 | CONCLUSIONS

A wide range of pre-clinical evidence supports a role for SCFA as a modulator of colonic and overall health. However, fundamental differences between animal and human physiology dictate caution in extrapolating animal effects to human diseases and create a need to conduct clinical trials to validate these effects in humans. A major issue relates to differences in the dose of SCFA that can be delivered to the target, whether it be, for example, the distal colon or systemic circulation. Additional factors might include inter-individual variability in microbiota and metabolism. Major limitations in progress have included inability to measure SCFA production in vivo other than in peripheral sites of faeces or blood. However, emerging technologies may provide insight into this dynamic process. Furthermore, development of novel targeted approaches for colonic SCFA delivery represents a desirable strategy that might achieve more consistent and reliable dosing. These may also be combined with dietary supplementation of SCFA to maximise delivery. With these emerging technologies at hand, this gives enormous potential for SCFA to finally be translated therapeutically in a wide range of intestinal, metabolic and inflammatory diseases.

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AUTHORSHIP

Guarantor of the article: P.R. Gibson.

Author contributions: P.A Gill performed the literature search. P.A Gill and P.R Gibson wrote the manuscript. J.G Muir, M.C van Zelm and P.R Gibson critical reviewed the manuscript. All authors approved of the final version of the manuscript.

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