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SMALL INTESTINE (D SACHAR AND AC STEIN, SECTION EDITORS)

Probiotics, Nutrition, and the Small Intestine

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Abstract

Purpose of Review Probiotics are promising remedial treatments for symptoms of small intestine (SI) diseases and promoters of overall good health. Probiotics play an important role in supporting a healthy SI microbiome (eubiosis), and in preventing establishment of unhealthy microbiota. SI eubiosis promotes optimal nutrient uptake, and optimal nutritional status maintains a healthy SI, reducing the likelihood of SI diseases. It is important to understand the advantages and limitations of probiotic therapies.

Recent Findings Microbial dysbiosis decreases the capacity of the small bowel to utilize and absorb dietary compounds. In some studies, probiotic supplements containing lactic acid bacteria and *Bifidobacterium* have been demonstrated effective in supporting beneficial microbes in the SI while improving barrier integrity and reducing nutrient malabsorption and SI disease-related pathology.

Summary Strain-specific probiotic therapy may be a natural and effective approach to restoring SI barrier integrity and eubiosis, resulting in improved nutrient absorption and better health, including reducing the incidence of and severity of SI diseases.

Keywords Small intestine · Probiotics · Nutrition · Microbiota · Dysbiosis, intestinal permeability

Introduction

The preponderance of nutrient absorption occurs in the small intestine (SI), and diseases affecting the SI may therefore disrupt nutrient absorption. Malnutrition occurs when adequate amounts of single or multiple nutrients cannot gain entry to the body compartment or gain entry in excessive or unbalanced amounts. This can be the result of SI disease pathology, or dysbiosis of the normal SI microbial flora, either of which may alter the structure and permeability of the SI epithelial barrier. Probiotics are live microorganisms that, when ingested in adequate amounts, confer a health benefit to the host [1]. Probiotics are generally regulated as either dietary supplements or medical foods (e.g., Visbiome®, a multi-strain probiotic formerly called VSL#3) in the USA. Most probiotics currently available are lactic acid bacteria (LAB), and *Bifidobacterium* spp., and certain yeasts such as *Saccharomyces boulardii* [2], which have a long history of safe use and are legally "generally recognized as safe" (GRAS). The effects of probiotics, and moreover studies on the microbial composition of the SI flora, have been hampered by limitations on access, as SI epithelial biopsies or aspirates via naso-ileal catheters are invasive procedures. Therefore, most microbiome analyses are conducted on stool which is influenced heavily by colonic microbiota. Nonetheless, recent animal studies and human clinical trials suggest that probiotics can have a restorative effect on gut integrity and nutrient uptake via promoting eubiosis in the SI.

This article is part of the Topical Collection on *Small Intestine*

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Small Intestine

Characteristics

The SI, comprised of the duodenum, jejunum, and ileum, is the major site of macro- and micronutrient digestion and absorption. Digestion is accomplished through a mixture of digestive enzymes (pancreatic lipases, SI brush-border disaccharidases, etc.) as well as other secretions (i.e., bile salts and bicarbonate) active in digestive processes. Plicae circularis, transverse folds of submucosa covered by mucosa

predominantly in the duodenum and proximal jejunum, are covered by villi and microvilli to increase the surface area of the SI and optimize nutrient absorption.

The gastrointestinal (GI) tract is lined with the mucosal epithelium to act as a direct barrier between the environment and host. This intestinal barrier contains various components, such as commensal gut microbiota, mucus layer, antimicrobial peptides (AMPs), and junctional complexes (i.e., tight junctions (TJs), adherens junctions, and desmosomes). These dynamic components work together to maintain normal barrier integrity [3]. Permeability of the barrier can be increased through direct damage to the epithelial mucosa or changes to other components via dysbiosis, diet, or inflammation [4].

The duodenum, jejunum, and ileum experience unique luminal environmental factors that can change each section's microbial abundance. On average, the duodenum and jejunum contain up to 10^3–10^4 bacteria/mL followed by an increase to 10^8 bacteria/mL in the ileum. While the concentration of bacteria increases along the GI tract, in comparison, it is much lower than the typical concentration of the colon (10^{11} bacteria/mL) [5].

Modulators of SI Microbiota

SI microbiota abundance and composition can be modulated by oxygen availability, pH, transit time, AMPs, and intake of probiotics. Oxygen availability, on average, decreases from proximal to distal SI and microniches in the lumen create environments for aerobes and strict anaerobes alike to survive and metabolize.

The pH of SI regions and transit time of food content contribute to the changes in microbial density. The median pH of the proximal intestine is 6.7 with an increase to 7.5 in the terminal ileum [6]. Acidic chyme passes from the stomach into the duodenum and stimulates the hormone secretin, which in turn stimulates the liver and pancreas to release bicarbonate into the duodenum, thus increasing pH and allowing for optimal function of digestive enzymes. The basic pH within the terminal ileum may create a more favorable environment for SI microbiota to begin degradation of complex carbohydrates, ferment simple carbohydrates, and utilize energy. These processes are time-limited as food content is only in the SI for 2–5 h [7]. Unabsorbed nutrients and fiber enter the colon where they reside for 12–24 h [7], allowing for fermentation of complex carbohydrates and production of short chain fatty acids (SCFAs).

The microbial environment of the SI can also be shaped by AMPs that function as a part of the innate immune system and thus appear in greater amounts during inflammatory events triggered by dysbiosis or disease. In mice, reduced concentrations of cathelicidin-related AMP resulted in increased duodenal inflammation and permeability allowing for translocation of bacteria to the spleen, liver, and pancreas [8]. Normal AMP secretion is important for maintaining a eubiotic environment and healthy SI barrier.

Consumption of probiotics also impacts the microbial environment of the SI. Probiotics can provide 10^8–10^{12} colony forming units per day [9]. Assuming 10% survival of 10^{10} ingested probiotic bacteria, the relative abundance of ingested bacteria compared with resident bacteria in the SI can be 0.01- to 1-fold compared with 0.0001- to 0.00001-fold in the colon [9]. This suggests that probiotics may have a greater impact in the SI than in the colon. Ingested probiotic bacteria support the SI microbiota through cross-feeding and reducing or inhibiting pathogens [9]. However, ingested probiotics are considered transients, as they do not become integral members of the core microbiota [10]. Difficulties in sampling the human SI microbiota limit our knowledge of the relationship between additional factors (i.e., dietary components, medications, lifestyle) and the SI microbiota. Table 1 lists some of the factors affecting the composition of the SI microbiota.

SI Microbiota Products

Phyla present in the SI (Firmicutes, Bacteroidetes, and Actinobacteria) have the ability to produce B-vitamins through biosynthesis pathways [11•, 12]. It has been estimated that up to 60% of microbes can produce each of the B-vitamins [11•] used by either the human host or other microbiota. A eubiotic microbiome also produces butyrate through fermentation which helps maintains the SI epithelial barrier integrity, promotes villus development, and dampens excessive inflammation [13–17]. Thus, changes to the SI microbiota composition that directly or indirectly decrease butyrate

Table 1 Factors that may affect and influence the microbial profile of the small intestine

Nutrient availability
Macronutrients
Micronutrients
Fermentation substrates
Antimicrobial peptides
Gut motility
Gastric acid, bile, pancreatic enzymes
Temperature
pH
Resident microbes
Inflammation
Antibiotics or medications
Topography
Mucosal health
Small intestine surgery
Disease state
Probiotics (food and dietary supplements)

producers can impact nutrient absorption and gut health of individuals.

Probiotics

Probiotics are of growing interest due to their modulatory effects on markers of human health. Several meta-analyses demonstrate probiotic benefits in modulating symptoms of various GI diseases, such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and *Clostridium difficile* infections, as well as mood disorders such as depression [18–21]. Additional meta-analyses have been conducted in order to determine if probiotic efficacy is strain- and disease-dependent [22]. There is strong evidence that probiotics are strain-specific in mitigating symptoms associated with individual diseases [23]. When evaluating a probiotic supplement, the specific strain, the disease, and the individual should be considered, as should results of well-designed human clinical trials.

International consensus states that probiotics exert their benefits to the host by (i) interference with pathogenic bacteria by competing for nutrients and adhesion sites, (ii) improvement of the barrier function of the epithelial lining, (iii) immunomodulation, and (iv) influence on other organs of the body through the immune system and neurotransmitter production [1]. Probiotics also increase the production of vital compounds necessary for eubiosis and human health, including SCFAs such as butyrate [24]. Finally, these beneficial microbes also ensure an intestinal environment where optimal nutrient absorption may occur [24].

Probiotics and the Small Intestine

As stated previously, studying the microbiome of the SI is difficult, as invasive procedures are generally required. Traditional stool samples collected from humans will identify species indigenous to the colon, plus transient bacteria from food, or the oral, esophageal, or SI microbiota. Current understanding of how probiotics influence the SI is largely derived from animal models. Recently, one group administered three probiotic strains, *Lactobacillus salivarius* G1-1, *L. reuteri* G8-5, and *L. reuteri* G22-2, and an antibiotic control to groups of piglets and examined the ileal mucosa proteomics [25]. Piglets consuming the lactobacillus strains had expression of 32, 40, and 27 proteins that are associated with maintaining the integrity of cell structure, cell stability, and pathogen defenses, respectively. Another group administered *L. rhamnosus* GG (LGG) prophylactically to pigs prior to a *Salmonella Infantis* challenge [26••]. LGG taken prophylactically downregulated the *S. Infantis*-induced increase of $CD4^+$ $IFN\gamma^+$ T cells in Peyer's patches and IL-7Rα expression in the jejunum, demonstrating a probiotic benefit exerted through the immune system and the complexity of the interactions occurring. Probiotics, specifically LAB, may protect the SI by increasing microbial diversity, upregulating protein expression involved in homeostasis, and maintaining immune system integrity.

SI rotavirus diarrhea and antibiotic-associated diarrhea are both routinely treated with probiotics, particularly LGG [27]. LGG is further able to mechanically protect the mucosa and inhibit the attachment of certain pathogenic bacteria [27].

Probiotics and Intestinal Permeability

A healthy intestinal barrier is selectively permeable, permitting passage of essential nutrients and water while restricting absorption of toxins and pathogens [28]. The TJ, the main regulator of paracelluar permeability, is comprised of transmembrane proteins (claudins), scaffolding proteins (zonulin), and regulatory proteins [29]. Chronic disruption to the gut barrier over time may contribute to GI and autoimmune diseases by stimulating an overactive inflammatory response and may decrease nutrient bioavailability [30]. Probiotics are a potential approach to help maintain the intestinal barrier along the entire intestinal tract. In addition to contributing to butyrate production by a healthy, balanced microbiome, probiotics are effective in strengthening TJ proteins and preserving mucosal integrity, and as such also promote optimal nutrient absorption [31].

One study recently examined the effects of *L. reuteri* LR1 on intestinal permeability of the SI in weaned pigs [32•]. One hundred forty-four weaned pigs were divided into three intervention groups consisting of a control diet or the same diet plus *L. reuteri* LR1 or antibiotic treatments for 14 days. When compared with pigs on the antibiotic or control diet, those in the probiotic group had increased villus height to crypt depth ratio and increased TJ protein expression in the mucosa of the jejunum and ileum. Another study administered *L. reuteri* ZJ617 and LGG by oral gavage to mice who were injected with lipopolysaccharide (LPS) to induce barrier dysfunction [33]. LPS administration caused a reduction in abundance of occludin and claudin-3, and both probiotic strains were able to attenuate the reduction. In another investigation, high doses of kanamycin were administered to disturb the intestinal barrier in mice and study the effects of LAB on Peyer's patch cells in the ileum [34]. When compared with mice on the control diet, those receiving LAB had increased expression of zonulin-1 and occludin in the ileal tissue. They also had higher levels of serum immunoglobulin A in Peyer's patch cells, reflecting that Peyer's patches were protected from kanamycin by LAB. Based on evidence for LAB maintaining barrier integrity observed in recent animal studies, similar studies should be undertaken in humans [35, 36]. The yeast *S. boulardii* has been shown to be very effective in treatment of clinical disorders with associated intestinal barrier disruption in both animal studies and human clinical trials [2].

Diseases of the Small Intestine and Nutritional Impacts

Small Intestinal Bacterial Overgrowth

Small intestinal bacterial overgrowth (SIBO) has been implicated as a cause of chronic diarrhea and nutrient malabsorption. Estimates of prevalence of SIBO vary based on the testing methods used to diagnose this disease, and many testing methods, such as hydrogen breath tests, are imprecise [37]. Functional GI symptoms of SIBO do not correlate with quantitative SI bacterial culture profiles, but do correlate with dysbiosis as defined by 16S rRNA sequencing of the SI microbiota [38, 39]. Nutrient malabsorption can range from mild to profound, resulting in weight loss and vitamin deficiency–associated neuropathies [37].

Persons with SIBO have between 10^5 and 10^6 bacteria/mL luminal content, 2 to 3 $\log_{10}$/mL higher than healthy individuals [40]. The bacterial species contaminating the SI in SIBO patients are commonly identified oropharyngeal and colonic flora, including microaerophilic bacteria such as *Streptococcus*, *Escherichia coli*, *Staphylococcus*, *Micrococcus*, *Klebsiella*, and *Proteus*, and anaerobic bacteria such as *Lactobacillus*, *Bacteroides*, *Clostridium*, *Veillonella*, *Fusobacterium*, and *Peptostreptococcus* [41]. The most commonly prescribed treatment for SIBO is the broad-spectrum antibiotic rifaximin; however, this medication only has a 66.7% cure rate [42, 43]. Rifaximin also has the potential to disturb commensal bacterial populations and induce antibiotic-associated diarrhea and *C. difficile* infections. Therefore, other therapeutic options such as probiotics to mitigate bacterial overgrowth and repopulate the SI with beneficial bacteria are of interest [44]. Efficacy studies of probiotics in treating SIBO have yielded discordant results [45]. A meta-analysis and systematic review concluded that probiotics were effective at SIBO decontamination and symptom relief, but were ineffective in SIBO prevention [45]. It should be noted that consumption of certain probiotic strains (e.g., *Bifidobacterium infantis*) may increase methane gas levels suggestive of SIBO in response to the lactulose breath test [46].

Irritable Bowel Syndrome

Discussions about IBS are made difficult by proposed disparate symptomatic subtypes and etiologies [47]. IBS is characterized by abdominal pain associated with altered bowel habits in the form of constipation, diarrhea, or both [48]. SIBO may or may not be present concurrently with IBS. Evidence of a role for SI dysbiosis in IBS is strong, but treatment with probiotics, although yielding promising results, is hampered by not knowing the effectiveness of the specific probiotic strain(s), dose, or necessary duration of treatment [49]. However, treatment with probiotic *Bacillus* spp. spores reportedly improved measurements of the quality of life of IBS patients, probably owing to modification of the gut microbiota [50]. As with SIBO, altered SI permeability is present in IBS, but only in the diarrhea-predominant subtype [51]. It can safely be concluded that along with permeability changes and associated diarrhea with decreased transit time, nutrient uptake is negatively affected.

Crohn's Disease

The inflammatory state of Crohn's disease (CD) can affect SI permeability and reduce nutrient absorption, putting individuals at an increased risk of malnutrition. Immunohistochemical analyses of duodenal biopsies from active CD showed destruction and dilation of TJs compared with controls. This damage coincided with shortening of the microvilli and increased intervilli distance [52]. Damage to the mucosa, through villi blunting, can limit absorptive capabilities of the SI through loss of brush-border enzymes [53] and reduced surface area.

Nutrient absorption is highly dependent on the action of transporters at the apical surface of epithelial cell membranes. Transcriptional analysis of the ileal mucosa of CD individuals revealed alterations in the expression of 62 solute carrier transporters (SLC) and zinc transporters. The majority of the SLC transporters were downregulated, including those important for amino acid transport. Low levels of transporters and metallothioneins important for the absorption, storage, and export into circulation of zinc was also seen in CD mucosa [54••]. The low expression of the transporters limits the amount of nutrients that enter the enterocyte, ultimately lowering the concentrations in circulation. When the relationship between microbial species and transporter expression was examined by incubating human ileal mucosa with *L. casei*, only partial recovery of SLC transporter expression was shown [54••]. Although humans are more variable in both ileal microbial composition and physiological processes than an in vitro study, the study provides evidence for a role of intestinal microbiota in CD.

In individuals who have treatment-naïve CD, the SI microbiota is dysbiotic due to a decrease in butyrate producers [55–58]. The genera *Bacteroides* and *Clostridiales* are absent in CD individuals [56•, 58] and negative associations for CD severity were found with lower abundance of the genera: *Bacteroides*, *Faecalibacterium*, *Roseburia*, *Blautia*, *Ruminococcus*, and *Coprococcus* [56•], butyrate producers responsive to probiotic support. Decreased butyrate production could contribute to compromised SI barrier integrity, thus affecting nutrient absorption and increasing inflammation and disease severity. In an in vitro model of CD microbiota, the addition of six butyrate producing bacteria to monolayers of intestinal epithelium cells exposed to CD fecal-derived cultures improved epithelial barrier integrity as measured by transepithelial electrical resistance (TEER) and apparent permeability of the paracellular marker Lucifer yellow [59].

TEER is a widely accepted quantitative technique to measure the integrity of tight junctions in cell culture models of the intestinal epithelium. Colonization capacity in mucus- and lumen-associated CD microbiota was highest when a mixture of butyrate producers was used [59] suggesting that one species alone may not be able to establish within resident microbiota.

A systematic review of 9 studies found little benefit of probiotics in persons with CD [60]. However, many of these studies focused on the use of *Bifidobacterium* and *Lactobacillus*. Interestingly, these genera have been found to be at higher concentrations in gut mucosal biopsies in active CD patients [55]. Future probiotic studies should evaluate the use of combination butyrate producers not currently available as dietary supplement probiotics [61].

Nutrition and the Small Intestine Microbiome

Persons with SI diseases that demonstrate malabsorption exhibit distinctive microbiota profiles. A pilot study compared duodenal fluid between children recently diagnosed with IBD to healthy controls [62]. Children with IBD had decreases in total microbial counts of *Collinsella*, *Lactobacillus* and *Bacillus*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes.* This information is of value as patients with IBD are at risk of malabsorption with micronutrient deficiencies, perhaps related to the dysbiosis observed in the SI [63]. The SI microbiome also dictates how a host will digest and absorb dietary compounds, such as lipids, which may lead to over or under nutrition. One study provided a high-fat diet to germ-free (GF) mice and controls housed under standard conditions and found that GF mice had impaired lipid digestion compared with controls, suggesting an important role for microbiota in digestion/absorption [64].

Other studies have also demonstrated that dietary patterns influence the SI microbiota, which in turn may affect health status. In one study, pigs were fed a diet with a standard concentration of protein (16%), a diet that was moderately reduced in protein diet (13%), or a diet low in protein (10%) for 28 days [65]. Ileal samples were obtained at slaughter for microbiota analysis. Ileal bacteria richness decreased as dietary protein was decreased to 10%; however, TJ protein expression was highest in those receiving the 13% diet. This suggests that a diet that moderately restricted protein intake may actually promote a healthier pattern of ileal bacterial community. Future research may define optimal bacterial communities to promote health and divulge the dietary patterns to build those communities. Other dietary compounds such as sugar substitutes, food additives, and emulsifiers are associated with low microbial diversity and increased inflammation in the SI [66, 67]. Diets rich in polyphenols, fiber, and whole plant sources, however, are associated with increased biodiversity in fecal samples and the upregulation of commensal bacteria in the microbiome [68]. Unfortunately, typical western diets containing processed foods and acellular nutrients are more bioavailable in the SI [69]. This then provides ample nutrients that fuel adverse changes in microbiota composition of the SI [70]. When discussing nutrition and the SI, an interdependent relationship is observed. Beneficial microbes may allow for the optimal absorption and utilization of dietary nutrients while a proper diet will increase microbial diversity and abundances of valuable species to promote efficient nutrient absorption.

Conclusion

The SI is the major site of nutrient absorption, and disruption of normal SI function and integrity can lead to nutritional deficiencies and malnutrition [71, 72]. SI microbiota may be a significant contributor in the development of SI diseases such as SIBO, IBS, and CD, and overt or covert malnutrition. Beneficial microbes produce valuable compounds, such as butyrate, which support proper SI structure and physiology needed to optimally harness nutrients. Therefore, the composition of the SI microbiota plays a substantial role in predicting and influencing human health [73].

Probiotics could help maintain a eubiotic environment, correct dysbiosis, and ameliorate nutrient malabsorption issues within the SI. However, the use of probiotics is complicated as characterization of the SI microbiota in healthy adults, and clinical trials to evaluate probiotic efficacy are relatively scarce, likely due to the invasive sampling procedures required to examine SI contents. Future studies could utilize ex vivo models of SI such as enteroids, 3-dimensional organoids derived from SI stem cells to study probiotic interactions with the SI epithelium [74], and explore new technologies such as robotic sampling capsules to harvest SI microbiota. Non-invasive access to SI luminal contents will improve understanding of SI microbiota's profile in health and disease and enable more precise studies on the efficacy of probiotics in the SI. Research is also needed to determine efficacy of specific probiotic strains or combinations of strains in therapeutic applications in the SI.

Until new SI lumen sampling methods are available and verified, the use of biomarkers may be the key to determining the status of the SI microbiota, the SI epithelial barrier integrity, and even nutritional status. For example, blood serum analyses for zonulin and bacterial components such as lipopolysaccharide can allude to TJ integrity, and specific cytokines and immunoglobulins can reflect overall immune status of the SI [75]. Additionally, measuring sugar output in the urine is a promising technique that allows researchers to compare site-specific intestinal permeability during various interventions [76].

Eubiosis in the SI creates a homeostatic environment is which the digestive, immune, and endocrine systems collaborate to ensure proper nutrient absorption and utilization. Nutritional status of persons with SI dysbiosis or SI disease should be taken into consideration and probiotics considered as a therapeutic option.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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