Current Research in Microbial Sciences 6 (2024) 100219

Contents lists available at ScienceDirect

Current Research in Microbial Sciences

journal homepage: www.sciencedirect.com/journal/current-research-in-microbial-sciences

Gut microbiome derived short chain fatty acids: Promising strategies in necrotising enterocolitis

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ARTICLE INFO

Keywords:
Necrotising enterocolitis
Preterm neonates
Microbiome
Short-chain fatty acids

ABSTRACT

Necrotising enterocolitis (NEC) is a devastating condition that poses a significant risk of morbidity and mortality, particularly among preterm babies. Extensive research efforts have been directed at identifying optimal treatment and diagnostic strategies but results from such studies remain unclear and controversial. Among the most promising candidates are prebiotics, probiotics and their metabolites, including short chain fatty acids (SCFAs). Such metabolites have been widely explored as possible biomarkers of gut health for different clinical conditions, with overall positive effects on the host observed. This review aims to describe the role of gut microbiome derived SCFAs in necrotising enterocolitis. Until now, information has been conflicting, with the primary focus on the main three SCFAs (acetic acid, propionic acid, and butyric acid). While numerous studies have indicated the relationship between SCFAs and NEC, the current evidence is insufficient to draw definitive conclusions about the use of these metabolites as NEC biomarkers or their potential in treatment strategies. Ongoing research in this area will help enhance both our understanding of SCFAs as valuable indicators of NEC and their practical application in clinical settings.

Introduction

The human gut contains an astounding number of microorganisms that are at least as numerous as the human cells within the host and genetically there are 100-fold more microbial genes than those encoded by the human genome. This creates a complete ecosystem indispensable for the adequate health and nutrition of individuals. Small alterations in its balance can end in deleterious outcomes (short and long-term), especially in preterm neonates where the intestine is immature and more prone to inflammation (Jiang et al., 2017). Due to this immaturity, around 10 % of preterm infants under 1500 gs develop a condition called necrotising enterocolitis (NEC) (Zheng et al., 2020). Despite numerous studies, the precise mechanism underlying the development of this illness remains only partially understood, with certain aspects yet to be fully elucidated (Ahearn-Ford et al., 2022; Baldassarre et al., 2019; Pichler et al., 2020). As health care is improving survival rates in extremely preterm babies, this disease is becoming an increasing health problem due to its high morbi-mortality and economic impact (Masi and Stewart 2019; Pammi and Suresh 2017; Patel and Pammi 2021). This has encouraged interest in research exploring early diagnosis, preventive strategies and novel treatment options for this condition (Kaplina et al., 2023). Because of the fast instauration of this disease on infants, enteral feeding protocols and early administration of antibiotics have been widely established in the neonatal intensive care units (NICUs) (Neu 2020; Neumann et al., 2023).

Some of the main risk factors for NEC include prematurity, aberrant bacterial colonisation and a lack of breast milk (Claud et al., 2013; Huang et al., 2022). A decrease in stool microbial community diversity with enriched Proteobacteria has been most typically reported in NEC cases, but there are inconsistencies between studies (He et al., 2021; Murphy et al., 2021; Sajankila et al., 2023). Studies using tissue from the site of disease, rather than stool, have found similar associations (Stewart et al., 2019), but are limited to cross-sectional analysis and lack truly healthy controls (i.e., those infants absent of an indication for invasive surgery).

The gut microbiome has been described as a dynamic whole ecosystem with a significant role in human health (Murphy et al., 2021; Pichler et al., 2020). Considering the intricacies of this complex biosystem, each of its components functions harmoniously to collectively maintain its stability (Barbosa et al., 2022). The impact on health is related to the production of an array of elements such as antimicrobial

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https://doi.org/10.1016/j.crmicr.2024.100219

Available online 12 January 2024

Abbreviations

HMO	Human milk oligosaccharides
MOM	Mother's own milk
NEC	Necrotising enterocolitis
NICU	Neonatal intensive care unit
SCFA	Short chain fatty acids
BCFA	Branched chain fatty acids
LA	Lactic acid

peptides, conjugated linoleic acid, gamma aminobutyric acid and short-chain fatty acids (SCFAs), all of which are examples of microbially produced bioactive compounds (Murphy et al., 2021). Advances in "omics" technologies have allowed the deep characterisation of a wide variety of factors within the gut environment spanning microbiome, spatial and single cell transcription, proteomics, and metabolomics (Beck et al., 2021; Renwick and Stewart 2022). As technological advances play an ever more important role in early life research, it is vital that studies are appropriately designed, and samples collected with consideration for downstream analysis (reviewed in detail elsewhere (Beck et al., 2021)).

SCFAs are among the most investigated of all bacterially derived-metabolites. Cunningham et al. (2021) describe SCFAs including acetic, propionic and butyric acids as "chief by-products of bacterial prebiotic metabolism" that are recognised in the literature as facilitators of many prebiotics' effects (Cunningham et al., 2021). They have a range of activities in the host, including reducing pro-inflammatory cytokine production, intestinal barrier preservation and promoting colonocyte function, ultimately impacting the immune system, nervous system and metabolism (Cunningham et al., 2021; Murphy et al., 2021; Pichler et al., 2020). Representing the end processes of cellular reactions, SCFAs have been postulated to be a more informative health indicator than the abundance of any specific or combination of bacterial strains (Barbosa et al., 2022).

While the term SCFAs refers to a specific group of organic acids sharing similar characteristics, their metabolism and effects on the host vary. Acetic, butyric, and propionic acid are the main three metabolites that have been associated with positive effects on the host and are mostly studied, especially in conditions such as NEC due to their role as a source of energy for colonocytes and their anti-inflammatory effects (Alsharairi 2023). Although other SCFAs are present in lesser amounts, they are relevant for different metabolic processes. However, their role as health indicators requires further studies. Therefore, making a thoughtful selection is crucial when evaluating their potential clinical applications in certain conditions. This review is focused on SCFAs and their role in improving outcomes in preterm infants at risk of NEC, highlighting their modulation effects and their possible use in bed-side scenarios. Understanding the significance of this group of gut metabolites is crucial for identifying potential biomarkers and novel therapeutic strategies.

What are short chain fatty acids?

SCFAs are produced from the fermentation of dietary components by certain anaerobic bacteria (Wong et al., 2006). They are considered aliphatic carboxylic acids, based on saturated fatty acids with a range from 1 to 6 carbon molecules, which form an aliphatic tail. The main SCFAs described in literature are formic (C1) acetic (C2), propionic (C3), butyric (C4), pentanoic or valeric (C5), and hexanoic or caproic (C6) (Akhtar et al., 2022; Tan et al., 2014). Acetic, propionic and butyric acids are considered the main SCFAs present in the human gut, with adult individuals exhibiting a relative abundance ratio of 60:20:20 respectively (Wong et al., 2006). Although the exact ratio is dependent on gut microbiome composition and multiple host factors, such as diet.

While these molecules are produced through multiple metabolic pathways, their main source in humans is the microbiota resident in the colon. Within a healthy intestine, SCFAs are produced at the highest concentrations in the proximal colon (70–140 mM), followed by the distal colon (20–70 mM) and distal ileum (20–40 mM). Studies with unfavourable outcomes involving the supplementation of SCFAs have reported an increase of concentrations in an abnormal intestinal epithelium of up to 200–300 mM. These high concentrations were described as potentially toxic, with prolonged exposure causing intestinal mucosal injury in preterm infants (Lin 2004; Nafday et al., 2005).

The major metabolic precursors of SCFAs are non-digestible carbohydrates including oligosaccharides (Ramos Meyers et al., 2022). In human breast milk, the most relevant components described as SCFA precursors are human milk oligosaccharides (HMOs), which are being extensively researched as potential biomarkers and therapeutics for NEC (Autran et al., 2018; Masi et al., 2021). Preterm infants reportedly exhibit a deficiency of SCFAs during the initial days of life, but concentrations start to increase upon the introduction of milk into their diet (Frau et al., 2021). Further studies support the link between these metabolites and the microbiota and its substrates. For instance, *Bifidobacterium longum* has ATP binding cassette type carbohydrate (ABC) transporters that facilitate the uptake of substrates in order to produce acetic acid (Fukuda et al., 2011). Genomic analysis has also revealed expression of other transporters, such as the phosphotransferase system, which is involved in the transport of carbohydrates (Zoetendal et al., 2012).

Over the years, several pathways have been identified as crucial for the production of SCFAs. Production is determined by bacterial activity through the glycolytic acid pathway, although certain microbes, such as *Bifidobacteria*, are capable of also utilising the pentose phosphate pathway. Notable examples include the oxygen-sensitive Wood–Ljungdahl or acetyl-CoA pathway for acetic acid, the carbon dioxide fixation pathway for propionic acid, and the acetyl-S coenzyme A condensation pathway for butyric acid (Liu et al., 2022; Tan et al., 2014). The distribution of these pathways and their respective production is diverse across different bacterial genera (Liu et al., 2022; Ríos-Covián et al., 2016).

Importance of specific short chain fatty acids

The production of SCFAs relies on specific microorganisms, with variations in enzyme and protein expression among distinct bacterial groups, such as Firmicutes and Bacteroidetes, underscoring the intricacy of these metabolites. As a result, initial hypotheses emerged suggesting a symbiotic balance wherein Bacteroidetes contribute acetic acid which is subsequently utilised by Firmicutes to produce mainly butyric acid (Mahowald et al., 2009). Despite their similarities, acetic, propionic and butyric acid possess distinct characteristics, which are outlined below:

Acetic acid: Among the SCFAs, acetic is typically most abundant in faecal matter (Zhang et al., 2020). It is transported to the liver via the portal vein where it is stored and subsequently released into the systemic circulation, explaining its presence in the bloodstream (Tan et al., 2014).

Numerous studies provide support for its role in immune modulation, metabolism, and energy production. Moreover, it regulates the proliferation and differentiation of adipocytes, leading to a diverse range of positive outcomes in the host (Hernández et al., 2019). Further promising results have been reported in the literature showing the positive role of acetic acid in multiple body systems, such as immune, metabolic and endocrine. An illustrative example of this is its ability to upregulate the intestinal IgA response to the microbiota (Wu et al., 2017). Due to the multiple beneficial effects of acetic acid, recent research has focused on acetic acid-producing bacteria as possible probiotic candidates. Microorganisms involved in its production are mainly from the genera *Bacteroides, Bifidobacterium, Lactobacillus* and *Prevotella* (Liu et al. 2022). Multiple studies have been performed which focus on

specific bacteria, such as *Akkermansia muciniphila*, which has been shown to have both the ability to degrade mucin and a protective role in the intestinal mucosa. The results of some of these studies have shown an increase in acetic acid levels with positive effects on natural aging-related disorders and anti-inflammatory effects in chronic colitis in murine models (Ma et al., 2023; Zhai et al., 2019)

Propionic acid: This compound is transported to the liver where it is metabolised by hepatocytes (Tan et al., 2014). Its role is related to colonic epithelial cells, as shown in Fig. 2, and as a precursor for hepatic gluconeogenesis. It has also been involved with glucose homeostasis and satiety (Barbosa et al., 2022). Propionic acid is mainly produced by *Bacteroides, Veillonella* and *Propionibacterium* (Liu et al. 2022). Other specific bacteria such as *Anaerobutyricum hallii* and *Prevotella copri* have been involved and evaluated in the production of this metabolite as possible probiotic candidates (De Vadder et al. 2016).

Butyric acid: This SCFA is mostly metabolised by colonocytes and is considered to be the main metabolic substrate for these specific cells, fulfilling 60–70 % of their energy requirements (Barbosa et al., 2022; Liu et al., 2020). This is supported by the fact that germ-free mice with no SCFAs are energy deprived, with it being possible to correct this deficit by the addition of butyric acid (Donohoe et al., 2011). Evidence is robust regarding the multiple positive effects on the host, such as anti-carcinogenic, promotion of intestinal gluconeogenesis with subsequent improvement of metabolic syndrome and anti-inflammatory effects (Deleu et al., 2021).

This SCFA is mainly produced by bacteria within the Firmicutes and Bacteroidetes phyla, such as *Faecalibacterium prausnitzii, Anaerobutyricum hallii,* and *Butyricicoccus pullicaecorum* (Barbosa et al., 2022; Tan et al., 2014). As a consequence of the already mentioned effects in human health, butyric acid producing bacteria have also become new candidates for use as probiotics. It is important to take into consideration that some of these microbes require the presence of acetic acid in order to grow. Some others, such as *Anaerobutyricum hallii,* depend on other microorganisms, such as *Bifidobacterium* spp., to first metabolise complex oligosaccharides and polysaccharides into substrates that they themselves can then use to produce butyric acid (Schwab et al., 2017).

Acetic acid, Propionic acid, and Butyric acid are considered the most abundant SCFAs with a major impact on host health and, therefore, have been extensively studied in neonatal gut. Notwithstanding, there are other important SCFAs in lower concentrations, discussed below:

Formic acid: This is the smallest of the SCFAs with just 1 carbon atom. It is considered a bacterial fermentation end-product related to the conversion of pyruvate into acetyl-CoA by the enzyme pyruvate formate lyase. This SCFA has been linked with the abundance of Enterobacteriaceae, specifically *Enterobacter cloacae* and *Klebsiella pneumoniae* (Casaburi et al., 2022). Although it was presumed to have a minor role in human physiology, it can be relevant for metabolic acidosis during methanol metabolism (Akhtar et al., 2022). Formate deficiency has also been linked to a range of disease spanning cancer, neurological disorders, obesity, and cardiovascular disease (Pietzke et al., 2020). In contrast, in a recent study using a murine NEC model, luminal formate caused NEC-like injury in a dose dependant manor (Casaburi et al., 2022).

Valeric acid: Like formic acid, this SCFA is also considered as a major product of bacteria fermentation (Trefflich et al., 2021). Until recently, this SCFAs and caproic acid were considered a food component, but new studies have found that its production is linked with gut microbiota (Rios-Covian et al., 2020). Specific intestinal bacteria associated with its production include bacteria of the family Prevotellaceae (e.g., *Prevotella copri)* and *Megasphaera* spp. (Xu et al., 2020). Further studies are required to determine its role in health and disease.

Lactic acid (LA): While not technically a SCFA, LA has been studied as a colonic bacterial fermentation product. It is considered an organic acid produced by specific lactic acid-producing bacteria, such as bifidobacteria (Wang et al., 2007). In preterm neonates a significant proportion of lactose is digested by bacterial fermentation in the colon (Lin et al., 2002). While inconclusive, some studies have reported an increase in lactic acid-producing bacteria in breastfed neonates, coupled with a decrease in SCFAs (Lin et al., 2002; Wang et al., 2007). It is well known that breastmilk works as a protective factor for multiple conditions including allergies and NEC. Recent studies have shown the association between breast milk HMOs and specific *Bifidobacterium* strains such as *B. bifidum, B. breve, B. longum* subsp. *longum* and *B. longum* subsp. *Infantis* in the production of aromatic lactic acids (Laursen et al., 2021). This topic is reviewed elsewhere (Stewart 2021). Notably, LA can increase due to reasons other than bacterial fermentation, such as tissue hypoxia inducing anaerobic respiration. d-Lactic acid is considered a potentially more specific marker for bacterial overgrowth due to gut permeability ((Henry et al., 2012; Howarth et al., 2022). The potential beneficial role of LA requires further study, but is not reported to induce intestinal mucosal injury at different concentrations, in contrast to some SCFAs (Lin et al., 2002).

Branched chain fatty acids (BCFAs): Although the main three SCFAs mentioned are considered the major components recognised as the primary products of carbohydrate fermentation by the gut microbiota, it's important to note that products of amino acid fermentation, such as isovalerate, isobutyrate, and 2-methylbutyrate, are also generated by gut bacteria. They have been linked to various health and disease outcomes, such as depression and obesity, among others (Ramos Meyers et al., 2022; Salazar et al., 2022; Trefflich et al., 2021). These main products are the result of dietary protein metabolism, HMOs, and fermentation of the branched-chain amino acids (BCAA) leucine, valine, and isoleucine (Macfarlane et al., 1992). Genes related to this have been found in certain groups of bacteria such as *Bacillus* spp., *Escherichia coli, Bacteroides* spp., *Lactobacillus* spp., and *Clostridium* spp. (Hong et al., 2017; Salazar et al., 2022).

Literature regarding BCFAs is scarce in the neonatal period. In a 2022 study, researchers examined the dynamics of SCFAs and BCAAs in the urine and stools of mothers, term, and preterm infants. The authors established potential reference values, indicating their necessity for assessing these metabolites in the context of other critical conditions, including NEC (Ramos-Garcia et al., 2022).

Neonates acquire initial BCFAs from the vernix, which is later suspended in amniotic fluid and swallowed by the fetus. This process typically occurs in the third trimester. Due to this late exposure to BCFAs, some authors have theorised their involvement in NEC which primarily affects preterm babies. Moreover, one recent study reported lower BCFAs in younger infants compared to adults suggesting a inverse correlation with age (Rios-Covian et al., 2020). Additionally, previous studies have found that bacteria producing BCFAs act as a protective factor for NEC, relating to increased expression of IL-10 (Ran-Ressler et al., 2013).

While significant differences can be observed in the gut microbiota composition, less is known about the variations in the production of SCFAs and other metabolites between preterm and term babies. Prematurity is associated with various challenges influenced by factors such as gestational age, gut immaturity, type of feeding (e.g., human milk versus bovine formula), increased antibiotic usage, inflammatory conditions like NEC, among others. These factors have been implicated in alterations to the neonatal gut ecosystem, consequently impacting the production of metabolites derived from gut bacteria (Kok et al., 2020; Wang et al., 2021). This topic remains an active area of research.

Short chain fatty acid producing bacteria and necrotising enterocolitis

The gut microbiome has been extensively studied, especially over the past two decades, owing to its clear links to human health and association with various health conditions, including diabetes, asthma, inflammatory bowel disease, necrotising enterocolitis, and more (Ahearn-Ford et al., 2022). The identification of a low incidence of inflammatory diseases in individuals with higher abundances of

SCFA-producing bacteria and accordingly higher SCFA concentrations, has led to a spike in scientific interest in this subject (De Filippo et al. 2010).

Moreover, recent studies have described the beneficial effects of SCFAs on intestinal barrier integrity, which is a critical factor in cases of NEC. A decrease in barrier effectiveness results in a "leaky gut" (Baldassarre et al., 2019), wherein the increased permeability of the intestine makes bacterial translocation more likely and heightens inflammation. Restoration of the mucus layer has been associated with the administration of SCFAs, shown by the addition of butyric acid to H4 epithelial cells and in mouse models, which resulted in increased transcription of genes involved in mucin production and tight junction formation, with a decrease in those encoding pro-inflammatory cytokines (Gao et al., 2021). The latter has intensified the interest in SCFA-producing bacteria. The description of major groups of fermentative microorganisms involved in SCFAs production are mentioned in Fig. 1.

Production of SCFAs begins upon the first interaction between breast milk and colonising bacteria (Zheng et al., 2020). Interestingly, the two main butyric acid-producing bacterial phyla (Firmicutes and Bacteroidetes) have been found to be persistently decreased in studies which evaluate the NEC microbiota. Although conflicting, in some of these studies a downward trend in specific microbes and SCFAs have been identified (He et al., 2021). In comparison, other studies of preterm stool have failed to find any significant associations between specific metabolites and NEC (Renwick and Stewart 2022; Stewart et al., 2016).

The ability of the *Bifidobacterium* genus to utilise substrates such as HMOs and to produce SCFAs as a metabolic by-product is well studied and of importance when considering the use of prebiotics and probiotics associated with SCFA production (Stewart 2021). In 2007, one study evaluated a *Bifidobacterium breve* probiotic and the amount of SCFAs in preterm neonates. The study suggested a reduction in NEC achieved through administering this probiotic, which was associated with a decrease in butyric acid and increase in acetic acid (Wang et al., 2007). However, more recent research reported an increase of acetic acid related to the colonisation of the specific strain *Bifidobacterium breve* BBG-001 in preterm neonates, but with no statistically significant difference on intestinal barrier function (Fleming et al., 2021). More recent studies, such as the one performed by Liu et al. (2022), indicated a decrease of *Bifidobacterium subs. infantis* associated with low levels of SFCAs in stools, 7 days prior to the diagnosis of NEC (Liu et al. 2022). While further work in larger, multi-centre studies are needed, emerging evidence supports the use of SCFAs in the early prediction of NEC. In addition, consideration for the optimal SCFA concentrations for preterm infants could help with the design of novel probiotic interventions.

Other functions potentially relevant for preterm infants at risk of necrotising enterocolitis

For decades, SCFAs have been associated with anti-inflammatory effects, having been shown to induce decreases in certain pro-inflammatory cytokines such as IL-6, IL-8 and TNF-α. This ability has been linked to their role as inhibitors of histone deacetylases, which are involved in the transcription of pro-inflammatory cytokines (He et al., 2021; Huang et al., 2022; Zheng et al., 2020). Furthermore, there have been reports of a reduction in TNF-α production from human blood cells following LPS stimulation and modulation of the immune system through T cells (He et al., 2021; Zhu et al., 2021). Additional associations have been outlined regarding their anti-inflammatory effects with modification of cytokines, neutrophils migration, enrichment of the mucus layer, release of neutralising IgA, among others. Regulatory effects linked to the metabolic and endocrine system, including satiety, glucose regulation, insulin production and fatty acid oxidation have also been described, as well as control of blood pressure and the nervous system, (Deleu et al., 2021; Hernández et al., 2019; Sun et al., 2021) (Fig. 2).

SCFAs have been used in the agricultural industry because of their intrinsic broad-spectrum antimicrobial effect. Important examples in food production and human studies include the addition of butyric acid to control *Salmonella* (Liu et al. 2022) and the ability of SCFAs to reduce the growth and virulence of pathogenic *E. coli* in a pH dependant manor (Zhang et al., 2020). Another relevant study related to this antimicrobial effect described that the administration of *B. longum* was associated with significant acetic acid production, which resulted in a decreased translocation of the Shiga toxin from *E. coli* O157:H7 in mice (Fukuda et al., 2011). The underlying antimicrobial mechanisms of SCFAs likely relate to their impact on microbial physiology, through altering environmental and intracellular conditions such as pH, osmotic balance and energy generation (Kumar et al., 2020). Thus, SCFAs can be beneficial in protecting against numerous pathogens. These antimicrobial properties may be relevant in the control of NEC associated infections.

Furthermore, owing to the many immune functions linked to NEC pathogenesis, SCFAs could prove to be important in the prevention and treatment of NEC.

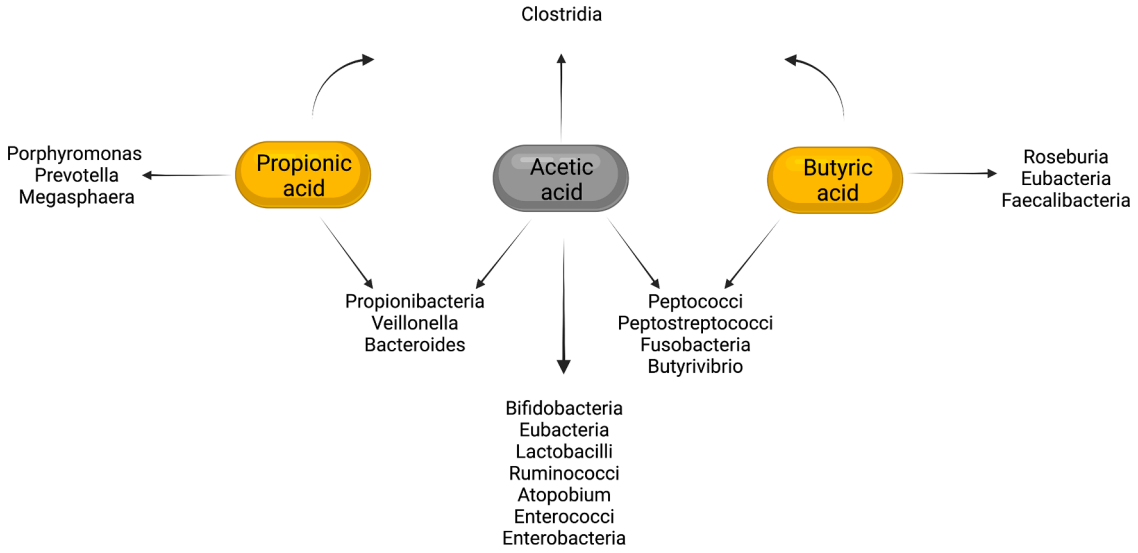

Fig. 1. Schematic showing gut microbes involved in the production of short chain fatty acids. The data underpinning the figure was derived from: (Macfarlane and Macfarlane 2012).

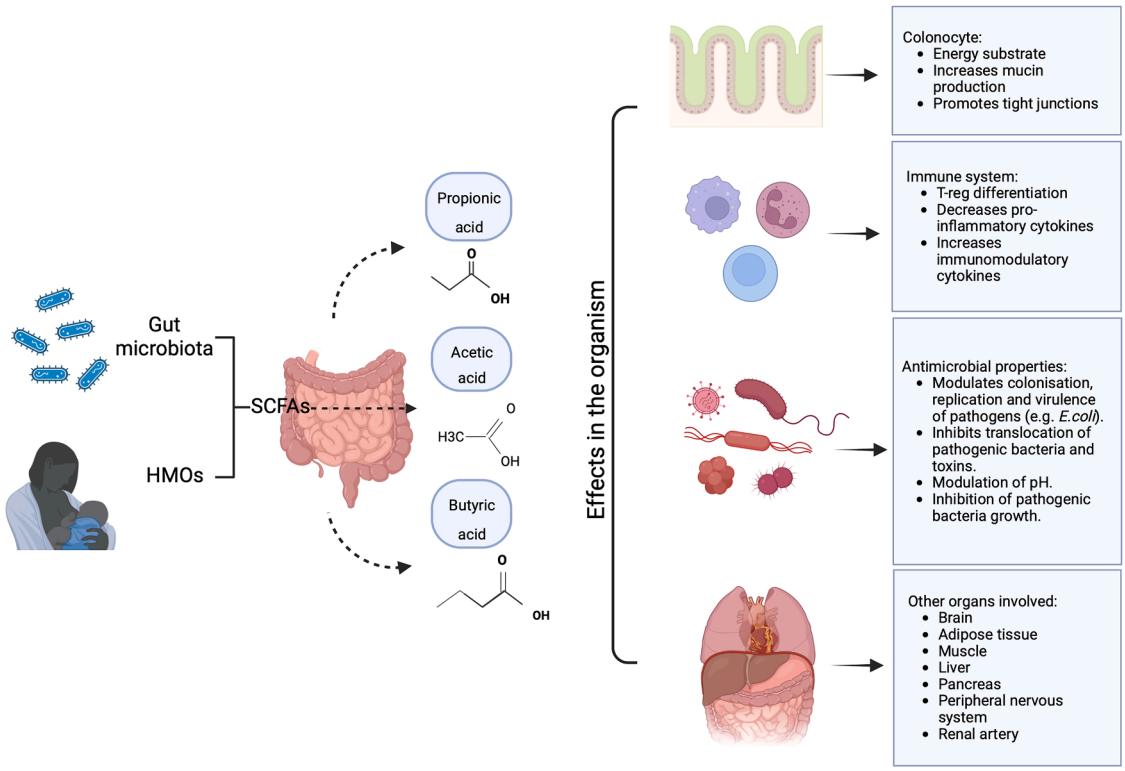

Fig. 2. Short chain fatty acids: Production and main effects.

Biomarkers or treatments for NEC?

A wide range of prevention and treatment strategies have been discussed regarding NEC. Overall, the significance of mother's own milk (MOM) as a primary protective factor is evident (Stewart 2023). However, other approaches like the use of prebiotics and probiotics have also shown promise in promoting gut health and overall well-being. Recent evidence suggests that the synergistic effect resulting from a combination of these strategies can amplify their individual beneficial outcomes (Zheng et al., 2020). Moreover, with the advancements in metabolomics, SCFAs and other immunomodulatory small molecules are gaining significant attention as novel biomarkers and therapeutics. Detection and quantification of SCFAs have been proposed as possible early diagnostic tools for NEC and indicators of general gut health in infants (Ramos Meyers et al., 2022). However, the evidence remains unclear.

Previous studies have shown deleterious effects related to the administration of SCFAs in an altered and immature gut with a low microbial diversity. Lin et al. (2002) evaluated their activities on the intestine. In animal models, researchers used 150 mM and 300 mM of butyric acid, acetic acid and LA to determine their impact on the mucosa. Their results showed a dose dependent intestinal mucosal injury with butyric and acetic acid, in contrast with LA. The authors suggested that their findings may be supported by the fact that breast-fed (protector factor for NEC) infants produced more LA due to the presence of a lactic acid-producing bacteria community, while formula-fed infants had elevated SCFAs in their stool (Lin et al., 2002). Preterm babies have an abnormal initial gut microbiome that may impact the production and/or metabolism of SCFAs, resulting in abnormally high levels of these compounds and a further alteration of the gut environment, such as lower pH (Ferraris et al., 2023; Nafday et al., 2005). High luminal levels of SCFAs may also be secondary to low intestinal motility and carbohydrate malabsorption (Lin 2004).

In the time since the study by Lin et al. (2002), others have been published supporting the theory of the butyric acid dose dependent paradoxical effect, where lower doses are linked to protecting the epithelium and higher doses upregulate inflammation (Liu et al., 2020; Peng et al., 2007). More recently, using a gnotobiotic quail model of NEC, colonisation by strains impaired in butyric acid production was found to reduce intestinal lesions when compared to wild-type butyric acid producing strains (Ferraris et al., 2023). Such findings are contrary to the results of He et al. (2021), whose study combined clinical samples (stool from infants with NEC) and germ-free mice and showed butyric acid to be decreased in the NEC group in contrast to the controls (He et al., 2021). Intestinal injury was also reduced through the replenishment of butyric acid. Additional murine studies have reported favourable outcomes in mice who received sodium butyrate (150 mM) (Sun et al., 2021). The evidence suggests a dose dependent effect (Jiang et al., 2022), but due to the contradictory findings of previously described studies, it is not possible to make an affirmation related to this issue. Ultimately, use of different analytical methods, lack of well-powered preterm infant cohorts and inconsistencies in the experimental models (e.g., cell lines, mice, or quail) hinder direct comparisons between different studies and contribute to the conflicting results. Further work in large human cohorts, combined with relevant preterm models such as preterm intestinal-derived organoids (Masi et al., 2022; Stewart et al., 2020), will help to disentangle the role of SCFAs in preterm infant health and move closer to understanding the optimal therapeutic doses. Studies performed evaluating SCFAs in NEC are summarised in Table 1.

Randomised control trials have evaluated the administration of probiotics in preterm babies, measuring SCFAs and shifts in microbiota, with variables such as feeding type and specific strain probiotic supplementation. In 2022, one study evaluated the effect of multiple vs. single strain probiotics in extremely preterm infants, with the time to achieve full feeds as the major outcome. Additionally, they assessed the gut microbiota and SCFA concentrations. Interestingly, both groups reported similar levels of these metabolites, except for butyric acid, which was significantly higher in the triple-strain group (Athalye-Jape et al., 2022). Among other studies, Underwood et al. (2009) compared two multiple-strain probiotics plus prebiotic vs. placebo. Results suggested

Table 1
Studies investigating short chain fatty acids in necrotising enterocolitis.

References	Study model	Sample	Results	Method used for evaluation of SCFAs
Lin et al., 2002	In vivo (Animal model)	Newborn Sprague-Dawley rats $n = 72$ Control $n = 10$: NSS Group 2: $n = 11$: 150 mM acetic acid Group 3: $n = 11$: 300 mM acetic acid Group 4: $n = 10$: 150 mM butyric acid Group 5: $n = 11$: 300 mM butyric acid Group 6: $n = 7$: 150 mM lactic acid Group 7: $n = 12$: 300 mM lactic acid	Acetic acid and butyric acid induced dose-dependent intestinal mucosa injury.	–
Nafday et al., 2005	In vivo (Animal model)	Newborn Sprague-Dawley rats $n = 170$ Control: NSS Groups randomly divided: 300 mM acetic acid, butyric acid, propionic acid and a mixture of the above.	The administration of SCFAs caused colonic mucosal injury that decreased with age.	–
Peng et al., 2007	In vitro	Caco-2 cells monolayer model of intestinal barrier	Sodium butyrate*** at low concentrations (2 mM) demonstrated a positive effect on intestinal barrier function, leading to a significant increase in transepithelial electrical resistance (TER) and a reduction in permeability. However, at high concentrations (8 mM), it resulted in increased permeability, decreased TER, and induced apoptosis.	–
Wang et al., 2007	In vivo (Human model)	Preterm infants: $n = 66$ 3 groups: $n = 22$ (ELBW, VLBW and LBW) Each group was subdivided in 2: Group control: $n = 11$ Group with B. breve supplementation $n = 11$	Over time, in the control groups, there was an increase in SCFAs. However, in the intervention group, there was a decrease observed, primarily in butyric acid.	High performance liquid chromatography.
Underwood et al., 2009	In vivo (Human model)	Preterm infants: $n = 90$ Placebo: $n = 29$ CUL (*Lactobacillus. rhamnosus GG* as well as inulin): $n = 30$ PBP (several species of lactobacilli and bifidobacteria plus inulin): $n = 31$	Authors compared different outcomes between dietary supplements. No significant difference of SCFAs were found between the groups.	High performance liquid chromatography.
Stewart et al., 2016	In vivo (Human model)	Preterm neonates: $n = 19$ Cases: $n = 10$ Controls: $n = 9$ Stool samples: $n = 39$	No correlation between SCFAs and NEC/LOS (Late onset sepsis).	Liquid chromatography mass spectrometry (LCMS)
Liu et al., 2020	In vivo (Animal model) In vitro	In vivo (Mice): $n = 8$ NEC Control: $n = 7$ NEC plus Butyric acid=6 In vitro: IEC-18	In vitro results: Low levels (1 mM) of butyric acid where protective whereas high doses (16 mM) upregulated pro-inflammatory cytokines (IL-6). In-vivo results: NEC mice who received butyric acid had reduced intestinal damage, reduced inflammatory cytokines expression and increased tight junction marker (Claudin-7)	–
Zheng et al., 2020	In vivo (Animal model) Ex vivo In vitro.	H4 cell Animal model C57BL6 Mouse fetal intestine and human intestinal organoids	The administration of SCFAs resulted in the inhibition of IL-1B induced IL-8 secretion, and HDACs protein expression. (Sodium acetate and propionate*** showed a dose dependent effect)	–
Fleming et al., 2021	In vivo (Human model)	Preterm babies: $n = 94$ Randomised controlled trial of *B. breve* BBG-001 (the PiPS trial) Probiotic *B. breve* BBG-001 vs placebo	The organic acids evaluated in the study included succinic acid, lactic acid, and acetic acid. In terms of SCFAs, the authors observed an elevation of acetic acid in babies colonised by *B. breve* BBG-001. However, there were no differences noted in intestinal barrier function, and no evidence of efficacy on reducing NEC was found.	High-performance liquid chromatography
Sun et al., 2021	In vivo (Animal model)	C57BL/6 neonatal mice Groups: Control, untreated NEC and sodium butyrate pre-treated NEC.	Sodium butyrate (150 mM) pre-treated NEC models had overall favourable outcomes. Decreases intestinal inflammation, decreased the opportunistic *Clostridium_sensu_stricto_1* and *Enterococcus.* Increased the proportion of *Lactobacillus* and *Firmicutes.*	–
He et al., 2021	In vivo (Human and animal model) Ex vivo In vitro	Human model: NEC: $n = 81$ Controls: $n = 81$ H4 cell and animal model C57BL/6 mice	Levels of butyric acid were lower in NEC samples compared to the control group. However, no significant differences were observed between the groups concerning acetic acid, propionic acid or isobutyric acid.	Gas chromatography-mass spectrometry (GC–MS)
Frau et al., 2021	In vivo (Human model)	Preterm babies <32weeks: $n = 51$ Stool samples: $n = 152$	Number of volatile organic compounds (VOC) limited in the first 5 days of life (SCFAs evaluated: Acetic acid, Propionic acid and Butanoic acid). Acetic acid present since birth. Gradual progression in the first week of life when exposure to enteral feeding.	Gas chromatography-mass spectrometry (GC–MS)
Gao et al., 2021	In vivo (Animal model) In vitro	H4 cell Animal model C57BL/6 mice	Butyric acid inhibited IL-1B inflammation. It increased the expression of tight junction and mucin levels	–

(*continued on next page*)

Table 1 (*continued*)

References	Study model	Sample	Results	Method used for evaluation of SCFAs
Athalye- Jape et al. 2022	In vivo (Human model)	Randomised trial Extremely preterm (Gestational age <28 weeks): $n = 173$ Single Strain probiotic: $n = 86$ Triple Strain Probiotic: $n = 87$	SCFAs were evaluated as secondary outcomes: Faecal propionic and butyric acid increased significantly after 3 weeks of probiotic supplementation.	Modified gas chromatography–mass spectrometry
Huang et al., 2022	Ex vivo In vitro	Fetal mouse jejunum cultures Human fetal intestinal epithelial FHs 74 Int cells	The addition of SCFAs (Acetic, butyric and propionic acid) decreased the inflammatory response induced by IL-1B through inhibiting ERK1/2 pathway; NF-κB p65, JNK1/2, and ERK1/2 pathways, or NF-κB p65 and ERK1/2 pathways.	
Liu et al. 2022	In vivo (Human model)	Preterm infants <34 weeks: $n = 34$ NEC: $n = 17$ Control: $n = 17$	SCFAs (acetic acid, propanoic acid, and butyric acid) were significantly lower in the NEC group both before and at the moment of diagnosis ($p < 0.05$). The area under the curve (AUC) between the control group and pre-NEC samples was 0.73, 0.70, and 0.68, respectively. The authors concluded that the increase of *Streptococcus salivarius* and *Rothia mucilaginosa,* coupled with the decrease in *Bifidobacterium_animals* subsp._*lactis* and SCFAs, might aid in the early prediction of NEC.	Gas chromatography–mass spectrometry (GC–MS)
Xiong et al., 2022	In vivo (Human model)	Neonates: $n = 43$ NEC: $n = 22$ FPIAP: $n = 21$	In NEC, there was an increased proportion of Actinobacteria and reduced Bacteroidetes compared to FPIAP. Additionally, children with NEC had significantly lower levels of SCFAs with higher levels of hexanoic acid compared with FPIAP.	Gas chromatography-mass spectrometry (GC–MS)
Casaburi et al., 2022	In silico In vivo (Animal model) In vitro	In silico: NEC: $n = 245$; healthy: $n = 1402$ In vivo: NEC onset ($n = 8$), During recovery ($n = 6$) Age matched controls ($n = 10$) In vitro: HIEC6 - human intestinal epithelial cells	Acid formate was elevated in the stool of NEC patients at disease onset ($P = 0.005$) decreasing during recovery ($P = 0.02$). There was a strong correlation with the degree of intestinal injury (r2 = 0.86). In vitro, this SCFAs caused enterocyte cytotoxicity in human cells through necroptosis ($P < 0.01$). In vivo, it caused significant dose and development dependent NEC-like injury in newborn mice.	Liquid chromatography-mass spectrometry (LC-MS)
Ferraris et al., 2023	In vivo (Animal model) In vitro	Gnotobiotic quail animal model. *C. butyricum* CB1002 and *C. neonatale* 250.09 hbd- knockout strains (Impaired butyrate production)	Significant lower production levels of butyric acid in KO than the WT strains. No statistical difference in acetic or propionic acids. Caecal injury score and NEC like lesions higher in the WT group.	Gas chromatography-mass spectrometry (GC–MS)
Neumann et al., 2023	In vivo (Human model)	Preterm infants <1500 gr: $n = 55$ 3 different prophylactic regimens evaluated (Probiotics**, Antibiotics, antifungal agents and feeding protocol).	The authors observed increasing levels of acetic acid, formic acid, valeric acid, and butyric acid over time. Interestingly, this trend was noted without a clear connection to changes in microbiome composition or probiotic supplementation. Additionally, a spike in propionic acid was reported at tp3 (days 5–8).	NMR-based metabolomics

**hbd* involved in the production of end-fermentation metabolites.
**Probiotic regimens: *Lactobacillus rhamnosus* LCR 35, *Bifidobacterium longum* subsp. *infantis* NCDO 2203 in combination with *Lactobacillus acidophilus NCDO 1748*.
*** *Sodium acetate: the sodium salt of acetic acid. Sodium butyrate: the sodium salt of butyric acid. Sodium propionate: the sodium salt of propionic acid.*
NMR: Nuclear magnetic resonance; WT: Wild type; FPIAP: Food-Induced Allergic Proctocolitis, NSS: Normal saline solution; ELBW: Extremely low birth weight; VLBW: Very low birth weight; LBW: Low birth weight.

an increase in acetic acid in samples of children who received *Lactobacilli* and *Bifidobacteria* plus fructooligosaccharides, although the difference was not significant (Underwood et al., 2009).

Other studies have investigated a broader spectrum of organic acids concerning NEC. Casaburi et al. evaluated the effects of specific metabolites, including organic acids like LA, SCFAs and BCFAs related to this condition. The authors identified *Enterobacter cloacae* and *Klebsiella pneumoniae* as the most discriminatory taxa associated with this disease. Notably, they found a statistically significant increase in formic acid during NEC onset, with a decrease in the recovery phase. In vitro and in vivo evaluations of formic acid effects resulted in an increase in enterocyte cytotoxicity and dose-dependent NEC-like injury (Casaburi et al., 2022). With advancements in new technologies, there is a growing potential to establish the role of these components, either as possible biomarkers or the specific bacteria producing them, as therapeutic strategies in various health conditions.

NEC can share similar symptoms with other disorders and recent work has begun to explore the sensitivity and specificity of NEC diagnoses. Xiong et al. (2022) evaluated the association between SCFAs in children with NEC compared to food protein-induced allergic proctocolitis. Both diseases have overlapping symptoms but very different treatments and outcomes. They showed a significant shift in microbiota and reduced levels of total SCFAs in NEC patients, but higher levels of hexanoic acid (Xiong et al., 2022). Due to the small sample sizes, heterogenieity and limitations of these studies, and considering that SCFAs were not evaluated as main outcomes, the clinical significance of these findings is yet to be established. Extensive human studies with specific probiotic strains are crucial to determining the strength of this association and clinical relevance of these metabolites. Conflicting results indicate the need for further investigation, but SCFAs hold exciting possibilities and appear to be amenable to a bedside point-of-care detection method, allowing diagnosis in a simple, quick, and cost-effective manor compared to e.g., sequencing of gut microbiota, making application in a clinical setting easier (Liu et al. 2022).

Summary and outlook

Despite the intense search for novel diagnostic and therapeutic strategies surrounding NEC, much is still unclear. Due to the advances in metagenomics and metabolomics, SCFAs are now widely recognised as indispensable for gut health. However, their exact concentrations, metabolism and impacts in a healthy preterm gut have not been completely elucidated and even less so when related to specific conditions such as NEC. Evidence supporting their use as a possible intervention strategy or as biomarkers is conflicting, although results are promising. Larger high-quality scientific studies in preterm neonates are required in order to determine the role of these metabolites in NEC. With the advancements in metabolomics and metagenomics, future research might take into consideration other variables such as non-bacterial microorganisms and optimal methods of SCFA identification. While the methods described in this review have high sensitivity and specificity, choosing between them for research purposes can pose challenges when

interpreting and comparing results.

Credit author statement

All authors contributed to planning the review and writing the review. All authors approved the final submission

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Christopher Stewart reports financial support was provided by Wellcome Trust. Christopher Stewart reports a relationship with Astarte Medical that includes: consulting or advisory. Dr Stewart declares performing consultancy for Astarte Medical and receiving lecture honoraria from Nestle Nutrition Institute. He also supervises a BBSRC collaborative training partnership PhD student for which Nestlé are involved (no salary or other personal payment is provided by Nestlé). He has no share options or other conflicts.

Data availability

No data was used for the research described in the article.

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