REVIEW

Mechanisms of Action of Probiotics and the Gastrointestinal Microbiota on Gut Motility and Constipation[1,2]

Eirini Dimidi,[3,4] Stephanos Christodoulides,[3,4] S Mark Scott,[4] and Kevin Whelan[3]*

[3]Faculty of Life Sciences and Medicine, Diabetes and Nutritional Sciences Division, King's College London, London, United Kingdom; and [4]Centre for Neuroscience and Trauma, Neurogastroenterology Group and GI Physiology Unit, Queen Mary University of London, London, United Kingdom

ABSTRACT

Constipation is a common and burdensome gastrointestinal disorder that may result from altered gastrointestinal motility. The effect of probiotics on constipation has been increasingly investigated in both animal and human studies, showing promising results. However, there is still uncertainty regarding the mechanisms of action of probiotics on gut motility and constipation. Several factors are vital to normal gut motility, including immune and nervous system function, bile acid metabolism and mucus secretion, and the gastrointestinal microbiota and fermentation; an imbalance or dysfunction in any of these components may contribute to aberrant gut motility and, consequently, symptoms of constipation. For example, adults with functional constipation have significantly decreased numbers of bifidobacteria (with one study showing a mean difference of 1 $\log_{10}$/g) and lactobacilli (mean difference, 1.4 $\log_{10}$/g) in stool samples, as well as higher breath methane, compared with control subjects. Modifying the gut luminal environment with certain probiotic strains may affect motility and secretion in the gut and, hence, provide a benefit for patients with constipation. Therefore, this review explores the mechanisms through which probiotics may exert an effect on gut motility and constipation. Nevertheless, the majority of current evidence is derived from animal studies, and therefore, further human studies are needed to determine the mechanisms through specific probiotic strains that might be effective in constipation. *Adv Nutr* 2017;8:484–94.

Keywords: constipation, gut motility, mechanisms, probiotics, gastrointestinal microbiome

Introduction

Motility of the gastrointestinal tract is an imprecise term embracing several measurable phenomena, including enteric contractile activity, gut wall biomechanical functions, and intraluminal flow responsible for the propulsion of gut contents. Sensitivity of the gastrointestinal tract, which refers both to conscious perception of gut stimuli and to afferent input within gastrointestinal sensory pathways, is inextricably linked, and hence, gut motility can be the consequence of interrelated sensory motor functions.

Gut transit is the functional consequence of tonic and phasic gut contractions and refers to the time taken for intraluminal contents to traverse the gastrointestinal tract (1). Despite a wide variation between individuals, normal whole-gut transit time is considered to be 30–40 h (2).

Several factors regulate gut motility (**Figure 1**). Afferent and efferent neural control is provided through interaction of the gut with the central nervous system (CNS)[5] via somatic or autonomic [autonomic nervous system (ANS)] neurons, and communication between different parts of the gut is achieved by the transmission of myogenic and neurogenic signals along the gut via the enteric nervous system (ENS) and by reflex arcs through autonomic neurons (3). The hierarchy of neural control of gut motility is as follows: the primary regulator is ENS, followed by ANS and then CNS. Simultaneously, the immune system, gut secretions, gastrointestinal microbiota, and products of fermentation interact and modulate gut motility.

Constipation may, in some cases, be regarded as a colonic motility disorder (4). Slow-transit constipation may be caused by the dysfunction of colonic smooth muscle or neural innervation, resulting in neural colonic motor abnormalities (5); the principal pathophysiological mechanism is

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*To whom correspondence should be addressed. E-mail: kevin.whelan@kcl.ac.uk.

[5] Abbreviations used: ANS, autonomic nervous system; CNS, central nervous system; ENS, enteric nervous system; IBS, irritable bowel syndrome; 5-HT, 5-hydroxytryptamine.

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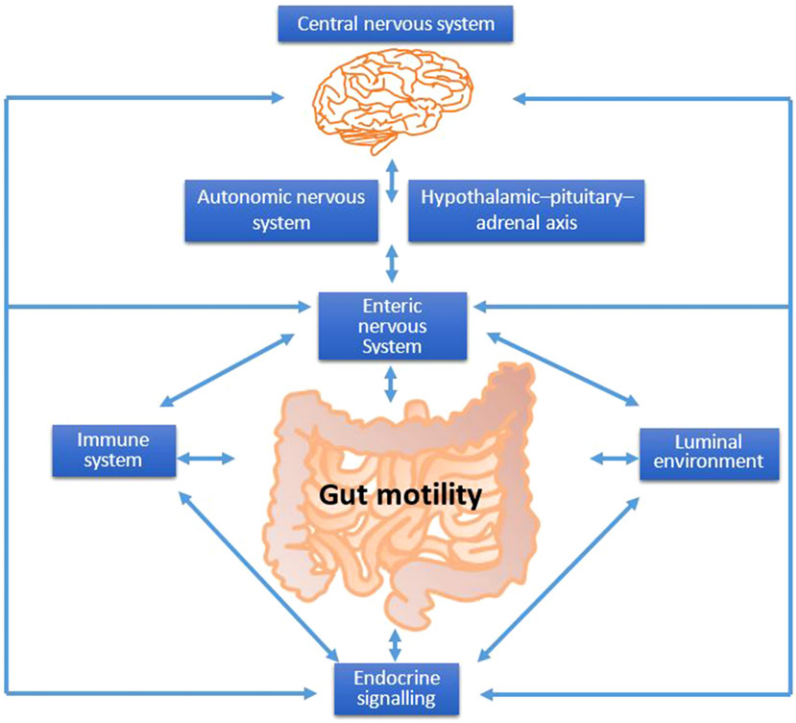


FIGURE 1 Factors that control gut motility. The gut luminal environment (including the gastrointestinal microbiota and fermentation), immune system, enteric nervous system, and central nervous system are highly interrelated and control gut motility; disturbance in any of these overlapping systems may contribute to symptoms of constipation.

believed to be dysregulated or deficient colonic propulsive motor patterns (5, 6), although abnormal visceral sensitivity may also be involved in some cases.

Patients with constipation have an increased gut transit time compared with healthy controls, with the upper limit of normal considered to be 70 h (2). Patients use a variety of treatments, including fiber supplements, laxatives, and prescription medication (7). However, nearly half of patients are dissatisfied with current treatments, mainly because of a lack of efficacy and concerns about adverse effects (7).

There has been increasing research regarding the importance of the gastrointestinal microbiota to gut function and the effect of probiotics on gut motility and constipation (**Table 1**). Probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (13). Studies have shown that specific probiotics may help decrease gut transit time in people with or without constipation (Table 1). A recent systematic review and meta-analysis also showed that administration of 10^8 to 3×10^{10} CFU/d of specific probiotic species and strains decreased gut transit time by 12 h, increased stool frequency by 1.5 stools/wk, and improved some constipation-related symptoms (14). The aim of this review was to summarize existing evidence on the mechanisms through which probiotics may exert an effect on gut motility and constipation.

Current Status of Knowledge

In order to understand the mechanisms through which probiotics exert an effect on gut motility and constipation, first the physiology of gut motility and its pathophysiology in constipation must be reviewed, including the role of the central and enteric nervous systems, the gastrointestinal microbiota and fermentation and immune system function.

CNS and ENS

ENS, CNS, gut motility, and constipation. The ENS can function independently of the CNS and contains the reflex pathways associated with normal motor and sensory function of the gut (15, 16). Studies in germ-free mice have shown that bacterial colonization of the gut is key to the development and maturation of the ENS (17). Furthermore, metabolic products from gastrointestinal microbiota fermentation, such as SCFAs, or peptides can stimulate the ENS and affect gut transit (17). The neuroendocrine system of the gut has also been shown to interact with the microbiota (18) via serotonin [5-hydroxytryptamine (5-HT)] (19). 5-HT is produced in both the ENS and CNS and is a key neurotransmitter that plays a pivotal role in mediating motor and secretory responses in the ENS (20). 5-HT stimulates local enteric nervous reflexes to initiate secretion and propulsive motility and acts on vagal afferents to modulate contractile activities (20).

CNS control of gastrointestinal tract motility is modulated via the ANS and the hypothalamus-pituitary-adrenal axis (21). Both the sympathetic and parasympathetic branches of the ANS regulate gut motility via influences on the circuits of the ENS (22). The gastrointestinal microbiota plays a crucial role in the normal development of the CNS (23) and seems to interact with both the CNS and gut (24) through microbiota–enterochromaffin cells–vagal afferent nerve signaling (25). There is now increasing evidence to support the existence of the bidirectional "microbiota-gut-brain axis" (26), which has a key role in regulating gut motility (27).

TABLE 1 A selection of human studies investigating the effect of probiotics specifically on gut transit time in healthy and constipated individuals[1]

Reference	*N*	Population	Intervention	Comparator	Duration	Method of gut transit time assessment	Outcome
Marteau et al., 2002 (8)	36	Healthy women	*B. animalis* DN-173010 (maximum 3.75 × 10^{10} CFU/d)	Fermented milk without probiotics	10 d	ROM technique (20 ROM/d for 3 d, X-ray on day 4)	Gut transit time was significantly lower in the probiotic group than in the placebo group (52 h vs. 61 h, respectively; $P < 0.005$).
Agrawal et al., 2009 (9)	41	Constipation (Rome III for IBS-C)	*B. lactis* DN-173 010 (2.5 × 10^{10} CFU/d)	Nonfermented dairy product	4 wk	ROM technique (24 ROM/d for 3 d, X-ray on day 4)	Gut transit time was significantly reduced in the probiotic group compared with the placebo group (mean difference: −12 h; $P = 0.026$).
Krammer et al., 2011 (10)	24	Constipation (gut transit time >72 h)	*L. casei* Shirota (6.5 × 10^{9} CFU/d)	Milk drink without probiotics	4 wk	ROM technique (20 ROM over 6 d, X-ray on day 7)	Gut transit time was decreased from 96 h at baseline to 77 h after the probiotic consumption ($P = 0.05$). No statistical comparisons were performed between the probiotic and placebo groups at the end of the treatment period.
Waller et al., 2011 (11)	88	Constipation (2–47 stool type at Bristol stool chart and 1–3 stool/wk)	*B. lactis* HN019 (17.2 × 10^{9} CFU/d or 1.8 × 10^{9} CFU/d)	Capsules with rice maltodextrin	14 d	ROM technique (24 ROM/d for 6 d, X-ray on day 7)	Change in WGTT was statistically significant across study groups (high dose: −28 h, low dose: −19 h, placebo: +1 h; $P < 0.001$).
Merenstein et al., 2014 (12)	68	Healthy women	*B. lactis* Bf-6 (5.6 × 10^{10} CFU/d)	Yogurt without probiotics	2 wk	ROM technique (24 ROM/d for 3 d, X-ray on day 4 and 8)	Gut transit time was not different between the probiotic and placebo periods (42 h vs. 43 h, respectively; $P > 0.69$).

[1] *B.*, *Bifidobacterium*; IBS-C, Constipation-predominant irritable bowel syndrome; *L.*, *Lactobacillus*; ROM, radio-opaque marker; WGTT, whole-gut transit time.

Impaired gut motility can develop through dysfunction of the control mechanisms of the ENS (1), potentially leading to gut symptoms, including constipation. Abnormal gastrointestinal microbiota composition may cause disruption in microbiota-gut-brain axis signaling, leading to changes in gut motility (23, 24). Alteration in gut motility can also be a result of a primary defect in CNS modulation (28), although impaired gut motility can develop through dysfunction of control mechanisms at any level from the gut to the CNS (1).

ENS, CNS, gut motility, and constipation: effects of probiotics. Modulation of microbiota gut-brain interactions with probiotics has been proposed as a novel therapeutic tool for the treatment of gut motility disorders (29). Administration of *Lactobacillus reuteri* has been shown to modulate neural-dependent motility reflexes that communicate with the brain in the mouse (30). Furthermore, *L. reuteri* has been shown to interact with the gut-brain axis in rats through the modulation of afferent sensory nerves that influence gut motility (31). However, although certain probiotic species and strains have been shown to modulate brain activity in humans (29), their effect on gut motility via CNS modulation has yet to be investigated in humans (22). *L. reuteri* has also been shown to selectively increase the excitability of myenteric neurons in rats, indicating that the mechanism of action of probiotics involves the ENS. Furthermore, supernatant from *Escherichia coli* Nissle increased the maximal tension forces of smooth muscle from the human colon in an in vitro study, although blockage of enteric nerves abolished these effects, suggesting that *E. coli* Nissle may potentially influence contractility by direct stimulation of smooth muscle cells (32). This effect was not attributed to fermentation end products, such as SCFAs, but to other unidentified contractility enhancing agents (32).

In summary, although the ENS appears to be the primary regulator of gut motility, both the ENS and CNS are involved in its control, and both interact with the gastrointestinal microbiota. Dysfunction or dysregulation of the ENS or CNS can lead to symptoms of constipation. A small number of studies have now shown that the beneficial effects of probiotics on gut motility are mediated through the nervous system, providing evidence that probiotics may help regulate the ENS or CNS to normalize gut motility.

Luminal factors

Microbiota, gut fermentation, and gut motility. The gastrointestinal microbiota play a vital role in gut motility, as highlighted by studies in germ-free mice showing that, in the absence of a gastrointestinal microbiota, gastric emptying and gut transit time are increased compared with in wild-type mice (33, 34). Colonization with a specific pathogen-free microbiota normalizes small-bowel migrating motor complexes (35), and colonization with *L. acidophilus*, *Bifidobacterium bifidum,* or *Clostridium tabificum* in germ-free rats also normalized the small-bowel migrating motor complexes and gut transit time, whereas colonization with *E. coli* inhibited intestinal myoelectric activity (36). In vitro and in vivo studies have shown that colonization with microbiota in conventionally raised and germ-free mice results in a 2- to 5-fold increase in mRNAs encoding L-glutamate transporter, L-glutamate decarboxylase, γ-aminobutyric acid (neuromodulator in enteric nerves), vesicle-associated protein 33 (protein involved in neurotransmitter release), enteric γ-actin, and cysteine-rich protein-2, indicating that the gastrointestinal microbiota affects ENS components crucial to motility (37–39). A murine study has also shown that colonic contractility was higher and gut transit time significantly decreased in mice colonized with gastrointestinal microbiota compared with germ-free controls (286 min compared with 457 min, respectively; $P < 0.005$) (40). In the same study, the administration of polyethylene glycol (a laxative) further decreased gut transit time and caused a decrease in the relative abundance of Peptococcaceae, Eubacteriaceae, and Anaeroplasmataceae, whereas the administration of loperamide (a constipating agent) increased gut transit time and resulted in an increase in the ratio of Firmicutes to Bacteroides and a decrease in relative abundance of Lachnospiraceae (40). Administration of cellulose (insoluble dietary fiber) led to a decrease in gastrointestinal transit, an effect that was independent of the presence of a gastrointestinal microbiota (277 min compared with 502 min in germ-free controls and 225 min compared with 300 min in colonized mice; $P < 0.05$). The authors concluded that dietary-induced changes in microbial composition may be partly mediated by changes in gut transit time and that the effect of diet on gut transit time may be in part caused by altered functionality of the gastrointestinal microbiota resulting from dietary change (40). It is important to note that there is no perfect animal model that exhibits anatomical and functional defects consistent with constipation. Therefore, caution is needed when extrapolating conclusions from animal studies to humans (41) but also when extrapolating from in vitro studies to in vivo effects.

End products of bacterial fermentation can also affect gut motility. For example, the chemotactic peptides produced by the gastrointestinal microbiota, such as formyl-methionyl-leucyl-phenylalanine, stimulate the ENS and primary afferent nerves (42), whereas the bacterial endotoxin LPS may promote gut dysmotility via the ENS and intestinal smooth muscle contractions (43). SCFAs also affect gut contractility, with an in vitro study in the rat colon showing that propionate, butyrate, and valerate induced concentration-dependent phasic contractions in the middle and distal colon (44), although other studies contradict these findings (45), possibly because of variations in animal models, experimental methods, and the nature and form of SCFA used. There are several mechanisms for the effect of SCFAs on gut motility, which are not completely understood; for example, in vitro studies have shown that propionate, butyrate, and valerate stimulate the mucosal receptors connected to enteric and/or vagal nerves (44) and act directly on the colonic smooth muscle (46). Intraluminal administration of a blend of acetate, propionate, and butyrate in rats was also shown to lead to increased 5-HT concentrations and, hence, decreased colonic transit time (47).

SCFAs have been shown to affect gut motility independent of pH. Colonic infusion of acids does not impact gut motility, whereas infusion of a solution containing acetate, propionate, and butyrate (while maintaining an intraluminal pH 6.2–6.4) reduced gut transit time (45). Furthermore, human in vivo studies have shown that infusion of boluses containing a blend of SCFAs stimulate ileal motility to a greater degree than air or saline (48), although these effects have not been consistently demonstrated (49).

Other products of microbiota fermentation are methane and hydrogen (50). Methane is a gas produced by gastrointestinal microbiota and acts as a neuromuscular transmitter affecting gut motility (50). Methane increased small intestinal transit time in a canine in vivo model, whereas exposure to methane increased in vitro ileal circular muscle contractility in guinea pigs (51). Conversely, another ex vivo study showed that methane decreased contractility of ileal muscle, whereas a hydrogen infusion increased it and decreased colonic transit time (52). The effects of microbiota-derived fermentation gases on contractility and gut motility thus remain uncertain.

The microbiota also indirectly influence gut motility via gut immune responses, mediated through pattern-recognition receptors, such as toll-like receptors (**Figure 2**). In mice, antibiotic-induced depletion of the microbiota resulted in low-grade gut inflammation, decreased gut transit time, and reduced amplitude of spontaneous contractions (58). More specifically, activation of toll-like receptor 4 expression by LPSs restored these effects, suggesting that the presence of bacteria containing LPSs (such as proteobacteria) may contribute to maintaining normal gut motility (58).

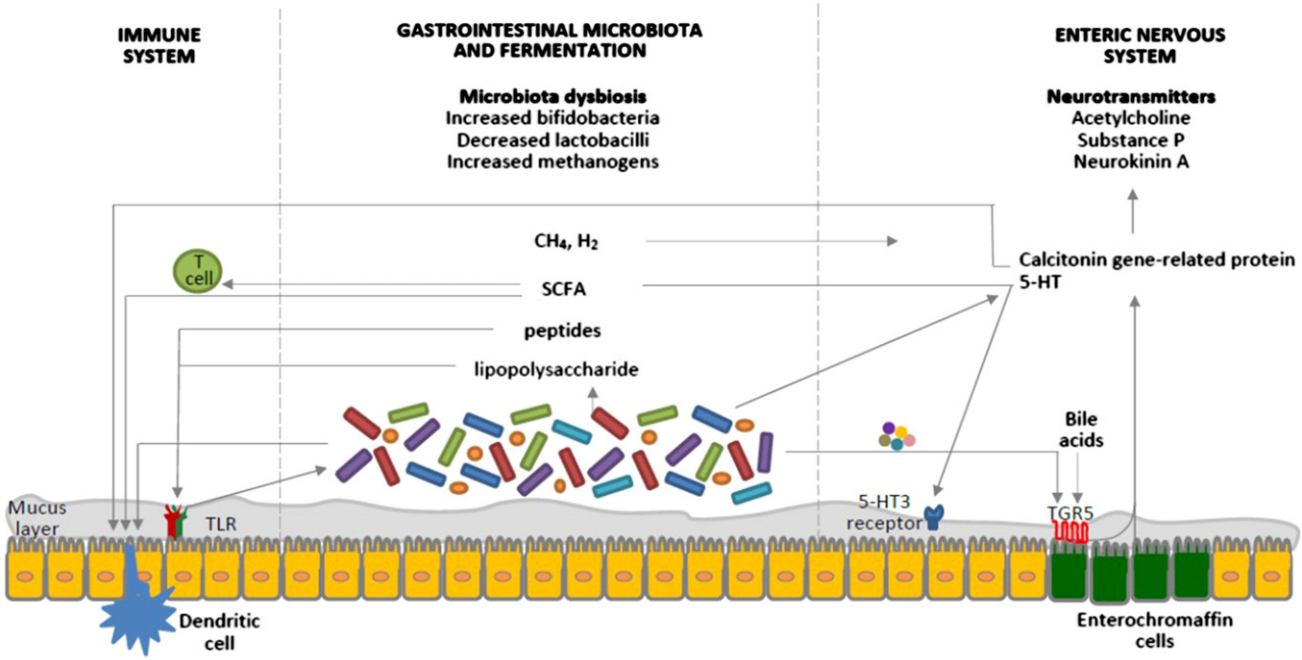


FIGURE 2 Interrelated factors involved in the pathophysiology of constipation as potential targets for the therapeutic role of probiotics. Probiotics affect the gastrointestinal microbiota composition, the byproducts of which interact with pattern-recognition receptors, such as TLRs, as well as with dendritic cells. SCFAs increase intestinal regulatory T cells, which limit intestinal inflammation, by reducing histone deacetylase 9 gene expression (53). The gastrointestinal microbiota regulates 5-HT production by elevating its synthesis by host enterochromaffin cells via the release of metabolites, such as deoxycholate, which activates TGR5, expressed by enterochromaffin cells (54). 5-HT is also released from enterochromaffin cells in response to SCFAs produced by the gastrointestinal microbiota and stimulates 5-HT3 receptors located on the vagal afferent fibers, resulting in muscle contractions (47). Gases produced by the gastrointestinal microbiota seem to affect gut motility via the ENS, rather than the brain-gut axis; however, the exact mechanisms are still unknown (50). Moreover, the gastrointestinal microbiota is key to the development of the ENS, which is the primary regulator of gut motility, and certain bacteria are known to produce 5-HT. Calcitonin gene–related protein, a sensory neuropeptide, modulates dendritic cell function and may signal the presence of gastrointestinal microbiota to the brain (55). Components of the gastrointestinal microbiota also act via intestinal dendritic cells to influence the inflammatory process (56). TLRs signaling controls ENS structure and neuromuscular function and hence motility (57). Bile acids activate TGR5 expressed by enterochromaffin cells and myenteric neurons and release 5-HT and calcitonin genre–related peptide. Furthermore, probiotics appear to interact with the gut-brain axis via the modulation of afferent sensory nerves that influence gut motility. CH_4, methane; ENS, enteric nervous system; H_2, hydrogen; TGR5, a G protein–coupled receptor; TLR, toll-like receptor; 5-HT, 5-hydroxytryptamine; 5-HT3, 5-hydroxytryptamine type 3.

Altered microbiota and gut fermentation in constipation. Several studies have investigated the gastrointestinal microbiota in constipation, as well as in constipation-predominant irritable bowel syndrome (IBS) (**Table 2**). In adults, these studies consistently demonstrate decreased bifidobacteria and lactobacilli and increased Bacteroidetes compared with controls (61, 63, 65, 66), although this has not been confirmed in pediatric studies (60, 64). Parthasarathy et al. (66), using a case-control study design, showed that fecal microbiota composition was correlated with colonic transit time, and the colonic mucosal microbiota composition was associated with constipation status even after adjusting for age, BMI (in kg/m^2), diet, and transit time (Table 2). More specifically, the abundance of Actinobacteria, *Bacteroides*, *Lactococcus*, and *Roseburia* were correlated with faster gut transit time, whereas *Faecalibacterium* was directly correlated to slower gut transit time (66). However, whether dysbiosis causes constipation or is merely a consequence of it remains unclear.

Little evidence exists regarding differences in SCFAs and stool pH between constipated and healthy subjects; SCFAs have been shown to be lower in constipation-predominant IBS than in healthy controls (63); however, this may result from slower gut transit time increasing SCFA absorption (67). No difference was observed in stool pH between constipated children and controls (60).

One study showed that more individuals with slow-transit constipation (59%) have a positive methane breath test compared with normal-transit constipation (13%) and healthy controls (12%) ($P < 0.01$) (68). It has also been shown that methane production is higher in functional constipation than in constipation-predominant IBS (69). A recent case-control study showed that breath methane production was associated with the composition of the fecal microbiota but not with colonic transit time (66). In an

TABLE 2 Summary of human case-control studies investigating the gastrointestinal microbiota composition and fermentation profiles in constipation[1]

Study	Population group		Comparison group		Method of microbiota characterization	Summary of main findings in constipation (compared to comparison groups)
	N	Characteristics	*N*	Characteristics		
Shimoyama et al., 1984 (59)	25	Patients with chronic constipation with several other diseases (not specified if adults)	37	Healthy controls without constipation	Unclear	Lower *Bifidobacteria*, Bacteroidaceae, and Clostridia Higher Micrococcaceae
Zoppi et al., 1998 (60)	42	Children with functional constipation	14	Healthy children without constipation	Culture methods	Higher Clostridia and *Bifidobacteria* Clostridia outnumber *Bacteroides* and *E. coli* in constipated children, whereas healthy children had similar mean counts for clostridia, *Bacteroides*, and *Bifidobacteria*
Khalif et al., 2005 (61)	57	Adults with chronic constipation	25	Healthy adults without constipation	Culture methods	Lower *Bifidobacterium* and *Lactobacillus* Higher *E. coli*, *Enterobacteria*, and *Staphylococcus aureus*
Attaluri et al., 2010 (62)	96	Adults with functional constipation ($n = 48$ with STC, $n = 48$ with normal-transit time)	106	Adults without constipation	Glucose breath test, which measured H_2 and CH_4 (used to define presence of methanogenic flora)	Significantly higher concentrations of methane in STC; the prevalence of methanogenic microbiota was 75%, 44%, and 28% in participants with STC and normal-transit constipation and in control participants, respectively
Chassard et al., 2012 (63)	14	Women with IBS-C	12	Healthy women without constipation	Strictly anaerobic cultural evaluation of functional groups of microbes and FISH	Lower *Bifidobacteria* (6.8 $\log_{10}$/g stool vs. 7.8 $\log_{10}$/g; $P < 0.001$), *Lactobacilli* (5.5 $\log_{10}$/g vs. 6.9 $\log_{10}$/g; $P = 0.0007$), and lactate-utilizing bacteria (7.9 $\log_{10}$/g vs. 9.3 $\log_{10}$/g; $P = 0.0046$) and the number of H_2-consuming populations, methanogens (8.3 $\log_{10}$/g vs. 9.7 $\log_{10}$/g; $P = 0.03$) and reductive acetogens Higher number of lactate- and H_2-utilizing sulfate-reducing population Lower *Roseburia* in the *E. rectale* group No difference in *F. prausnitzii* concentration
Zhu et al., 2014 (64)	8	Obese adolescents with constipation	14	Obese adolescents without constipation	16S rRNA pyrosequencing	Lower *Prevotella*, *Bacteroides* Higher Firmicutes (i.e., Lachnospiraceae and Ruminococcaceae) No difference in *Lactobacillus* and *Bifidobacteria* Absence of Acidobacteria, OP3 and TM7
Kim et al., 2015 (65)	30	Adults with functional constipation	30	Healthy controls without constipation	Real-time qPCR	Lower *Bifidobacterium* and *Bacteroides*
Parthasarathy et al., 2016 (66)	25	Adults with functional constipation, IBS-C, and mixed IBS	25	Healthy controls	16S-based sequencing	Higher abundance of Bacteroidetes (7% vs. 0.001%; $P < 0.01$), Flavobacteriaceae (7% vs. 0.001%; $P < 0.01$), and Caulobacteraceae (0.2% vs. 0.1%; $P < 0.04$) Lower Odoribacteraceae (0.01% vs. 1%; $P < 0.01$) and Comamonadaceae (0.2% vs. 12%; $P < 0.01$)

[1] CH_4, methane; *E.*, *Escherichia*; *F.*, *Faecalibacterium*; FISH, fluorescent in situ hybridization; H_2, hydrogen; IBS, irritable bowel syndrome; IBS-C, constipation-predominant irritable bowel syndrome; rRNA, ribosomal ribonucleic acid; STC, slow-transit constipation.

attempt to investigate the mechanisms of action of methane on gut motility, a small study of 18 patients with IBS showed that the postprandial blood 5-HT concentration in methane-producing patients was decreased compared with hydrogen producers, suggesting a possible, as yet unclear, interaction between methane and the enteric nervous function (70).

Altered microbiota, fermentation, gut motility, and constipation: effects of probiotics. The effect of probiotics on the microbiota in constipation is still relatively poorly understood. Some clinical trials that demonstrated improvements in stool frequency in constipation during supplementation with some probiotics have also reported alterations in gastrointestinal microbiota. Examples include *B. lactis* (10^{10} CFU/d for 2 wk) increasing total bifidobateria (71) and *L. casei* Shirota (4×10^9 CFU/d for 2 wk) increasing *Bifidobacteria*, *Lactobacilli*, and *Atopobium* (72); however, there are examples where little impact on the microbiota occurred, including VSL#3 (9×10^{11} CFU/d for 2 wk) having no impact on bifibdobacteria and Bacteroides despite affecting stool frequency (65).

One of the most popular theories on the mechanistic actions of probiotics in constipation is that some may increase SCFA concentration, thus normalizing gut motility (73). Several studies of various probiotic species and strains that demonstrate improvements in constipated-related outcomes (stool frequency, stool consistency) provide conflicting results regarding the effect of these probiotics on SCFA production; some show a change in acetate, propionate, and butyrate (72, 74, 75), and others show no change (76, 77), which could be attributed to the different strains tested. Nevertheless, these studies measured SCFA concentrations in stool samples (rather than the colonic lumen), which are not predictive of those found proximally (78) because >95% of SCFAs are absorbed during colonic transit (79).

In summary, the gastrointestinal microbiota and SCFAs, which influence gut motility through interaction with the ENS, have been shown to be imbalanced in patients with constipation. Preliminary results show that the administration of some probiotics affects the composition of some microbiota and SCFA production, but the mechanisms of the subsequent effect on motility and constipation are not yet fully understood.

Other luminal factors, gut motility, and constipation: effects of probiotics. In addition to the gastrointestinal microbiota and SCFAs, other luminal factors such as bile acids and mucus may also play a role in regulating gut motility. Bile acids act as physiological laxatives, altering luminal electrolyte and water transport (80, 81). Several decades ago, administration of deoxycholic acid in the rabbit colon was shown to increase circular smooth muscle contractile activity (81). In vitro studies have shown that bile acids influence gut motility via the ENS (82), as well as through endocrine and paracrine mechanisms (83); they appear to activate TGR5, a G protein-coupled receptor expressed by enterochromaffin cells and myenteric neurons, and release 5-HT and calcitonin gene–related peptide (84) (Figure 2). With regard to bile acid metabolism, reduced synthesis and/or concentrations of specific bile acids have been shown in patients with constipation (85), and administration of chenodeoxycholic acid or ileal bile acid–transporter inhibitors decrease gut transit time compared with placebo and improve constipation symptoms (86). Notably, bile acid metabolism and the gastrointestinal microbiota have been shown to interact because the gastrointestinal microbiota regulates hepatic bile acid synthesis and promotes deconjugation, dehydrogenation, and dihydroxylation of primary bile acids, increasing the chemical diversity of bile acids (87, 88).

The enterocytes of the gut are lined with a surface mucus gel that serves as a lubricant to protect the mucosa (89) but that also facilitates the passage of stools. A study in rats with drug-induced constipation reported a decreased mucus layer thickness, which might impede the stool passage (90). Reduction in mucus may be a consequence of cholinergic dysfunction, which has been recognized in some constipated patients (91). Conversely, a study of loperamide-induced constipation in a rat model showed that inducing gut motility with sulfated polysaccharides increased epithelial mucin production and mucus layer thickness, which is linked to an increase in stool excretion (92).

No effect of probiotic supplementation on stool bile acid concentrations (8) or stool water (72, 77) has been documented thus far in constipation. VSL#3 has been shown to induce colonic mucin production via upregulation of the MUC2 gene (93). The same study also included in vitro experiments assessing the effect of VSL#3, as well as that of its constituent individual bacterial species, on mucin secretion. Interestingly, *Lactobacillus* was as effective as VSL#3 in exerting a mucin secretion effect, whereas *Bifidobacterium* and *Streptococcus salivarius* had minimal effects (93). Furthermore, a human study (elderly nursing home residents) showed no effect of 4-wk supplementation of *L. reuteri* and *Propionibacterium freudenreichii* on mucin excretion (94). These data suggest that different probiotic species and strains have different effects on mucin production and may in part explain the variation in the effectiveness of probiotics in constipation reported in the literature.

In summary, bile acid metabolism and the mucus layer contribute to normal gut motility, and both appear to be altered in constipation, although the true cause-and-effect relation, or whether this represents an epiphenomenon, remains unclear. There is limited evidence supporting any impact of probiotics on these factors.

Immune System

Immune system, gut motility, and constipation

Evidence exists regarding a causal relation between gut mucosal inflammation and altered sensory and motor functions (95). The effect of enteropathogenic infection on the intestinal mucosal immune system and the increased gut motility and induction of diarrhea is well described. For example,

infection of rats with *Nippostrongylus brasiliensis* resulted in an increase in the contractile responses of the gut longitudinal muscle to agonists (96). This increased muscular contractile activity was diminished by suppressing the inflammatory response with the use of a corticosteroid (97). A study in mice infected with *Trichinella spiralis* showed an increased expression of the cytokine receptors IL-4Rα and TGF-β1, which mediate the effect of IL-4 or IL-13 and of TGF-1, respectively, and a subsequent upregulation of cyclooxygenase-2 (COX-2) and PGE_2 at the level of the smooth muscle cell, indicating that cytokines and their upregulation of COX-2 and PGE_2 are involved in the increased contractility (98). Furthermore, a strong correlation between the activation of mast cells via the immune response and the development of gut dysmotility in *T. spiralis* rats has been shown (99). In rats, intestinal mast cells have been shown to modify nervous reflexes and to modulate endocrine responses induced by intraluminal stimulus (100). It has also been suggested that the altered motility caused by inflammation may result from changes in the afferent nerve input into intrinsic and extrinsic neural circuits, as well as by imbalance of the ANS (95). Intestinal inflammation has been suggested to be linked to neurological changes (101). Indeed, in rectal biopsies from patients with slow-transit constipation decreased levels of the excretory substance P of the ENS of mucosa and submucosa have been found (102).

A microscopy study using surgically resected jejunal, ileal, or colonic specimens from patients with Crohn's disease showed that inflammation can result in morphological and functional changes in enteric nerves even at noninflamed sites (103), suggesting that inflammation at one site of the gut may possibly alter gut motility at another noninflamed site.

There are limited studies of immune system manifestations of constipation. One study revealed a disturbance in intestinal permeability as measured by ovalbumin serum concentration; whereas normalization of stool frequency with the use of bisacodyl (a stimulant laxative that increases water absorption and gut motility) resulted in restitution of normal intestinal permeability (61). The same study provided evidence of immune activation in functional constipation, with increased $CD3^+$, $CD4^+$, $CD8^+$, and $CD25^+$ T cells, as well as proliferation of lymphocytes, indicating the activation of T cell immunity (61).

Immune system, gut motility, and constipation: effect of probiotics

It has been shown that some probiotics, such as *L. rhamnosus* GG, can modulate the mucosal immune barrier and/or systemic immune barrier and normalize inflammation-related dysmotility in a small number of studies, albeit not specifically in constipation (104, 105). For example, *L. paracasei* has been shown to produce antagonistic metabolites, such as glutathionine, to stimulate immune cells in vitro (106), whereas *Saccharomyces boulardii* can improve gut epithelial cell restitution (107). Furthermore, subjects who consumed *B. lactis* (3×10^{11} CFU/d for 6 wk) had enhanced concentrations of IFN-α and polymorphonuclear cell phagocytic capacity compared with those who consumed a placebo (108).

In summary, it is well established that the immune system influences gut motility, and there is emerging evidence of an inflammatory response in some patients with constipation. Probiotics may have beneficial effects regarding some components of the immune system that could potentially influence gut motility, but the effect regarding constipation has not been investigated.

Clinical implication of probiotics in constipation

To this point, animal studies have suggested that various probiotic species and strains may have beneficial effects on gut motility and constipation; however, there are still limited data for human studies, and hence, it is difficult to extrapolate which probiotic strain is likely the most clinically efficacious. A systematic review and meta-analysis of 14 randomized controlled trials (n = 1182 participants) investigated the effect of probiotics in adults with functional constipation and revealed that overall probiotics reduced whole-gut transit time by 12 h and increased stool frequency by 1.3 bowel movements/wk (14). Most importantly, this study showed that the effect of probiotics were species- and strain-specific, with various *B. lactis* strains improving gut transit time, stool frequency and consistency, and flatulence, whereas the strain *L. casei* Shirota did not confer any beneficial results (14). However, caution is needed with the interpretation of these results because of the high heterogeneity and high levels of bias among the individual studies. Although there is no clear consensus to date on using probiotics for symptoms of constipation, a recent survey of 1830 health professionals in primary care showed that 18% recommend probiotics to patients with constipation, showing that clinicians have started incorporating probiotics as a management option in clinical practice (109).

Conclusion

The gut luminal environment, immune system, ENS, and CNS are highly interrelated and control gut motility; disturbance in any of these overlapping systems may contribute to symptoms of constipation. Modifying the gut luminal environment with certain probiotic species and strains may affect motility and secretion in the gut and hence provide benefit for patients with constipation. However, the majority of the current evidence is derived from animal studies, as their effect in humans is unclear because of a paucity of human studies. Further research of high methodological quality is required to fully establish the complex interactions of the luminal environment, immune system, and nervous system on gut motility and constipation and how different probiotic species and strains affect them. Further studies are needed to determine which probiotic species and strains, dose, and treatment duration are particularly effective in constipation, as well as to examine any potential probiotic-diet interactions and interindividual variability that may lead to differential responses to probiotics.

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