Review article

European Journal of Microbiology and Immunology
DOI: 10.1556/EuJMI-D-14-00030

REEVALUATING THE HYPE: FOUR BACTERIAL METABOLITES UNDER SCRUTINY

E. E. Fröhlich[*,], R. Mayerhofer[*] and P. Holzer**

Research Unit of Translational Neurogastroenterology, Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Universitätsplatz 4, 8010 Graz, Austria

Received: October 31, 2014; Accepted: November 6, 2014

With microbiome research being a fiercely contested playground in science, new data are being published at tremendous pace. The review at hand serves to critically revise four microbial metabolites widely applied in research: butyric acid, flagellin, lipoteichoic acid, and propionic acid. All four metabolites are physiologically present in healthy humans. Nevertheless, all four are likewise involved in pathologies ranging from cancer to mental retardation. Their inflammatory potential is equally friend and foe. The authors systematically analyze positive and negative attributes of the aforementioned substances, indicating chances and dangers with the use of pre- and probiotic therapeutics. Furthermore, the widespread actions of microbial metabolites on distinct organs and diseases are reconciled. Moreover, the review serves as critical discourse on scientific methods commonly employed in microbiome research and comparability as well as reproducibility issues arising thereof.

Keywords: microbiota, microbial metabolites, flagellin, lipoteichoic acid, butyric acid, propionic acid

Introduction

> "With every day, and from both sides of my intelligence, the moral and the intellectual, I thus drew steadily nearer to the truth, by whose partial discovery I have been doomed to such a dreadful shipwreck: that man is not truly one, but truly two."
>
> *Robert Louis Stevenson,* The strange case of Dr. Jekyll and Mr. Hyde

Throughout the history of mankind, tides have turned many a time on the concept of "health" [1]. While in ancient civilizations the importance of hygiene to avoid devastating diseases was already a well-known truth, this knowledge faded during medieval times yet to reemerge with full force owing to the life's work of luminaries such as Ignaz Semmelweis [2], Louis Pasteur [3], and Alexander Fleming [4]. At the turn of the millennium, yet a new chapter has been opened allowing for undreamt-of possibilities by virtue of newly developed sequencing methods granting research in never before seen depth and width [5, 6]. The understanding of the importance of the microbial kingdom for human health has reached an unprecedented climax.

The microbial residents inhabiting a human, termed microbiota, in their entirety outnumber the eukaryotic cells by several decimal powers. The human gut alone is home to ten times more cells than the human body. The colon is by far the most heavily colonized organ in the body. Under physiological conditions, the host benefits from the intestinal microbiota as it fundamentally contributes to host health. The gut microbiota is processing nutrients, producing vitamins, acting as immunomodulator, and supersedes pathogens [7, 8].

The complex microbial community of the gut comprises a large and diverse population of bacteria, including symbionts, commensals, opportunists, as well as pathogenic parasites [5, 6]. Many of the compounds produced by these microbes enter circulation and thereby influence the function of distal organs and systems. Hence, many studies support the notion that the gut microbiota in itself is a distinct endocrine organ. Considering the vast amount

* Both authors contributed equally to this work.

** Corresponding author: Esther Eleonore Fröhlich; Research Unit of Translational Neurogastroenterology, Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Universitätsplatz 4, 8010 Graz, Austria; E-mail: esther.froehlich@medunigraz.at

ISSN 2062-509X / $ 20.00 © 2014 Akadémiai Kiadó, Budapest

and varieties of metabolites stemming from the microbiota, it is not surprising that alterations in this complex community can impact human health. What is more, a large body of evidence indicates that the intestinal microbiota not only has an impact on physical health, such as colitis or inflammation in general [9, 10], but also on behavior, cognition, and psychological health [11, 12]. In animal models, probiotic strains have been shown to alleviate depression-related behavior [13, 14]. Just recently, Dinan et al. [12] introduced the term psychobiotics for probiotics that have a beneficial impact on mental health. This further strengthens the concept of the microbiota–gut–brain axis, describing the bidirectional interaction of gut microbiota and the central nervous system [15]. The microbial metabolites present in the gut are vital for the microbiota as well, serving as surface protection, enabling movement or adhesion to surfaces, energy production, exchanging signals with the environment, and many more [16].

The body of research elucidating the characteristics of microbial substances is ever increasing. The use of these compounds ranges from the induction of disease models to the study of their therapeutic and even preventative value. Evidence has shown that countless microbial metabolites influence health and well-being of the host [17–19]. Hence, the number of microbial metabolites under study is vast and exceeds the capacities of this review. In the work at hand, four distinct metabolites, namely, flagellin, lipoteichoic acid (LTA), butyric acid (BA), and propionic acid (PA) are considered. They have gained interest in distinct areas of research, the most prominent ones will be described herein, and some have already been applied in clinical trials. This review is intended to give an impression on the wide array of bacterial metabolites used in research and highlight the ample potential they harbor for the understanding of disease and more importantly for the improvement of health.

Flagellin

Flagellin is the primary structural component of protofilaments that build the flagellum, the whiplike organelle that enables the bacterium to directed motility. The flagellin protein forms monomers of about 28–65 kDa that polymerize to protofilaments [20]. Gram-negative as well as gram-positive bacteria can express the genes encoding flagellin as part of the chemotactic regulon [21, 22]. Depending on the bacterium, the genes encoding the flagellin protein have been named differently. Flagellin has four domains, namely, D0, D1, D2, and D3. D0 and D1 are largely helical and mainly responsible for polymerization. For formation of polymers, the D0 domain of one monomer interacts with the D1 domain of the other monomer. Hence, D0 and D1 are required for motility and highly conserved even among widely diverse bacterial species. D1 also contains the amino acids that have been shown to be crucial for toll-like receptor 5 (TLR5) recognition and binding [21, 23–26]. D2 and D3 on the other hand contain rather variable regions that vary even between closely related bacteria. These domains are in the center of the flagellin protein and exposed at the outer flagellum surface [24].

Extracellular flagellin is recognized by TLR5, which is a pattern-recognition receptor and able to initiate innate immune responses in a MyD88-dependent pathway. This further leads to the activation of the transcription factor nuclear factor-κB (NF-κB). TLR5 is expressed on several immune cells, like dendritic cells, macrophages, and T cells, and on many types of epithelial cells [21, 27]. Noteworthy, activation of TLR5 by flagellin in epithelial cells not only leads to pro-inflammatory gene expression but also mediates antiapoptotic effects which help to maintain the integrity of the epithelial barrier [28]. The expression patterns of TLR5 are adapted to the normal localization of bacterial colonization. In the highly colonized colon, TLR5 is expressed on the basolateral surface of epithelial cells whereas in the lower airways, where bacterial colonization is low, TLR5 is expressed at the apical surface [29, 30]. TLR5 recognizes flagellin by residues in the conserved region of the protein. These residues are largely hidden in the protofilaments but easily accessible in the flagellin monomer. Hence, monomeric flagellin was shown to elicit much higher TLR5 agonism than flagellin polymers [24], and it is likely that monomeric flagellin operates as native TLR5 agonist because flagellated bacteria deliberately release it [31]. Interestingly, *Helicobacter pylori* [32] and *Campylobacter jejuni* [33] produce a mutated form of flagellin. The mutations are located in the conserved regions affecting only TLR5 activation but not polymerization and motility [34].

Intracellular flagellin, on the other hand, activates the intracellular receptors interleukin-converting enzyme protease inhibitor (Ipaf) [35, 36] and Nod-like receptor apoptosis-inhibitory protein-5 (Naip5) [37–39]. This leads to the activation of caspase-1 via the inflammasome. For activation of Ipaf and Naip5 by flagellin, a sequence of 35 highly conserved C-terminal amino acids is required [39]. The effects of flagellin on Ipaf and Naip5 were mainly studied in macrophages. Collectively, the available evidence indicates that flagellin stimulates various immune cells via Ipaf/Naip5 and TLR5, which leads to activation of the innate and adaptive immune system. Flagellin is thus highly immunogenic [40–42].

Salmonella strains are frequently used models in the research on flagellin. Studies using *Salmonella* strains that had lost the entire flagellum machinery showed that these strains are even more virulent compared to the flagellated strains *in vivo* [43]. Hence, the presence of flagellin seems to protect against bacterial dissemination by early immune-cell recruitment. Hawn et al. [44] observed that TLR5-lacking mice show a delayed recruitment of neutrophils after *Legionella pneumophila* infection, which results in a greater inflammatory pathology of the lung tissue at a later time point. In a murine model of *Escherichia coli* urinary tract infection, Andersen-Nissen et al. [45] reported a similar effect of TLR5 knockout. In this model, TLR5-knockout mice exhibited decreased inflammation

in bladder tissue 2 days postinfection whereas, 5 days postinfection, they developed prominent inflammation in the bladder, and bacteria were detected in bladder and kidney. The beneficial effect of flagellin was also demonstrated by Vijay-Kumar et al. [46] in their study in which mice were orally infected with wildtype or aflagellated *Salmonella typhimurium*. The absence of flagellin led to a more severe clinical phenotype which was markedly reduced when animals were treated with intraperitoneal injection of flagellin 2 h before infection. The authors reported that aflagellated *Salmonella* greatly increased apoptosis in the mucosal epithelium, which may play a role in increased tissue injury and systemic invasion. In another study by the same authors, prophylactic administration of flagellin to mice was able to protect against oral dextran sodium sulfate (DSS)-induced colitis and against oral infection with rotavirus and γ irradiation [47].

Flagellin has attracted considerable interest in the field of immunology and vaccine development. Already in 1991, Newton et al. [48] constructed a flagellin carrying a protective epitope of the M protein of *Streptococcus pyogenes* type 5. *Salmonella* expressing this construct was used as live vaccine and were able to protect mice against *S. pyogenes* type 5 challenge. Cuadros et al. [49] used a flagellin-enhanced fluorescence protein fusion protein to show that flagellin is able to stimulate murine antigen presenting cells and induced cytokine secretion *in vitro*. In addition, immunization of mice with this fusion protein induced specific T-cell responses. The authors proposed flagellin fusion proteins to be potential constructs for the development of adjuvants and vaccines. Honko et al. [50] administered flagellin as adjuvant together with an antigen of *Yersinia pestis* intranasally to mice. Plasma immunoglobulin G (IgG) titers were greatly increased by flagellin treatment, and mice were protected against intranasal challenge with a virulent *Y. pestis* strain. The group also reported that the previous existence of antiflagellin antibodies had no negative impact on flagellin's adjuvant activity. This and other studies showed that the advantages of flagellin as vaccine are extensive: it is effective at low doses [51], it does not promote IgE responses [50], the antigen sequences can be inserted at various regions in the protein [52–54], and some fusion proteins were already shown to be safe and well-tolerated in human clinical trials [51, 55]. Even in aged mice, flagellin was shown to promote the adaptive immune response when used as adjuvant or vaccine although to a lower extent than in young animals [56, 57]. Asadi Karam et al. [58] demonstrated in mice that subcutaneous administration of flagellin–antigen fusion protein was more potent than flagellin admixed with the antigen to induce humoral and cellular immune responses against urinary tract infection.

Flagellin has also garnered considerable interest in the area of cancer research. Sfondrini et al. [59] showed that administration of flagellin to mice can influence tumor growth depending on the time of administration and tumor implantation, while early treatment with flagellin accelerated tumor growth, late treatment reduced tumor growth. Rhee et al. [60] used a mouse xenograft model of human colon cancer and reported that peritumoral administration of flagellin was able to increase tumor necrosis and thereby suppressed tumor growth. Lack of TLR5 or MyD88 expression on the other hand inhibited tumor necrosis and increased tumor growth. This was supported by the study of Cai et al. [61] in 2011 who reported that peritumoral administration of flagellin was able to inhibit tumor growth and increase tumor necrosis and neutrophil infiltration in a mouse xenograft model of human breast cancer. In contrast, Song et al. [62] observed that growth of gastric cancer cells was enhanced by flagellin treatment *in vitro*.

As already mentioned, TLR5 is polarized to the basolateral surface of the colon, so it should only be reached by flagellated bacteria which breach the epithelium or which are able to translocate their flagellin across the epithelium (e.g., *S. typhimurium* [63]). Hence, a disruption of the mucosal epithelial barrier, which can be seen in inflammatory bowel diseases (IBD), may lead to enhanced TLR5 activation [64]. Crohn's disease is an IBD characterized by an aberrant and uncontrolled immune reaction against the normal gut microbiota [65]. Lodes et al. [66] reported that, unlike colitis patients, Crohn's disease patients show a high antibody response to flagellin of commensal bacteria. Wallis et al. [67] also observed increased antiflagellin antibodies in the serum of patients with ankylosing spondylitis, a pathology frequently associated with IBD. Rhee et al. [68] found that intracolonic administration of flagellin to either DSS-treated mice or mice with injured colonic mucosa initiated and aggravated inflammatory responses. The hypothesis of a decisive role of TLR5 in this pathology is corroborated by data from humans with dominant negative TLR5 polymorphism (TLR5-stop). Healthy TLR5-stop carriers were shown to have lower flagellin specific antibodies, and a negative association of TLR5-stop with Crohn's disease, but not ulcerative colitis (UC), was observed [69]. Hence, reduced TLR5 function may offer some protection against Crohn's disease development.

Flagellin has also gained growing interest in the area of allergy research due to its ability to promote Th2 but not IgE responses [50]. Schülke et al. [70, 71] reported that intraperitoneal administration of flagellin–ovalbumin fusion protein prevented intestinal allergy in mice. On the other hand, Wilson et al. [72] instilled flagellin together with ovalbumin into the airways of mice in which it induced strong allergic airway responses to ovalbumin. They also found high titers of antiflagellin antibody in sera of asthmatics.

For research purposes, flagellin has been administered by various routes (e.g., oral, intranasal, and intraperitoneal) and in various forms such as purified flagellin, fusion-proteins, or whole flagellin-expressing bacteria. Most research on flagellin has been conducted in mice, but in recent years, flagellin has also been tested in clinical trials [51, 55, 73, 74]. Furthermore, new combinations and fusion proteins with flagellin are being tested in animals, especially in poultry vaccination [75]. The search for new vaccines and tolerable adjuvants is ongoing, and flagellin

seems to have great potential for the use in this area. Also the role of flagellin in cancer and allergies is still unclear, and further research is needed to elucidate the elusive properties of this protein.

Lipoteichoic acid

LTA is a major cell wall constituent of gram-positive bacteria, a so called surface-associated adhesion amphiphile [76]. The basic structure is composed of a soluble polymer attached to the cell membrane via a diacylglycerol. The polymer consists of polyhydroxy alkane units, such as ribitol and glycerol. The sequence of glycerol and ribitol varies between species [77]. LTA is mainly released after bacteriolysis, an important matter of concern with the use of β-lactam antibiotics. It specifically binds to cluster of differentiation 14 (CD14) or Toll-like receptor 2 (TLR2) and, thus, triggers the release of pro-inflammatory mediators and cytokines such as tumor necrosis factor α, interferon γ, and interleukins 1, 5, 6, and 8 [76, 78]. However, it can also nonspecifically bind to membrane phospholipids. Albeit having less pro-inflammatory potential than lipopolysaccharide, LTA has been reported to induce arthritis, nephritis, toxic shock syndrome, uveitis, encephalomyelitis, meningeal inflammation, and periodontal lesions in animal studies. Moreover, it can trigger cascade reactions resulting in septic shock and multi-organ failure. Strikingly, 50% of cases of sepsis and septic shock are caused by gram-positive bacteria [76], with *Staphylococcus aureus* being one of the most prevailing organisms causing nosocomial infections. More importantly, the emergence of multidrug resistant clones of methicillin-resistant *S. aureus* (MRSA) is an ongoing issue far from being under control [79]. LTA is furthermore prone to act synergistically with other microbial compounds such as endotoxin and peptidoglycan, thereby either enhancing or diminishing the inflammatory response [80].

In the past, issues have been raised concerning the purity of LTA preparations used in research [81]. Commercial preparations were found to contain trace amounts of endotoxin, yielding them inappropriate for experiments [82]. While commercial suppliers indicate purity of their LTA products, researchers are advised to verify their results via LPS blocking agents [83]. In 2001, Morath et al. [82] called attention to the issue of inactivation of LTA over the course of the purification process. The group demonstrated an improved purification process, yielding pure (>99%) biologically active LTA. Seo et al. [84] furthermore demonstrated how improved purification methods preserve LTA activity. Using this preparation, the group was able to induce a distinct immune response via LTA, an effect not evoked by LTA preparations inactivated via three different methods.

Recent reports suggest an ability of *S. aureus* to adhere to and invade the blood brain barrier (BBB), leading to meningeal inflammation and brain abscess formation including significant levels of brain bacterial counts [85]. Considering the high prevalence of infections caused by gram-positives and the capacity of such species to trespass the BBB, an analysis of possible consequences on host brain and mental health is desirable [79, 85]. Calling to mind the widespread use of gram-positive bacteria in probiotic formulations, the reevaluation of LTA regarding brain health and behavior should gain even more precedence.

Butyric acid

BA is a short chain fatty acid (SCFA) with four carbon atoms. BA and other SCFAs are produced by the resident gastrointestinal microbiota via fermentation of dietary fibers [86]. Resistant starch [87], inulin [88], and oligofructose [89] have been shown to increase the fecal content of BA-producing bacteria. Therefore, they have been suggested to be very efficient butyrogenic substrates. Besides colonic production, BA is also frequently used as a food additive [90]. As BA is the primary energy source for colonocytes, the majority is metabolized in the gut and only small amounts are processed in the liver. Thus, concentrations in the general circulation are comparatively low [91]. The majority of BA-producing bacteria are anaerobic gram-positives. Fecal sample analyses indicate members of clostridiales clusters IV and XIVa as the most important and abundant anaerobic BA-producing bacteria [92]. Transport of BA is cell type-specific; nonionic diffusion and monocarboxylated transporters (MCT) are two of the so far identified transport mechanisms of enterocytes [86]. The primary receptors for SCFAs are the G protein-coupled receptors (GPR) 41 and 43, though BA exhibits stronger affinity to GPR41 [93]. GPRs are cell surface receptors that sense extracellular molecules and activate signal transduction pathways in the cell upon binding. Both GPR41 and GPR43 are not only expressed in enterocytes but also in adipocytes. GPR41 is furthermore expressed in the sympathetic nervous system [94] whereas GPR43 is highly abundant in immune cells [93].

While BA has been in the spotlight of various areas of research by virtue of its touted beneficial effects, one should be aware of the controversies of data in this regard. Under healthy conditions BA stimulates growth and differentiation of epithelial cells and colonocytes. In addition, BA decreases apoptosis of normal colonocytes [95]. Contrary to healthy conditions, BA induces apoptosis in human colonic carcinoma cells. Due to these controversial actions, the term “butyrate paradox” was coined [96]. Evidence suggests that the concentration-dependent effect of BA on carcinoma cells is mainly exerted by the histone deacetylase inhibiting properties of BA. Histone deacetylases are important epigenetic regulators of gene transcription and the target for many chemostatic drugs [97]. BA sparked the interest of cancer research already in the 1980s when sodium butyrate was used for *in vitro* studies in human cancer cells [98]. In various cancer cell lines, obtained from colon [99], breast [100], bladder [101], and

prostate [102] cancer, sodium butyrate and other derivatives of BA exerted anticancerogenic effects. The studies indicate that the effects of BA on cancer cell lines are due to induction of apoptosis, tumor cell differentiation or simple prevention of cell proliferation. Weir et al. [103] reported recently that BA-producing bacteria are reduced in the stool of colorectal cancer patients. Noteworthy, not all studies observed a beneficial role of BA in colorectal cancer. Therefore, it was suggested that the time point of administration or the state of the cancerous cells plays an important role in the anticancerogenic actions of BA [104]. Nevertheless, BA has reached prominent interest in cancer research, and studies on this topic are ongoing.

Oxidative stress is a further factor contributing to carcinogenesis and inflammation. Hamer et al. [105] showed that daily sodium butyrate enemas in healthy patients increased the amount of the antioxidant glutathione, which points towards an antioxidative effect of BA.

BA has also been proposed to improve intestinal barrier function by upregulating mucin expression [106] and proliferation of epithelial cells. An impaired integrity of the gut barrier plays an important role in numerous infectious and gut diseases [107]. In UC patients, butyrate concentrations are lower than in healthy controls [108]. It has even been implied that butyrate metabolism is impaired in these patients [109]. Butyrate enemas have been reported to exert beneficial effects in UC and experimental colitis [110, 111]. Recently, Vieira et al. [112] showed that oral administration of sodium butyrate in a mouse colitis model is able to ameliorate mucosal damage and decrease inflammation. Hence, oral butyrate supplementation could be beneficial in UC patients. An additional anti-inflammatory property of BA is the inhibition of transcription factor NF-κB activation. Due to this action BA reduces the expression of pro-inflammatory genes [113]. It has been shown that NF-κB is frequently activated in epithelial cells of the inflamed intestinal mucosa and in macrophages, suggesting a role in IBD, such as Crohn's disease and UC [114]. In line with this contention, luminal administration of BA has been proposed to ameliorate symptoms in UC patients and reduce inflammation [115].

Besides these local effects, BA has in addition been shown to exert beneficial systemic actions. In the 1990s, clinical trials with orally administered sodium phenylbutyrate were performed in sickle cell disease and β-thalassemia patients. The reports showed that sodium phenylbutyrate was able to increase fetal hemoglobin production [116, 117]. Although novel butyrate derivatives are currently investigated in clinical trials, an optimal treatment regimen has not yet been identified [118, 119]. Furthermore, sodium phenylbutyrate has been tested in patients with urea cycle disorders where it acted as an ammonia scavenger [120].

The field of neurology has shown growing interest in the use of BA as well. In 2007, Schroeder et al. [121] demonstrated that daily intraperitoneal injections of 1.2 g/kg sodium butyrate to mice exert antidepressant-like effects in the tail suspension test. The same dose of BA was able to improve neurological parameters in a mouse model of Huntington's disease [122]. In rats subjected to cerebral ischemia, Kim et al. [123] observed that subcutaneously injected sodium butyrate enhanced incorporation of bromo-2′-deoxyuridine in the brain. Hence, sodium butyrate was suggested to stimulate neurogenesis after ischemic injury. Interestingly, it has been reported that fecal SCFA levels including BA are elevated in children with autism spectrum disorder [124]. Takuma et al. [125] showed that autism-like behavioral abnormalities induced prenatally in mice could be ameliorated by chronic intraperitoneal treatment with sodium butyrate. It should not go unnoticed, though, that BA has also been reported to enhance colonic pain sensitivity in rats [126].

The data concerning obesity and BA are still controversial [127]. In 2009, Gao et al. [128] reported that, in a mouse model of metabolic syndrome, oral supplementation of a high-fat diet with butyrate was able to attenuate the development of obesity and insulin resistance. Further studies demonstrated that, even in humans, diets that elevated fecal butyrate concentrations proved beneficial [129, 130]. Recently, Fernandes et al. [131] reported that fecal SCFA concentrations, including BA and PA, were significantly higher in overweight or obese patients than in lean subjects. This supports the hypothesis that colonic fermentation is altered in overweight or obese adults. However, the role of BA in obesity is still not fully elucidated [131] although an increasing number of studies points towards a beneficial effect of BA in obesity-related factors [132–135]. Interestingly enough, BA-producing bacteria were found to be decreased in fecal samples of type-2 diabetes patients [136].

For research purposes, especially in the field of neurology, BA is often administered via a parenteral route to exert stronger effects at smaller doses [121–123, 125]. However, in experimental models of colitis, the oral [112] and rectal [111, 113] route of application proved to be most successful. Therefore, supplementation of the diet with BA as well as prebiotic supplementation to promote BA production could be of interest to other research fields as well. Currently, in the majority of therapeutic attempts, BA is administered orally [137] or as an enema [115, 138]. Although the handling of BA is easy and there is an abundance of BA salts available, the taste and odor of BA if given in acidic form are unpleasant, complicating its use particularly in children. Nevertheless, the body of evidence concerning the beneficial effects of BA is continuously growing and, according to the data available, BA has great potential for future therapeutic applications.

Propionic acid

BA's "little brother" is the SCFA PA, bearing only three carbon atoms. While a lot of research focused on the role of BA, little has been done to let PA emerge from the shadow of his elder and reveal its potential. PA is a common constituent of the modern human diet owing to its ac-

tions as a food preservative due to its antifungal [139] and antimicrobial [140] effects. However, the majority of PA present in humans, like BA, is produced by the colonic microbiota through fermentation of dietary fibers [141]. Currently, not all PA-producing bacteria have been identified, though the ability of PA production seems not to be limited to one phylum. PA producers are for instance *Bacteroides fragilis* [142] and *Propionibacterium* [143]. The transport of PA by enterocytes is similar to that of BA and involves, for instance, MCT transporters [86]. Amidst SCFAs, PA is the prime ligand for GPR41 and GPR43 [93]. As already stated, GPR41 is expressed by enterocytes, adipocytes, and the sympathetic nervous system [94]. GPR43 is expressed in immune cells, adipocytes, and the intestine [93]. These expression patterns concord with the main field of action of PA. While the majority of BA is metabolized in the intestine, PA proves less favorable as an energy source for colonocytes. Consequently, much higher concentrations of PA are being taken up and metabolized by the liver [91]. Contrary to humans, PA is used as a major gluconeogenic substrate in ruminants [144]. Such a diversity of action modes scattered across species complicates the interpretation of findings, although it also sheds light on contradictory results obtained in various PA studies. As a substantial body of research conducted on PA converges on its effects on satiety, energy intake, and other obesity-related factors, the information presented herein focuses on studies in non-ruminants.

Animal experiments indicate that PA is playing a role in decreasing cholesterol and fatty acid levels in the liver [145]. Lin et al. [146] compared the PA concentrations required to lower cholesterol in rat and human hepatocytes. The susceptibility of human hepatocytes to PA was markedly smaller than that of rat hepatocytes, and the PA concentrations needed to inhibit cholesterol synthesis were around 10–20 mmol/l. However, it requires consideration that the physiological concentration of PA in the portal vein of about 0.1 mmol/l is substantially lower than the concentration required to attenuate cholesterol levels [141]. In 2004, Xiong et al. [147] reported increased plasma leptin levels in mice following acute oral administration of sodium propionate. Enhanced expression of this anorexigenic hormone in human adipose tissue was also reported by Al-Lahham et al. [148] in 2010. Both studies suggest an involvement of GPR41 in this process. Furthermore, Ge et al. [149] demonstrated reduced lipolysis in murine adipocytes treated with PA. In addition, GPRs are expressed by enteroendocrine cells which are capable of transducing information via gut hormones such as PYY or GLP-1. In line with this hypothesis, Psichas et al. [150] recently demonstrated increased PYY and GLP-1 concentrations in portal vein plasma of mice and rats subsequently to intracolonic administration of PA. In GPR43-deficient mice, PA treatment was unable to enhance the release of PYY and GLP-1.

Todesco et al. [151] reported in 1991 that the daily addition of 9.9 g sodium propionate to bread for 1 week lowered the blood glucose response. This was in line with Liljeberg et al. [152], who observed a beneficial effect of sodium propionate on satiety in humans. Supplementing whole-meal bread with sodium propionate prolonged satiety and lowered postprandial blood glucose as well as plasma insulin levels.

The data at hand suggest satietogenic actions exerted by PA and join the chorus of praise for food high in dietary fiber [153–155]. However, the unphysiologically high concentrations of sodium propionate applied in these studies advise caution. Bodinham et al. [156] recently studied the effects of increased resistant starch consumption in type-2 diabetes patients. While insulin sensitivity did not improve, a beneficial effect on meal handling behavior was observed. Furthermore, PA and BA were lowered in fasting serum. Bodinham and colleagues also demonstrated beneficial effects of resistant starch on peripheral insulin resistance in patients with metabolic syndrome.

A link between inflammation and obesity has been confirmed by many studies [157]. Hence, the impact of PA on inflammation could be linked to particular effects on obesity. Al-Lahham et al. [148] observed that incubation of human adipose tissue with PA reduces the expression of resistin which is a pro-inflammatory factor, the action of which is probably mediated by GPR43 on macrophages. Moreover, PA was reported to inhibit activated human lymphocyte proliferation in a concentration-dependent manner [158]. However, concentrations higher than 2.5 mmol/l were required to induce this effect, clearly exceeding the physiological portal vein concentration of 0.1 mmol/l PA [141].

To date, it remains elusive whether PA exerts beneficial effects on IBD. As far as we can tell, no study has been performed using enemas with PA as the sole active ingredient, PA being tested only in SCFA mixtures. The treatment of UC with SCFA enemas appeared promising in some studies [159, 160], albeit others saw only small trends towards an improvement [115, 161]. Since the available studies used varying concentrations, volumes, and treatment regimes, the data at hand are inconclusive, let alone a number of intersubject differences. Anyhow, Tana et al. [162] observed high fecal PA and acetic acid concentrations in irritable bowel syndrome patients; these parameters correlate with the severity of symptoms and negative emotions.

PA has also been reported to have beneficial effects on colon cancer cell growth [163] and to induce apoptosis in colorectal cancer cells, albeit to a lesser degree than BA [164]. In 2002, Jan et al. [165] and Hinnebusch et al. [166] observed in independent studies antiproliferative effects of PA in colon cancer cells. Jan et al. [165] found that supernatants of three strains of the *Propionibacterium* genus caused colorectal cancer cells to die. The cytotoxic effect was attributed mainly to PA and acetic acid which elicited apoptosis in the colorectal cancer cell line. Hinnebusch et al. [166] treated colon carcinoma cells with SCFAs including BA and PA. While BA and PA both induced significant histone hyperacetylation, the effect was more pronounced with BA. The same pattern was observed regarding the inhibition of growth rate of cancer cells, that

is, BA was more effective than PA. However, induction of cell differentiation was more pronounced with PA treatment. Yet in contrast to Jan et al. [165], Hinnebusch and colleagues [166] observed an induction of apoptosis only in cells treated with BA.

In the context of PA, attention has to be drawn to propionic acidemia, which is a metabolic disease characterized, among others, by very high PA concentrations in the body. The disease is caused by malfunction of propionyl-CoA carboxylase and involves symptoms such as vomiting and mental retardation [167, 168]. Those symptoms can also be seen in other organic acidemias [169]. Though liver transplantation is able to alleviate many of these symptoms, it has little effect on PA concentrations [170].

Adverse effects of PA have also been observed with respect to behavior and mental health. In 2007, MacFabe et al. [171] demonstrated induction of some symptoms of autism spectrum disorder following intraventricular infusion of PA in rats. Furthermore, they found increased oxidative stress markers in the brain and reported the presence of neuroinflammation indicators. Similar experimental setups support these findings and provide more evidence for abnormalities in brain and behavior [172–174]. These data fit well to the observation that the fecal SCFA concentrations are elevated in children with autism spectrum disorder [124]. In an experimental model of chemically induced propionic acidemia, young rats were treated subcutaneously with buffered propionate for three weeks. This treatment caused a delay in some attributes of physical development. In addition, a higher number of rearings in a repeated open field test and an aggravated avoidance response in the shuttle-avoidance task were observed 3 weeks after discontinuation of treatment [175]. Hence, the authors hypothesize that chronic PA treatment causes long-term behavioral deficits in aversive memory and habituation to novelty.

Remarkably enough, PA has also been demonstrated to have pro-inflammatory effects. In gingival inflammation, periodontal bacteria release high concentrations of SCFAs and induce inflammation of gum tissue. There is evidence that PA is the major SCFA produced in this process in humans [176]. Direct application of a SCFA mixture including PA, acetic, lactic, and formic acid to healthy patients elevated subgingival temperature and neutrophil emigration. The same effect was observed following supplementation of food with high amounts of the aforementioned mixture [177].

Most of the PA effects proposed are based on animal experiments only. Moreover, many studies performed in humans used a SCFA mix [161] or prebiotics such as resistant starch [156]. In consequence, it is not possible to separate the effects of the SCFAs and to analyze any synergistic or antagonistic actions. Considering the current state of knowledge, further studies in humans and with human-derived cells are necessary to elucidate the effects of PA in health and disease.

Conclusion

The amount of data generated by contemporary microbiome research is vast and ever evolving. To date, the microbiome's pivotal impact on host health is unequivocal. Even more so, the complexity of the microbiota–host interactions exceeded the expectations of most. Certainly, the founding fathers of today's pro- and prebiotic therapeutic approaches would be proud. Alfred Nissle, who developed the *E. coli* Nissle 1917 probiotic Mutaflor® in 1917 [178], or the Austrian researcher Friedrich Petuely, who in 1960 filed a patent for the discovery of lactulose as "bifido factor," a prebiotic specifically enhancing growth of Bifidobacteria strains [179], would be very content.

Vital questions, however, remain. Even though modern sequencing methods allow for the generation of massive amounts of data, the definition of a healthy or physiological microbiome is still pending. While the enterotype hypothesis seemed promising, evidence suggests that the picture is not as clear-cut as initially hoped for. Recent research indicates that function rather than phylogenetic aspects is the key factor for a healthy microbiota. Furthermore, the interleaved crosstalk between host and microbiota via microbial metabolites is still little understood. The review at hand serves to demonstrate how a single metabolite can act as the good, the bad, and the ugly, all depending on the circumstances and location of its action.

As long as the scientific community stays attentive, is not blinded by the light of possibilities, and stays focused on the rationality of the approaches pursued, research in this area offers great opportunities to further the understanding of health and disease. Moreover, it proposes never before seen chances for the prevention and treatment of diseases within and beyond the gastrointestinal tract.

Acknowledgements

The authors would like to thank Eleonore Fröhlich and Keith D. Voss for their careful reading of the paper and their valuable comments.

References

1. Pappas G, Kiriaze IJ, Falagas ME: Insights into infectious disease in the era of Hippocrates. Int J Infect Dis 12, 347–350 (2008)
2. Hach W: Puerperal sepsis in the 19th century and Trendlenburg's ligature of the internal iliacal vein. Hamostaseologie 27, 111–116 (2007)
3. Pasteur L, Chamberland, Roux: Summary report of the experiments conducted at Pouilly-le-Fort, near Melun, on the anthrax vaccination, 1881. Yale J Biol Med 75, 59–62 (2002)
4. Wennergren G, Lagercrantz H: "One sometimes finds what one is not looking for" (Sir Alexander Fleming): the most important medical discovery of the 20th century. Acta Paediatr 96, 141–144 (2007)

5. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Dore J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, MetaHIT Consortium, Bork P, Ehrlich SD, Wang J: A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464, 59–65 (2010)
6. Schloissnig S, Arumugam M, Sunagawa S, Mitreva M, Tap J, Zhu A, Waller A, Mende DR, Kultima JR, Martin J, Kota K, Sunyaev SR, Weinstock GM, Bork P: Genomic variation landscape of the human gut microbiome. Nature 493, 45–50 (2013)
7. Guarner F, Malagelada JR: Gut flora in health and disease. Lancet 361, 512–519 (2003)
8. Quigley EM: Gut bacteria in health and disease. Gastroenterol Hepatol (N Y) 9, 560–569 (2013)
9. Huang XZ, Zhu LB, Li ZR, Lin J: Bacterial colonization and intestinal mucosal barrier development. World J Clin Pediatr 2, 46–53 (2013)
10. Ferreira CM, Vieira AT, Vinolo MA, Oliveira FA, Curi R, Martins FD: The central role of the gut microbiota in chronic inflammatory diseases. J Immunol Res 2014, 689492 (2014)
11. Diaz Heijtz R, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, Hibberd ML, Forssberg H, Pettersson S: Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci U S A 108, 3047–3052 (2011)
12. Dinan TG, Stanton C, Cryan JF: Psychobiotics: a novel class of psychotropic. Biol Psychiatry 74, 720–726 (2013)
13. Desbonnet L, Garrett L, Clarke G, Bienenstock J, Dinan TG: The probiotic Bifidobacteria infantis: An assessment of potential antidepressant properties in the rat. J Psychiatr Res 43, 164–174 (2008)
14. Desbonnet L, Garrett L, Clarke G, Kiely B, Cryan JF, Dinan TG: Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. Neuroscience 170, 1179–1188 (2010)
15. Bienenstock J, Collins S: 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: psycho-neuroimmunology and the intestinal microbiota: clinical observations and basic mechanisms. Clin Exp Immunol 160, 85–91 (2010)
16. Sanz Y, Nadal I, Sanchez E: Probiotics as drugs against human gastrointestinal infections. Recent Pat Antiinfect Drug Discov 2, 148–156 (2007)
17. Nyangale EP, Mottram DS, Gibson GR: Gut microbial activity, implications for health and disease: the potential role of metabolite analysis. J Proteome Res 11, 5573–5585 (2012)
18. Russell WR, Hoyles L, Flint HJ, Dumas ME: Colonic bacterial metabolites and human health. Curr Opin Microbiol 16, 246–254 (2013)
19. Joyce SA, Gahan CG: The gut microbiota and the metabolic health of the host. Curr Opin Gastroenterol 30, 120–127 (2014)
20. Song WS, Yoon SI: Crystal structure of FliC flagellin from *Pseudomonas aeruginosa* and its implication in TLR5 binding and formation of the flagellar filament. Biochem Biophys Res Commun 444, 109–115 (2014)
21. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM, Aderem A: The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 410, 1099–1103 (2001)
22. Aldridge P, Hughes KT: Regulation of flagellar assembly. Curr Opin Microbiol 5, 160–165 (2002)
23. Jacchieri SG, Torquato R, Brentani RR: Structural study of binding of flagellin by Toll-like receptor 5. J Bacteriol 185, 4243–4247 (2003)
24. Smith KD, Andersen-Nissen E, Hayashi F, Strobe K, Bergman MA, Barrett SL, Cookson BT, Aderem A: Toll-like receptor 5 recognizes a conserved site on flagellin required for protofilament formation and bacterial motility. Nat Immunol 4, 1247–1253 (2003)
25. Murthy KG, Deb A, Goonesekera S, Szabo C, Salzman AL: Identification of conserved domains in *Salmonella muenchen* flagellin that are essential for its ability to activate TLR5 and to induce an inflammatory response *in vitro*. J Biol Chem 279, 5667–5675 (2004)
26. Zhang L, Pan Z, Kang X, Yang Y, Kang H, Zhang N, Rosati JM, Jiao X: Amino acids 89–96 of *Salmonella typhimurium* flagellin represent the major domain responsible for TLR5-independent adjuvanticity in the humoral immune response. Cell Mol Immunol (2014)
27. Means TK, Hayashi F, Smith KD, Aderem A, Luster AD: The Toll-like receptor 5 stimulus bacterial flagellin induces maturation and chemokine production in human dendritic cells. J Immunol 170, 5165–5175 (2003)
28. Zeng H, Wu H, Sloane V, Jones R, Yu Y, Lin P, Gewirtz AT, Neish AS: Flagellin/TLR5 responses in epithelia reveal intertwined activation of inflammatory and apoptotic pathways. Am J Physiol Gastrointest Liver Physiol 290, G96–G108 (2006)
29. Zhang Z, Louboutin JP, Weiner DJ, Goldberg JB, Wilson JM: Human airway epithelial cells sense *Pseudomonas aeruginosa* infection via recognition of flagellin by Toll-like receptor 5. Infect Immun 73, 7151–7160 (2005)
30. Ortega-Cava CF, Ishihara S, Rumi MA, Aziz MM, Kazumori H, Yuki T, Mishima Y, Moriyama I, Kadota C, Oshima N, Amano Y, Kadowaki Y, Ishimura N, Kinoshita Y: Epithelial toll-like receptor 5 is constitutively localized in the mouse cecum and exhibits distinctive down-regulation during experimental colitis. Clin Vaccine Immunol 13, 132–138 (2006)
31. Gewirtz AT, Simon PO, Jr, Schmitt CK, Taylor LJ, Hagedorn CH, O'Brien AD, Neish AS, Madara JL: *Salmonella typhimurium* translocates flagellin across intestinal epithelia, inducing a proinflammatory response. J Clin Invest 107, 99–109 (2001)
32. Gewirtz AT, Yu Y, Krishna US, Israel DA, Lyons SL, Peek RM, Jr: *Helicobacter pylori* flagellin evades toll-like receptor 5-mediated innate immunity. J Infect Dis 189, 1914–1920 (2004)
33. Johanesen PA, Dwinell MB: Flagellin-independent regulation of chemokine host defense in *Campylobacter jejuni*-infected intestinal epithelium. Infect Immun 74, 3437–3447 (2006)
34. Andersen-Nissen E, Smith KD, Strobe KL, Barrett SL, Cookson BT, Logan SM, Aderem A: Evasion of Toll-like receptor 5 by flagellated bacteria. Proc Natl Acad Sci U S A 102, 9247–9252 (2005)

35. Franchi L, Amer A, Body-Malapel M, Kanneganti TD, Ozoren N, Jagirdar R, Inohara N, Vandenabeele P, Bertin J, Coyle A, Grant EP, Nunez G: Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in salmonella-infected macrophages. Nat Immunol 7, 576–582 (2006)
36. Miao EA, Alpuche-Aranda CM, Dors M, Clark AE, Bader MW, Miller SI, Aderem A: Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1beta via Ipaf. Nat Immunol 7, 569–575 (2006)
37. Wright EK, Goodart SA, Growney JD, Hadinoto V, Endrizzi MG, Long EM, Sadigh K, Abney AL, Bernstein-Hanley I, Dietrich WF: Naip5 affects host susceptibility to the intracellular pathogen *Legionella pneumophila*. Curr Biol 13, 27–36 (2003)
38. Molofsky AB, Byrne BG, Whitfield NN, Madigan CA, Fuse ET, Tateda K, Swanson MS: Cytosolic recognition of flagellin by mouse macrophages restricts *Legionella pneumophila* infection. J Exp Med 203, 1093–1104 (2006)
39. Lightfield KL, Persson J, Brubaker SW, Witte CE, von Moltke J, Dunipace EA, Henry T, Sun YH, Cado D, Dietrich WF, Monack DM, Tsolis RM, Vance RE: Critical function for Naip5 in inflammasome activation by a conserved carboxy-terminal domain of flagellin. Nat Immunol 9, 1171–1178 (2008)
40. Honko AN, Mizel SB: Effects of flagellin on innate and adaptive immunity. Immunol Res 33, 83–101 (2005)
41. Crellin NK, Garcia RV, Hadisfar O, Allan SE, Steiner TS, Levings MK: Human CD4+ T cells express TLR5 and its ligand flagellin enhances the suppressive capacity and expression of FOXP3 in CD4+CD25+ T regulatory cells. J Immunol 175, 8051–8059 (2005)
42. Hruz P, Eckmann L: Caspase recruitment domain-containing sensors and adaptors in intestinal innate immunity. Curr Opin Gastroenterol 24, 108–114 (2008)
43. Schmitt CK, Ikeda JS, Darnell SC, Watson PR, Bispham J, Wallis TS, Weinstein DL, Metcalf ES, O'Brien AD: Absence of all components of the flagellar export and synthesis machinery differentially alters virulence of *Salmonella enterica* serovar Typhimurium in models of typhoid fever, survival in macrophages, tissue culture invasiveness, and calf enterocolitis. Infect Immun 69, 5619–5625 (2001)
44. Hawn TR, Berrington WR, Smith IA, Uematsu S, Akira S, Aderem A, Smith KD, Skerrett SJ: Altered inflammatory responses in TLR5-deficient mice infected with *Legionella pneumophila*. J Immunol 179, 6981–6987 (2007)
45. Andersen-Nissen E, Hawn TR, Smith KD, Nachman A, Lampano AE, Uematsu S, Akira S, Aderem A: Cutting edge: Tlr5−/− mice are more susceptible to *Escherichia coli* urinary tract infection. J Immunol 178, 4717–4720 (2007)
46. Vijay-Kumar M, Wu H, Jones R, Grant G, Babbin B, King TP, Kelly D, Gewirtz AT, Neish AS: Flagellin suppresses epithelial apoptosis and limits disease during enteric infection. Am J Pathol 169, 1686–1700 (2006)
47. Vijay-Kumar M, Aitken JD, Sanders CJ, Frias A, Sloane VM, Xu J, Neish AS, Rojas M, Gewirtz AT: Flagellin treatment protects against chemicals, bacteria, viruses, and radiation. J Immunol 180, 8280–8285 (2008)
48. Newton SM, Kotb M, Poirier TP, Stocker BA, Beachey EH: Expression and immunogenicity of a streptococcal M protein epitope inserted in Salmonella flagellin. Infect Immun 59, 2158–2165 (1991)
49. Cuadros C, Lopez-Hernandez FJ, Dominguez AL, McClelland M, Lustgarten J: Flagellin fusion proteins as adjuvants or vaccines induce specific immune responses. Infect Immun 72, 2810–2816 (2004)
50. Honko AN, Sriranganathan N, Lees CJ, Mizel SB: Flagellin is an effective adjuvant for immunization against lethal respiratory challenge with *Yersinia pestis*. Infect Immun 74, 1113–1120 (2006)
51. Turley CB, Rupp RE, Johnson C, Taylor DN, Wolfson J, Tussey L, Kavita U, Stanberry L, Shaw A: Safety and immunogenicity of a recombinant M2e-flagellin influenza vaccine (STF2.4xM2e) in healthy adults. Vaccine 29, 5145–5152 (2011)
52. Song L, Zhang Y, Yun NE, Poussard AL, Smith JN, Smith JK, Borisevich V, Linde JJ, Zacks MA, Li H, Kavita U, Reiserova L, Liu X, Dumuren K, Balasubramanian B, Weaver B, Parent J, Umlauf S, Liu G, Huleatt J, Tussey L, Paessler S: Superior efficacy of a recombinant flagellin:H5N1 HA globular head vaccine is determined by the placement of the globular head within flagellin. Vaccine 27, 5875–5884 (2009)
53. Camacho AG, Teixeira LH, Bargieri DY, Boscardin SB, Soares IS, Nussenzweig RS, Nussenzweig V, Rodrigues MM: TLR5-dependent immunogenicity of a recombinant fusion protein containing an immunodominant epitope of malarial circumsporozoite protein and the FliC flagellin of *Salmonella typhimurium*. Mem Inst Oswaldo Cruz 106 Suppl 1, 167–171 (2011)
54. Tanomand A, Farajnia S, Najar Peerayeh S, Majidi J: Cloning, expression and characterization of recombinant exotoxin A-flagellin fusion protein as a new vaccine candidate against *Pseudomonas aeruginosa* infections. Iran Biomed J 17, 1–7 (2013)
55. Treanor JJ, Taylor DN, Tussey L, Hay C, Nolan C, Fitzgerald T, Liu G, Kavita U, Song L, Dark I, Shaw A: Safety and immunogenicity of a recombinant hemagglutinin influenza-flagellin fusion vaccine (VAX125) in healthy young adults. Vaccine 28, 8268–8274 (2010)
56. Ben-Yedidia T, Abel L, Arnon R, Globerson A: Efficacy of anti-influenza peptide vaccine in aged mice. Mech Ageing Dev 104, 11–23 (1998)
57. Bates JT, Honko AN, Graff AH, Kock ND, Mizel SB: Mucosal adjuvant activity of flagellin in aged mice. Mech Ageing Dev 129, 271–281 (2008)
58. Asadi Karam MR, Oloomi M, Mahdavi M, Habibi M, Bouzari S: Vaccination with recombinant FimH fused with flagellin enhances cellular and humoral immunity against urinary tract infection in mice. Vaccine 31, 1210–1216 (2013)
59. Sfondrini L, Rossini A, Besusso D, Merlo A, Tagliabue E, Menard S, Balsari A: Antitumor activity of the TLR-5 ligand flagellin in mouse models of cancer. J Immunol 176, 6624–6630 (2006)
60. Rhee SH, Im E, Pothoulakis C: Toll-like receptor 5 engagement modulates tumor development and growth in a mouse xenograft model of human colon cancer. Gastroenterology 135, 518–528 (2008)
61. Cai Z, Sanchez A, Shi Z, Zhang T, Liu M, Zhang D: Activation of Toll-like receptor 5 on breast cancer cells by flagellin suppresses cell proliferation and tumor growth. Cancer Res 71, 2466–2475 (2011)
62. Song EJ, Kang MJ, Kim YS, Kim SM, Lee SE, Kim CH, Kim DJ, Park JH: Flagellin promotes the proliferation of

gastric cancer cells via the Toll-like receptor 5. Int J Mol Med 28, 115–119 (2011)
63. Lyons S, Wang L, Casanova JE, Sitaraman SV, Merlin D, Gewirtz AT: *Salmonella typhimurium* transcytoses flagellin via an SPI2-mediated vesicular transport pathway. J Cell Sci 117, 5771–5780 (2004)
64. McCole DF: IBD candidate genes and intestinal barrier regulation. Inflamm Bowel Dis 20, 1829–1849 (2014)
65. Comalada M, Peppelenbosch MP: Impaired innate immunity in Crohn's disease. Trends Mol Med 12, 397–399 (2006)
66. Lodes MJ, Cong Y, Elson CO, Mohamath R, Landers CJ, Targan SR, Fort M, Hershberg RM: Bacterial flagellin is a dominant antigen in Crohn disease. J Clin Invest 113, 1296–1306 (2004)
67. Wallis D, Asaduzzaman A, Weisman M, Haroon N, Anton A, McGovern D, Targan S, Inman R: Elevated serum antiflagellin antibodies implicate subclinical bowel inflammation in ankylosing spondylitis: an observational study. Arthritis Res Ther 15, R166 (2013)
68. Rhee SH, Im E, Riegler M, Kokkotou E, O'Brien M, Pothoulakis C: Pathophysiological role of Toll-like receptor 5 engagement by bacterial flagellin in colonic inflammation. Proc Natl Acad Sci U S A 102, 13610–13615 (2005)
69. Gewirtz AT, Vijay-Kumar M, Brant SR, Duerr RH, Nicolae DL, Cho JH: Dominant-negative TLR5 polymorphism reduces adaptive immune response to flagellin and negatively associates with Crohn's disease. Am J Physiol Gastrointest Liver Physiol 290, G1157–1163 (2006)
70. Schülke S, Burggraf M, Waibler Z, Wangorsch A, Wolfheimer S, Kalinke U, Vieths S, Toda M, Scheurer S: A fusion protein of flagellin and ovalbumin suppresses the TH2 response and prevents murine intestinal allergy. J Allergy Clin Immunol 128, 1340–1348, e12 (2011)
71. Schulke S, Wolfheimer S, Gadermaier G, Wangorsch A, Siebeneicher S, Briza P, Spreitzer I, Schiller D, Loeschner B, Uematsu S, Ryffel B, Akira S, Waibler Z, Vieths S, Toda M, Scheurer S: Prevention of intestinal allergy in mice by rflaA:Ova is associated with enforced antigen processing and TLR5-dependent IL-10 secretion by mDC. PLoS One 9, e87822 (2014)
72. Wilson RH, Maruoka S, Whitehead GS, Foley JF, Flake GP, Sever ML, Zeldin DC, Kraft M, Garantziotis S, Nakano H, Cook DN: The Toll-like receptor 5 ligand flagellin promotes asthma by priming allergic responses to indoor allergens. Nat Med 18, 1705–1710 (2012)
73. Talbot HK, Rock MT, Johnson C, Tussey L, Kavita U, Shanker A, Shaw AR, Taylor DN: Immunopotentiation of trivalent influenza vaccine when given with VAX102, a recombinant influenza M2e vaccine fused to the TLR5 ligand flagellin. PLoS One 5, e14442 (2010)
74. Taylor DN, Treanor JJ, Sheldon EA, Johnson C, Umlauf S, Song L, Kavita U, Liu G, Tussey L, Ozer K, Hofstaetter T, Shaw A: Development of VAX128, a recombinant hemagglutinin (HA) influenza-flagellin fusion vaccine with improved safety and immune response. Vaccine 30, 5761–5769 (2012)
75. Gupta SK, Bajwa P, Deb R, Chellappa MM, Dey S: Flagellin a toll-like receptor 5 agonist as an adjuvant in chicken vaccines. Clin Vaccine Immunol 21, 261–270 (2014)
76. Ginsburg I: Role of lipoteichoic acid in infection and inflammation. Lancet Infect Dis 2, 171–179 (2002)
77. Schneewind O, Missiakas D: Lipoteichoic acids, phosphate-containing polymers in the envelope of gram-positive bacteria. J Bacteriol 196, 1133–1142 (2014)
78. Hermann C, Spreitzer I, Schroder NW, Morath S, Lehner MD, Fischer W, Schutt C, Schumann RR, Hartung T: Cytokine induction by purified lipoteichoic acids from various bacterial species – role of LBP, sCD14, CD14 and failure to induce IL-12 and subsequent IFN-gamma release. Eur J Immunol 32, 541–551 (2002)
79. Otto M: MRSA virulence and spread. Cell Microbiol 14, 1513–1521 (2012)
80. Kim H, Jung BJ, Kim JY, Chung DK: Differential effects of low and high doses of lipoteichoic acid on lipopolysaccharide-induced interleukin-6 production. Inflamm Res 63, 419–428 (2014)
81. Gao JJ, Xue Q, Zuvanich EG, Haghi KR, Morrison DC: Commercial preparations of lipoteichoic acid contain endotoxin that contributes to activation of mouse macrophages *in vitro*. Infect Immun 69, 751–757 (2001)
82. Morath S, Geyer A, Hartung T: Structure–function relationship of cytokine induction by lipoteichoic acid from *Staphylococcus aureus*. J Exp Med 193, 393–397 (2001)
83. Lysakova-Devine T, Keogh B, Harrington B, Nagpal K, Halle A, Golenbock DT, Monie T, Bowie AG: Viral inhibitory peptide of TLR4, a peptide derived from vaccinia protein A46, specifically inhibits TLR4 by directly targeting MyD88 adaptor-like and TRIF-related adaptor molecule. J Immunol 185, 4261–4271 (2010)
84. Seo HS, Michalek SM, Nahm MH: Lipoteichoic acid is important in innate immune responses to gram-positive bacteria. Infect Immun 76, 206–213 (2008)
85. Sheen TR, Ebrahimi CM, Hiemstra IH, Barlow SB, Peschel A, Doran KS: Penetration of the blood–brain barrier by *Staphylococcus aureus*: contribution of membrane-anchored lipoteichoic acid. J Mol Med (Berl) 88, 633–639 (2010)
86. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM: The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res 54, 2325–2340 (2013)
87. Martinez I, Kim J, Duffy PR, Schlegel VL, Walter J: Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. PLoS One 5, e15046 (2010)
88. Ramirez-Farias C, Slezak K, Fuller Z, Duncan A, Holtrop G, Louis P: Effect of inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. Br J Nutr 101, 541–550 (2009)
89. Morrison DJ, Mackay WG, Edwards CA, Preston T, Dodson B, Weaver LT: Butyrate production from oligofructose fermentation by the human faecal flora: What is the contribution of extracellular acetate and lactate? Br J Nutr 96, 570–577 (2006)
90. Compendium of food additive specifications. Addendum 8. World Health Organization: Food and Agriculture Organization of the United Nations, Rome, 2000
91. Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ: Colonic health: Fermentation and short chain fatty acids. J Clin Gastroenterol 40, 235–243 (2006)
92. Hold GL, Pryde SE, Russell VJ, Furrie E, Flint HJ: Assessment of microbial diversity in human colonic samples by 16S rDNA sequence analysis. FEMS Microbiol Ecol 39, 33–39 (2002)

93. Bindels LB, Dewulf EM, Delzenne NM: GPR43/FFA2: physiopathological relevance and therapeutic prospects. Trends Pharmacol Sci 34, 226–232 (2013)
94. Inoue D, Tsujimoto G, Kimura I: Regulation of energy homeostasis by GPR41. Front Endocrinol (Lausanne) 5, 81 (2014)
95. Daly K, Shirazi-Beechey SP: Microarray analysis of butyrate regulated genes in colonic epithelial cells. DNA Cell Biol 25, 49–62 (2006)
96. Lupton JR: Microbial degradation products influence colon cancer risk: The butyrate controversy. J Nutr 134, 479–482 (2004)
97. Chen YX, Fang JY, Lu J, Qiu DK: Regulation of histone acetylation on the expression of cell cycle-associated genes in human colon cancer cell lines. Zhonghua Yi Xue Za Zhi 84, 312–317 (2004)
98. Kim YS, Tsao D, Siddiqui B, Whitehead JS, Arnstein P, Bennett J, Hicks J: Effects of sodium butyrate and dimethylsulfoxide on biochemical properties of human colon cancer cells. Cancer 45, 1185–1192 (1980)
99. Hague A, Manning AM, Hanlon KA, Huschtscha LI, Hart D, Paraskeva C: Sodium butyrate induces apoptosis in human colonic tumour cell lines in a p53-independent pathway: Implications for the possible role of dietary fibre in the prevention of large-bowel cancer. Int J Cancer 55, 498–505 (1993)
100. Coradini D, Biffi A, Costa A, Pellizzaro C, Pirronello E, Di Fronzo G: Effect of sodium butyrate on human breast cancer cell lines. Cell Prolif 30, 149–159 (1997)
101. Miyake H, Hara S, Arakawa S, Kamidono S, Hara I: Overexpression of Bcl-2 regulates sodium butyrate- and/or docetaxel-induced apoptosis in human bladder cancer cells both *in vitro* and *in vivo*. Int J Cancer 93, 26–32 (2001)
102. Kuefer R, Hofer MD, Altug V, Zorn C, Genze F, Kunzi-Rapp K, Hautmann RE, Gschwend JE: Sodium butyrate and tributyrin induce *in vivo* growth inhibition and apoptosis in human prostate cancer. Br J Cancer 90, 535–541 (2004)
103. Weir TL, Manter DK, Sheflin AM, Barnett BA, Heuberger AL, Ryan EP: Stool microbiome and metabolome differences between colorectal cancer patients and healthy adults. PLoS One 8, e70803 (2013)
104. Bordonaro M, Lazarova DL, Sartorelli AC: Butyrate and Wnt signaling: A possible solution to the puzzle of dietary fiber and colon cancer risk? Cell Cycle 7, 1178–1183 (2008)
105. Hamer HM, Jonkers DM, Bast A, Vanhoutvin SA, Fischer MA, Kodde A, Troost FJ, Venema K, Brummer RJ: Butyrate modulates oxidative stress in the colonic mucosa of healthy humans. Clin Nutr 28, 88–93 (2009)
106. Willemsen LE, Koetsier MA, van Deventer SJ, van Tol EA: Short chain fatty acids stimulate epithelial mucin 2 expression through differential effects on prostaglandin E(1) and E(2) production by intestinal myofibroblasts. Gut 52, 1442–1447 (2003)
107. Canani RB, Costanzo MD, Leone L, Pedata M, Meli R, Calignano A: Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. World J Gastroenterol 17, 1519–1528 (2011)
108. Vernia P, Gnaedinger A, Hauck W, Breuer RI: Organic anions and the diarrhea of inflammatory bowel disease. Dig Dis Sci 33, 1353–1358 (1988)
109. Roediger WE: The colonic epithelium in ulcerative colitis: An energy-deficiency disease? Lancet 2, 712–715 (1980)
110. Scheppach W, Sommer H, Kirchner T, Paganelli GM, Bartram P, Christl S, Richter F, Dusel G, Kasper H: Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. Gastroenterology 103, 51–56 (1992)
111. Butzner JD, Parmar R, Bell CJ, Dalal V: Butyrate enema therapy stimulates mucosal repair in experimental colitis in the rat. Gut 38, 568–573 (1996)
112. Vieira EL, Leonel AJ, Sad AP, Beltrao NR, Costa TF, Ferreira TM, Gomes-Santos AC, Faria AM, Peluzio MC, Cara DC, Alvarez-Leite JI: Oral administration of sodium butyrate attenuates inflammation and mucosal lesion in experimental acute ulcerative colitis. J Nutr Biochem 23, 430–436 (2012)
113. Venkatraman A, Ramakrishna BS, Shaji RV, Kumar NS, Pulimood A, Patra S: Amelioration of dextran sulfate colitis by butyrate: Role of heat shock protein 70 and NF-kappaB. Am J Physiol Gastrointest Liver Physiol 285, G177–184 (2003)
114. Atreya I, Atreya R, Neurath MF: NF-kappaB in inflammatory bowel disease. J Intern Med 263, 591–596 (2008)
115. Scheppach W: Treatment of distal ulcerative colitis with short-chain fatty acid enemas. A placebo-controlled trial. German–Austrian SCFA Study Group. Dig Dis Sci 41, 2254–2259 (1996)
116. Dover GJ, Brusilow S, Charache S: Induction of fetal hemoglobin production in subjects with sickle cell anemia by oral sodium phenylbutyrate. Blood 84, 339–343 (1994)
117. Collins AF, Pearson HA, Giardina P, McDonagh KT, Brusilow SW, Dover GJ: Oral sodium phenylbutyrate therapy in homozygous beta thalassemia: A clinical trial. Blood 85, 43–49 (1995)
118. Patthamalai P, Fucharoen S, Chaneiam N, Ghalie RG, Chui DH, Boosalis MS, Perrine SP: A phase 2 trial of HQK-1001 in HbE-beta thalassemia demonstrates HbF induction and reduced anemia. Blood 123, 1956–1957 (2014)
119. Reid ME, El Beshlawy A, Inati A, Kutlar A, Abboud MR, Haynes J, Jr, Ward R, Sharon B, Taher AT, Smith W, Manwani D, Ghalie RG: A double-blind, placebo-controlled phase II study of the efficacy and safety of 2,2-dimethylbutyrate (HQK-1001), an oral fetal globin inducer, in sickle cell disease. Am J Hematol 89, 709–713 (2014)
120. Burlina AB, Ogier H, Korall H, Trefz FK: Long-term treatment with sodium phenylbutyrate in ornithine transcarbamylase-deficient patients. Mol Genet Metab 72, 351–355 (2001)
121. Schroeder FA, Lin CL, Crusio WE, Akbarian S: Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. Biol Psychiatry 62, 55–64 (2007)
122. Ferrante RJ, Kubilus JK, Lee J, Ryu H, Beesen A, Zucker B, Smith K, Kowall NW, Ratan RR, Luthi-Carter R, Hersch SM: Histone deacetylase inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington's disease mice. J Neurosci 23, 9418–9427 (2003)
123. Kim HJ, Leeds P, Chuang DM: The HDAC inhibitor, sodium butyrate, stimulates neurogenesis in the ischemic brain. J Neurochem 110, 1226–1240 (2009)
124. Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA: Elevated fecal short chain fatty acid and ammonia concentrations in children with autism spectrum disorder. Dig Dis Sci 57, 2096–2102 (2012)

125. Takuma K, Hara Y, Kataoka S, Kawanai T, Maeda Y, Watanabe R, Takano E, Hayata-Takano A, Hashimoto H, Ago Y, Matsuda T: Chronic treatment with valproic acid or sodium butyrate attenuates novel object recognition deficits and hippocampal dendritic spine loss in a mouse model of autism. Pharmacol Biochem Behav 126C, 43–49 (2014)
126. Bourdu S, Dapoigny M, Chapuy E, Artigue F, Vasson MP, Dechelotte P, Bommelaer G, Eschalier A, Ardid D: Rectal instillation of butyrate provides a novel clinically relevant model of noninflammatory colonic hypersensitivity in rats. Gastroenterology 128, 1996–2008 (2005)
127. Harris K, Kassis A, Major G, Chou CJ: Is the gut microbiota a new factor contributing to obesity and its metabolic disorders? J Obes 2012, 879151 (2012)
128. Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, Cefalu WT, Ye J: Butyrate improves insulin sensitivity and increases energy expenditure in mice. Diabetes 58, 1509–1517 (2009)
129. Kamphuis MM, Lejeune MP, Saris WH, Westerterp-Plantenga MS: The effect of conjugated linoleic acid supplementation after weight loss on body weight regain, body composition, and resting metabolic rate in overweight subjects. Int J Obes Relat Metab Disord 27, 840–847 (2003)
130. Lecerf JM, Depeint F, Clerc E, Dugenet Y, Niamba CN, Rhazi L, Cayzeele A, Abdelnour G, Jaruga A, Younes H, Jacobs H, Lambrey G, Abdelnour AM, Pouillart PR: Xylo-oligosaccharide (XOS) in combination with inulin modulates both the intestinal environment and immune status in healthy subjects, while XOS alone only shows prebiotic properties. Br J Nutr 108, 1847–1858 (2012)
131. Fernandes J, Su W, Rahat-Rozenbloom S, Wolever TM, Comelli EM: Adiposity, gut microbiota and faecal short chain fatty acids are linked in adult humans. Nutr Diabetes 4, e121 (2014)
132. Lin HV, Frassetto A, Kowalik EJ, Jr, Nawrocki AR, Lu MM, Kosinski JR, Hubert JA, Szeto D, Yao X, Forrest G, Marsh DJ: Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. PLoS One 7, e35240 (2012)
133. Jakobsdottir G, Xu J, Molin G, Ahrne S, Nyman M: High-fat diet reduces the formation of butyrate, but increases succinate, inflammation, liver fat and cholesterol in rats, while dietary fibre counteracts these effects. PLoS One 8, e80476 (2013)
134. Li HP, Chen X, Li MQ: Butyrate alleviates metabolic impairments and protects pancreatic beta cell function in pregnant mice with obesity. Int J Clin Exp Pathol 6, 1574–1584 (2013)
135. Yadav H, Lee JH, Lloyd J, Walter P, Rane SG: Beneficial metabolic effects of a probiotic via butyrate-induced GLP-1 hormone secretion. J Biol Chem 288, 25088–25097 (2013)
136. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, Peng Y, Zhang D, Jie Z, Wu W, Qin Y, Xue W, Li J, Han L, Lu D, Wu P, Dai Y, Sun X, Li Z, Tang A, Zhong S, Li X, Chen W, Xu R, Wang M, Feng Q, Gong M, Yu J, Zhang Y, Zhang M, Hansen T, Sanchez G, Raes J, Falony G, Okuda S, Almeida M, LeChatelier E, Renault P, Pons N, Batto JM, Zhang Z, Chen H, Yang R, Zheng W, Li S, Yang H, Wang J, Ehrlich SD, Nielsen R, Pedersen O, Kristiansen K, Wang J: A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 490, 55–60 (2012)
137. Assisi RF, GISDI Study Group: Combined butyric acid/mesalazine treatment in ulcerative colitis with mild-moderate activity. Results of a multicentre pilot study. Minerva Gastroenterol Dietol 54, 231–238 (2008)
138. Steinhart AH, Brzezinski A, Baker JP: Treatment of refractory ulcerative proctosigmoiditis with butyrate enemas. Am J Gastroenterol 89, 179–183 (1994)
139. Palou L, Usall J, Smilanick JL, Aguilar MJ, Vinas I: Evaluation of food additives and low-toxicity compounds as alternative chemicals for the control of *Penicillium digitatum* and *Penicillium italicum* on citrus fruit. Pest Manag Sci 58, 459–466 (2002)
140. Taniguchi M, Nakazawa H, Takeda O, Kaneko T, Hoshino K, Tanaka T: Production of a mixture of antimicrobial organic acids from lactose by co-culture of *Bifidobacterium longum* and *Propionibacterium freudenreichii*. Biosci Biotechnol Biochem 62, 1522–1527 (1998)
141. Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT: Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 28, 1221–1227 (1987)
142. Macy JM, Ljungdahl LG, Gottschalk G: Pathway of succinate and propionate formation in *Bacteroides fragilis*. J Bacteriol 134, 84–91 (1978)
143. Hara T, Kashihara D, Ichimura A, Kimura I, Tsujimoto G, Hirasawa A: Role of free fatty acid receptors in the regulation of energy metabolism. Biochim Biophys Acta 1841, 1292–1300 (2014)
144. Bergman EN: Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol Rev 70, 567–590 (1990)
145. Demigne C, Morand C, Levrat MA, Besson C, Moundras C, Remesy C: Effect of propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated rat hepatocytes. Br J Nutr 74, 209–219 (1995)
146. Lin Y, Vonk RJ, Slooff MJ, Kuipers F, Smit MJ: Differences in propionate-induced inhibition of cholesterol and triacylglycerol synthesis between human and rat hepatocytes in primary culture. Br J Nutr 74, 197–207 (1995)
147. Xiong Y, Miyamoto N, Shibata K, Valasek MA, Motoike T, Kedzierski RM, Yanagisawa M: Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. Proc Natl Acad Sci U S A 101, 1045–1050 (2004)
148. Al-Lahham SH, Roelofsen H, Priebe M, Weening D, Dijkstra M, Hoek A, Rezaee F, Venema K, Vonk RJ: Regulation of adipokine production in human adipose tissue by propionic acid. Eur J Clin Invest 40, 401–407 (2010)
149. Ge H, Li X, Weiszmann J, Wang P, Baribault H, Chen JL, Tian H, Li Y: Activation of G protein-coupled receptor 43 in adipocytes leads to inhibition of lipolysis and suppression of plasma free fatty acids. Endocrinology 149, 4519–4526 (2008)
150. Psichas A, Sleeth ML, Murphy KG, Brooks L, Bewick GA, Hanyaloglu AC, Ghatei MA, Bloom SR, Frost G: The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. Int J Obes (Lond) (2014)
151. Todesco T, Rao AV, Bosello O, Jenkins DJ: Propionate lowers blood glucose and alters lipid metabolism in healthy subjects. Am J Clin Nutr 54, 860–865 (1991)
152. Liljeberg HG, Lonner CH, Bjorck IM: Sourdough fermentation or addition of organic acids or corresponding salts to

bread improves nutritional properties of starch in healthy humans. J Nutr 125, 1503–1511 (1995)
153. Anderson JW, Chen WJ: Plant fiber. Carbohydrate and lipid metabolism. Am J Clin Nutr 32, 346–363 (1979)
154. Robertson MD, Bickerton AS, Dennis AL, Vidal H, Frayn KN: Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. Am J Clin Nutr 82, 559–567 (2005)
155. Robertson MD, Wright JW, Loizon E, Debard C, Vidal H, Shojaee-Moradie F, Russell-Jones D, Umpleby AM: Insulin-sensitizing effects on muscle and adipose tissue after dietary fiber intake in men and women with metabolic syndrome. J Clin Endocrinol Metab 97, 3326–3332 (2012)
156. Bodinham CL, Smith L, Thomas EL, Bell JD, Swann JR, Costabile A, Russell-Jones D, Umpleby AM, Robertson MD: Efficacy of increased resistant starch consumption in human type 2 diabetes. Endocr Connect 3, 75–84 (2014)
157. Cox AJ, West NP, Cripps AW: Obesity, inflammation, and the gut microbiota. Lancet Diabetes Endocrinol (2014)
158. Wajner M, Santos KD, Schlottfeldt JL, Rocha MP, Wannmacher CM: Inhibition of mitogen-activated proliferation of human peripheral lymphocytes *in vitro* by propionic acid. Clin Sci (Lond) 96, 99–103 (1999)
159. Senagore AJ, MacKeigan JM, Scheider M, Ebrom JS: Short-chain fatty acid enemas: A cost-effective alternative in the treatment of nonspecific proctosigmoiditis. Dis Colon Rectum 35, 923–927 (1992)
160. Vernia P, Marcheggiano A, Caprilli R, Frieri G, Corrao G, Valpiani D, Di Paolo MC, Paoluzi P, Torsoli A: Short-chain fatty acid topical treatment in distal ulcerative colitis. Aliment Pharmacol Ther 9, 309–313 (1995)
161. Breuer RI, Soergel KH, Lashner BA, Christ ML, Hanauer SB, Vanagunas A, Harig JM, Keshavarzian A, Robinson M, Sellin JH, Weinberg D, Vidican DE, Flemal KL, Rademaker AW: Short chain fatty acid rectal irrigation for left-sided ulcerative colitis: A randomised, placebo controlled trial. Gut 40, 485–491 (1997)
162. Tana C, Umesaki Y, Imaoka A, Handa T, Kanazawa M, Fukudo S: Altered profiles of intestinal microbiota and organic acids may be the origin of symptoms in irritable bowel syndrome. Neurogastroenterol Motil 22, 512–519, e114–115 (2010)
163. Scheppach W, Bartram HP, Richter F: Role of short-chain fatty acids in the prevention of colorectal cancer. Eur J Cancer 31A, 1077–1080 (1995)
164. Hague A, Elder DJ, Hicks DJ, Paraskeva C: Apoptosis in colorectal tumour cells: induction by the short chain fatty acids butyrate, propionate and acetate and by the bile salt deoxycholate. Int J Cancer 60, 400–406 (1995)
165. Jan G, Belzacq AS, Haouzi D, Rouault A, Metivier D, Kroemer G, Brenner C: Propionibacteria induce apoptosis of colorectal carcinoma cells via short-chain fatty acids acting on mitochondria. Cell Death Differ 9, 179–188 (2002)
166. Hinnebusch BF, Meng S, Wu JT, Archer SY, Hodin RA: The effects of short-chain fatty acids on human colon cancer cell phenotype are associated with histone hyperacetylation. J Nutr 132, 1012–1017 (2002)
167. Ando T, Rasmussen K, Nyhan WL, Donnell GN, Barnes ND: Propionic acidemia in patients with ketotic hyperglycinemia. J Pediatr 78, 827–832 (1971)
168. Hsia YE, Scully KJ, Rosenberg LE: Inherited propionyl-Coa carboxylase deficiency in "ketotic hyperglycinemia." J Clin Invest 50, 127–130 (1971)
169. Henriquez H, el Din A, Ozand PT, Subramanyam SB, al Gain SI: Emergency presentations of patients with methylmalonic acidemia, propionic acidemia and branched chain amino acidemia (MSUD). Brain Dev 16 Suppl, 86–93 (1994)
170. Yorifuji T, Muroi J, Uematsu A, Nakahata T, Egawa H, Tanaka K: Living-related liver transplantation for neonatal-onset propionic acidemia. J Pediatr 137, 572–574 (2000)
171. MacFabe DF, Cain DP, Rodriguez-Capote K, Franklin AE, Hoffman JE, Boon F, Taylor AR, Kavaliers M, Ossenkopp KP: Neurobiological effects of intraventricular propionic acid in rats: Possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders. Behav Brain Res 176, 149–169 (2007)
172. Shultz SR, MacFabe DF, Ossenkopp KP, Scratch S, Whelan J, Taylor R, Cain DP: Intracerebroventricular injection of propionic acid, an enteric bacterial metabolic end-product, impairs social behavior in the rat: Implications for an animal model of autism. Neuropharmacology 54, 901–911 (2008)
173. Shultz SR, Macfabe DF, Martin S, Jackson J, Taylor R, Boon F, Ossenkopp KP, Cain DP: Intracerebroventricular injections of the enteric bacterial metabolic product propionic acid impair cognition and sensorimotor ability in the Long-Evans rat: further development of a rodent model of autism. Behav Brain Res 200, 33–41 (2009)
174. Thomas RH, Foley KA, Mepham JR, Tichenoff LJ, Possmayer F, MacFabe DF: Altered brain phospholipid and acylcarnitine profiles in propionic acid infused rodents: Further development of a potential model of autism spectrum disorders. J Neurochem 113, 515–529 (2010)
175. Brusque AM, Mello CF, Buchanan DN, Terracciano ST, Rocha MP, Vargas CR, Wannmacher CM, Wajner M: Effect of chemically induced propionic acidemia on neurobehavioral development of rats. Pharmacol Biochem Behav 64, 529–534 (1999)
176. Niederman R, Buyle-Bodin Y, Lu BY, Robinson P, Naleway C: Short-chain carboxylic acid concentration in human gingival crevicular fluid. J Dent Res 76, 575–579 (1997)
177. Kashket S, Zhang J, Niederman R: Gingival inflammation induced by food and short-chain carboxylic acids. J Dent Res 77, 412–417 (1998)
178. Jacobi CA, Malfertheiner P: *Escherichia coli* Nissle 1917 (Mutaflor): New insights into an old probiotic bacterium. Dig Dis 29, 600–607 (2011)
179. Mayerhofer F, Petuely F: Untersuchungen zur Regulation der Darmtätigkeit des Erwachsenen mit Hilfe der Lactulose (Bifidus-Faktor). Wiener Wschr 71, 865–869 (1959)