4

Gut Microbiota

Gail A.M. Cresci, PhD, RD, LD, CNSC, and Kristin M. Izzo, MS, RD, LD, CNSC

CONTENTS

Necrotizing Enterocolitis

Inflammatory Bowel Disease

Clinical Evidence with Prebiotic Provision

Prebiotic Therapy and Enteral Nutrition

References

Objectives

Present an overview of the complexity of the human gut microbiota and the factors that influence its development and balance.

Provide working definitions for probiotic and prebiotic.

Review available evidence for the application of probiotics and prebiotics in clinical practice relating to common nutrition therapy issues.

Test Your Knowledge Questions

1. Which of the following statements best describes the human gut microbiota?

A. The human gut microbiota is established by the age of 3 years and few factors influence it.
B. Trillions of bacteria currently comprise the human gut microbiota.
C. The human gut microbiota is highly dependent on the host for survival but provides little benefit to the host.
D. The human gut microbiota is not influenced by the mode of infant delivery.

2. Which of the following statements best describes a probiotic?

A. A probiotic is a live organism used to make yogurt.
B. A probiotic is a "live nonpathogenic organism (bacteria or yeast) which when administered in adequate amounts confers a health benefit on the host."
C. Probiotics are on the Generally Recognized As Safe (GRAS) list and therefore can be safely provided to all humans receiving nutrition support therapy.
D. The mechanisms of probiotics are well known, making probiotic therapy a great addition to nutrition support therapy.

3. Which of the following statements best describes a prebiotic?

A. All fibers are considered prebiotics.
B. Prebiotics are synthetic compounds.
C. Prebiotics are dietary polysaccharides that escape digestion by the host enzymes, are fermented by the gut microbiota, and influence the gut microbiota pattern in a beneficial manner.

D. All prebiotics are fermented to yield the same short-chain fatty acids (SCFAs).

Test Your Knowledge Answers

1) The correct answer is B. Humans are sterile in utero and are first colonized depending on the mode of delivery. The gut microbiota development increases as the diet increases in complexity. The colonization of gut microbiota is influenced primarily by undigested polysaccharides that ferment to produce SCFAs. The SCFAs serve many biological functions in the host's body. The gut microbiota also has many other positive benefits to the host.
2) The correct answer is B. While the starter cultures used to make yogurt, Lactobacillus bulgaricus and Streptococcus thermophilus, are on the GRAS list and therefore considered safe for human consumption, they are not probiotics. There are specific criteria that a bacterium must meet to be considered a probiotic. The mechanisms of action of probiotics are still being researched, but what is known is that each strain of bacteria behaves differently, particularly in different environments. Therefore, a general application of probiotic therapy for nutrition support patients is not recommended.
3) The correct answer is C. Prebiotics are naturally occurring substances, predominantly dietary polysaccharides, which escape digestion by the host enzymes. They reach the distal intestine where they are fermented by the host gut microbiota to yield SCFAs and beneficially influence the gut microbiota pattern. Prebiotics are commonly thought of as dietary fiber; however, not all fibers are fermentable, and not all fermentable polysaccharides yield the same molar ratios of SCFAs.

Overview of the Gut Microbiota

The gut microbiome, a phrase coined by the molecular biologist Joshua Lederberg in 2001, is defined as the totality of microorganisms and their collective genetic material present in the intestine. The gut microbiota comprises all the microorganisms—commensal, symbiotic, and pathogenic—residing in the intestine. While mucosal surfaces of humans are colonized with bacteria, most bacteria reside within the gastrointestinal (GI) tract.1 An estimated 800 different bacterial species with more than 7000 strains and approximately 100 trillion total bacteria are present in the human gut; these bacteria are predominantly anaerobic bacteria based on the sequencing of the highly conserved 16S region of ribosomal ribonucleic acid RNA (rRNA).2 rRNA, the RNA component of the ribosome, provides a mechanism for decoding messenger RNA into amino acids and interacts with transfer RNAs during translation. Bacterial ribosomes contain about 60% rRNA and 40% protein by weight and consist of 2 subunits of unequal size; the larger subunit has a sedimentation coefficient of 50S whereas the sedimentation coefficient of the smaller one is 30S. The 30S subunit contains 1 molecule of 16S rRNA and 21 proteins. Multiple sequences of 16S rRNA can exist within a single bacterium.3 The density of bacteria is 108 colony-forming units (CFUs) per mL in the distal small intestine and 1011 to 1012 CFU/mL in the colon. In comparison, low densities of bacteria (102 CFU per mL luminal contents) are located in the proximal

intestinal tract (ie, stomach, duodenum, and jejunum). This lower density is due to the acidic gastric environment, presence of bile, and peristalsis.2

The human host has a mutualistic relationship with its gut microbiota. The gut microbiota plays a role in energy balance,4–7 metabolism of xenobiotics,8,9 resistance to pathogen colonization,10,11 and the maturation of the intestinal and immune systems.12,13 A xenobiotic is a chemical that is found in an organism but is not normally produced or expected to be present within it.

Recent studies have challenged the traditional understanding that the human GI tract is sterile until the birthing process. Although further study is needed, there is increasing evidence that microorganisms inhabit the placenta, amniotic fluid, and umbilical cord; thus, by swallowing amniotic fluid in utero, the fetus begins to colonize the developing GI tract.14 Colonization continues with the birthing process, with the gut microbiota of newborns closely resembling the microbes encountered during birth. The colonization of neonates differs among those born vaginally compared to those born via cesarean section (C-section). The primary flora of vaginally delivered babies includes Lactobacillus, Prevotella, or Sneathia spp. In contrast to vaginally delivered neonates, babies delivered by C-section harbor bacterial communities that resemble those of the skin, comprising Staphylococcus, Corynebacterium, and Propionibacterium spp.15,16 Analysis of these communities finds that they do not resemble the mother's flora more closely than those of other women's flora, indicating that the baby is in contact with microbes from humans other than the mother.17 Because babies delivered by C-section do not receive that first vaginal maternal inoculum, their development of the GI microbiota is affected,18,19 which may explain the increased vulnerability these babies have to certain pathogens.17 Compared to vaginally delivered babies, C-section–delivered babies have higher incidences of atopic diseases,20 allergies and asthma,21,22 and skin infection with methicillin-resistant Staphylococcus aureus (among hospitals in the United States, 64% to 82% of readmissions of babies diagnosed with MRSA were delivered by C-section).23

While the newborn's gut microbiota is comprised of relatively few species and lineages, diversity rapidly increases within the first few years of life.24,25 The exact reason for the increase in diversity is unknown, but it is hypothesized to be from (1) an enlarging intestinal tract that provides a larger habitat for more bacteria, (2) increased exposure to environmental bacteria, and/or (3) dietary changes.17 Breastfeeding has been shown to enrich vaginally acquired, lactic acid–producing bacteria in the baby's intestine.26 Breast milk contains bifidobacteria, with Bifidobacterium longum the most widely found species followed by B. animalis, B. bifidum, and B. catenulatum; all are Bifidobacterium species that may promote healthy microbiota development.27

Advancing the diet from simple solid foods (eg, rice cereal) to one including more complex plant polysaccharides (eg, peas) changes the relative ratio of bacterial phyla in the GI tract from an unstable community dominated by Actinobacteria and Proteobacteria to a stable mixture dominated by Firmicutes and Bacteroidetes, like that of an adult-like microbiota.24 Analysis of the gut microbiota from African and Italian children found that African children had an increased abundance and diversity of Bacteroidetes.28 This raises the possibility that the gut microbiota is adapted by local diets, and that the

gut microbiota can adapt via genomic changes to changes in the host's diet; this adaption can be achieved by incorporating genes from bacteria in the environment.29

The composition of the gut microbiota among human family members tends to be more similar than the composition in unrelated individuals,30 and the same bacterial strains can be shared among family members.31–33 The gut microbiota pattern varies geographically with the host,28 as well as with age—the gut microbiota in older adults varies from that found in younger adults.34–36 Organisms have defined life spans and play different roles throughout their life span. Nature has created biological clocks to monitor the position of an individual in the aging spectrum; these clocks are phylogenetically deep and well conserved.37 One type of clock includes bifunctional genes, which are genes beneficial to young individuals but costly in aging individuals.38 Subsets of endogenous microbiota may have bifunctional genes,17 and there is evidence for selection of microbes that coevolve with their host.39

These familial, geographic, and age-related variations in microbiota could have an impact on drug and nutrient metabolism, making it even more important to perform drug and nutrition trials in age- and location-matched populations to avoid the effects of differences in the metabolic capabilities of the human gut microbiota.34 The variations also could influence outcomes after probiotic supplements are taken. Species that are beneficial to the young age group (eg, Lactobacillus and Bifidobacterium) might be harmful to older adults. These issues are important topics for study. In addition to diet, age, and geographical residence of the host, the gut microbiota can be altered by other factors such as medications and both physiological and psychological stress.39

Gut Dysbiosis

Attempts have been made to define a "core microbiome," as it is speculated that deviation from a core structure may lead to pathological states.30 Because levels of interindividual variation are high, a conserved core microbiome structure is difficult to identify; however, community function may be conserved within this variation.40 Both human and animal studies have increased our understanding of the delicate symbiosis between the gut microbiota and the host. Disruption of this mutual tolerance, or gut dysbiosis, may have a significant role in modulating host physiology. Environmental influences such as diet, exercise, and early life exposures can significantly affect the composition of the microbiota, potentially creating dysbiosis, which is associated with several autoimmune and metabolic diseases, including inflammatory bowel disease (IBD), type 2 diabetes, irritable bowel syndrome, metabolic syndrome, obesity, and cancer. The exact mechanisms of gut dysbiosis are uncertain, but, in most cases, they are linked with inflammation and alterations in immune responses and metabolism.14

Gut Microbiota in Select Patient Populations

Obesity

The gut microbiota has genetic and metabolic attributes that may play a role in human obesity. By analyzing the bacterial 16S rRNA gene, scientists have gained a better understanding of variations in the gut microbiota of lean vs obese animals and humans. The gut microbiota of obese individuals exhibits less taxonomic richness and potentially diminished metabolic capacity than the microbiota of lean

individuals. In a mouse model colonized with the human gut microbiota, the mice became obese when their diet was manipulated from a high-carbohydrate diet to a high-fat “Western” diet; this change in diet also provided a rapid switch of the microbe community.41 When this modified gut microbiota was transferred to germ-free mice known to be lean, they adapted the obese phenotype. In humans, analysis of the gut microbiota of lean vs obese adults has yielded conflicting results. The first study analyzing stool samples of obese vs matched lean individuals showed a shift in bacterial phyla (lower Bacteroidetes and more Firmicutes).42 When the human subjects lost weight by following a fat-restricted or carbohydrate-restricted low-calorie diet, the ratio of Bacteroidetes to Firmicutes approached a lean-type profile after 52 weeks.42 However, later work by other investigators indicates that the ratio of Bacteroidetes to Firmicutes is not altered in obese humans,43 that more Bacteroidetes were detected in obese humans compared to normal-weight humans,44 and that surgical manipulation via gastric bypass surgery decreased Firmicutes.44 Discrepancies in the results could be related to variations in the methodologies used for bacterial analysis among the studies.45 A large study identifying 3 robust clusters, referred to as enterotypes, identified in people from different countries and continents did not reveal any correlation between body mass index (BMI) and Firmicutes/Bacteroidetes ratio.46 A set of 3 gene modules have been strongly correlated with the host’s BMI; 2 are adenosine triphosphatase complexes, and this finding supports a conceptual link between gut microbiota capacity for energy harvest and obesity in a host.46 Evidence indicates that the microbiota of obese individuals exhibits less taxonomic richness and potentially diminished metabolic capacity than the microbiota of lean individuals.30,47,48

Inflammatory Bowel Disease

The underlying etiology of IBD remains unknown. A central hypothesis is that commensal gut bacteria generate an autoimmune response contributing to mucosal inflammation. Compared with the microbiota in the general population, the microbiota of individuals with IBD varies widely in number and diversity. In areas of inflammation, notably fewer Firmicutes, Bacteroidetes, and Clostridium are detected and the proportion of Proteobacteria and Actinobacteria bacterial counts increases.49 This finding is concerning because the diminished phyla contribute to the production of SCFAs and, in particular, butyrate, which is important for intestinal health.

Curative therapies for IBD are yet to be found. Current therapies for induction and maintenance of remission include immunosuppressive drugs (eg, corticosteroids, methotrexate) and anti-inflammatory drugs (eg, antitumor necrosis factor).50 Because approximately 30% to 50% of patients with IBD are nonresponders to these medications and require surgical intervention, alternative therapies are needed—and the concept of shifting the gut microbiota back to a “healthy” balance seems like a promising option. In a variety of cohort studies, fecal microbiota transplant (FMT) has provided therapeutic effects that alter the composition of gut microbiota and enhance the diversity and proliferation of beneficial bacteria. The shift in microbiota composition post-FMT provides ulcerative colitis (UC) patients with the opportunity to more adequately defend against pathogenic bacteria and subsequent inflammatory complications.51

The standard surgical treatment to help manage refractory UC, UC with dysplasia, or familial adenomatous polyposis (FAP), is reconstructive proctocolectomy with the creation of an ileal-pouch anal anastomosis (IPAA). This formed pouch takes place of the removed rectum and often has associated complications, including increased stool, urgent stool, rectal bleeding, incontinence, or fever. Additionally, endoscopic findings show that postsurgical patients are at risk for mucosal edema, granulation, decreased vasculature, and ulceration. Approximately 50% of individuals with UC who have undergone IPAA creation will experience acute pouchitis; in contrast, only a few patients who underwent the same procedure to address underlying FAP develop pouchitis. Of note, the risk of acute pouchitis seems to be related to the individual's genetic predisposition and altered gut microbiota of the conduit eliciting an immune response rather than the reconstruction itself.52

Small Intestinal Bacterial Overgrowth

Arising from a variety of underlying disease states, notably short-gut syndrome, small intestinal bacterial overgrowth (SIBO) is characterized by an increase in bacteria in the small intestine, which usually has a very low bacterial load. Clinical symptoms of SIBO include chronic diarrhea, foamy or frothy stool, nausea, vomiting, foul-smelling stool, bloating, and constipation. While definitive therapies for SIBO are not available, typical treatment includes broad-spectrum antibiotics, which can further alter the composition of the gut microbiota. In pilot studies, probiotic supplementation along with antibiotic regimens has shown promise, with reduction in clinical symptoms of chronic diarrhea, abdominal distention, pain, bloating, and belching. In one study, patients with SIBO supplemented with probiotics post–antibiotic therapy showed greater reduction in posttreatment hydrogen breath tests, suggesting more effective eradication of bacterial overgrowth, when compared with controls.53

Critical Illness

Critically ill patients are at risk for developing infections, and most patients receive antibiotics, which contribute to gut dysbiosis. Additionally, the therapies used to treat critically ill patients and the metabolic conditions these patients have can negatively alter the gut microbiota. Critical illness can cause redistribution of splanchnic perfusion, resulting in intestinal hypoperfusion, gut ischemia, and mucosal injury. Disruption of the intestinal barrier, an altered gut immune system, and dysfunctional metabolic activities of commensal bacteria make the intestine a major contributor to systemic infections.54 Negative shifts in gut microbiota have been demonstrated within 6 hours of a metabolic insult, prior to provision of antibiotics, with alterations not returning to baseline patterns during the hospital stay.55 A recent (2016) prospective study including several institutions demonstrates significant differences in the microbiome, including the gut microbiota, in critically ill patients compared with the microbiome of a healthy population, as demonstrated by analysis of fecal, oral, and skin sample collections.56 This rapidly occurring dysbiosis increased in magnitude throughout the length of stay in the intensive care unit (ICU).

Probiotics

The literal definition derived from Greek for probiotic is "for life." The Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) have defined probiotics as "live

non-pathogenic organisms (bacteria or yeast) which when administered in adequate amounts confer a health benefit on the host."57 Currently, the US regulatory framework does not include a definition of probiotics, nor has the federal government established any rules or regulations specific to probiotics. This lack of regulation is concerning because a product can be called a "probiotic" without having substantiated evidence demonstrating that it meets the minimum criteria set forth in guidelines issued by the FAO/WHO.57

Not all bacteria or live cultures found in foods and/or supplements can be characterized as probiotics. According to FAO/WHO, an organism must meet very strict criteria to be considered a probiotic (Table 4-1).57 Ideally, the live, viable organism with strain identification must be able to survive the proximal GI tract environment and reach the distal intestine. There it must function and/or adhere to the gut epithelial tissue, have a beneficial impact on the gut microbiota pattern, and be scientifically proven to exert a positive health effect. Lastly, the organism should be safe for human consumption.57 Even though conventional yogurt is a food rich in protein and calcium, the 2 starter cultures used to produce it, Streptococcus thermophilus and Lactobacillus bulgaricus, are not probiotics because they do not meet all probiotic criteria. Additionally, while other yogurt cultures may possibly be probiotic, scientific evidence must indicate that the yogurt product in question has high amounts of the probiotic strains and has demonstrated health benefits. These standards should also be applied to probiotic supplements.

TABLE 4-1 Probiotic Criteria

- Strain identification
- Human origin
- Live microorganism
- Viable
- Safe for human consumption
- Survive proximal gastrointestinal tract
- Reach distal intestine and colon
- Function/adhere to gut epithelial tissue
- Colonize distal gut, affecting microbiome composition
- Scientifically proven health benefits

To appropriately identify a probiotic, one should look at the genus, species, and strain names (eg, Bifidobacterium [genus] lactis [species] Bb-12 [strain]). Most manufacturers do not include this information in their labeling of products such as yogurts or other fermented milk foods and supplements. Often, manufacturers have not conducted research on the specific probiotic strain used in their fermented milk product or supplement; instead, companies typically generalize health claims from the other research sources for the genus and/or species included in the product. This lack of specific

information poses a challenge for the clinician and the consumer regarding whether the product confers a health benefit.

Mechanisms of Action

Elie Metchnikoff, a Russian Nobel Prize winner of the 20th century, was the first to propose beneficial effects of probiotics among aging Eastern Europeans. He hypothesized that proteolytic microbes in the colon produced toxic substances responsible for the aging process and proposed that consumption of fermented milk would coat the colon with Lactobacillus, thus decreasing luminal pH, suppressing proteolytic bacteria, and slowing the aging process.58 Recent advances in the field are beginning to sort out how probiotics exert their beneficial effects. Probiotic strains do not all exert the same mechanisms of action; instead, there is considerable interstrain diversity. Therefore, properties of one probiotic should not be extrapolated to another. One also cannot assume that probiotic actions in vitro reflect mechanisms of action in vivo or that the same probiotic strain exerts the same mechanism of action in different metabolic environments of the host, such as an ambulatory care setting vs an intensive care setting.

Probiotic strains may have multiple activities (Table 4-2) and therefore may be influential at various times.59 Various probiotics exert their predominant action in the lumen, at the mucosal surface, by engagement with the mucosal innate immune response, or by action beyond the gut with stimulation of the acquired immune response.60 For example, the probiotic Lactobacillus salivarius UCC118 provided protective luminal action by producing an antimicrobial, bacteriocin, against L. monocytogenes. The same probiotic was effective in protecting against salmonella infection but by a different mechanism.61 The luminal actions of probiotics can also cause metabolic alterations of commensal resident microbiota. For example, inoculation of germ-free mice with probiotic bacteria resulted in marked changes in gene expression within the resident commensal microbiome.62

TABLE 4-2 Probiotic Proposed Mechanisms of Action

Colonization resistance:

- Competitive exclusion

Intestinal barrier function maintenance:

- Maintain tight junctions
- Reduce macromolecular permeability and bacterial translocation

Enhancement of gut microbiome pattern:

- Enhance ratio of commensal bacteria

Modulation of inflammatory and immunoregulatory signaling:

- NF-κB
- Interleukin-10

- Toll-like receptor(s) innate/adaptive immune modulation

Increased mucin regulatory genes and mucin production:

- Defensin production
- Engagement with dendritic cells
- Immunoglobulin (IgA, IgG, IgM) production

Metabolic effects:

- Nutrient metabolism
- Bacteriocins
- Decrease luminal pH
- Quorum sensing

NF-κB, nuclear factor kappa–light-chain-enhancer of activated B cells.

Some probiotics have been shown to have action at the mucosal surface. These effects include induction of mucins (MUC2 and MUC3),63 with the resultant enhanced mucus layer overlying the gut epithelial lining then serving as an antibacterial shield that prevents the binding of enteric pathogens to mucosal surfaces and increases the clearance of pathogens from the gut.64 Additionally, mucin-producing cells secrete trefoil factors in response to microbial pathogens, which act together with mucins to prevent their binding to mucosal surfaces.65 Certain probiotics may also enhance gut integrity and its barrier function by preventing changes in tight junction proteins (eg, occludins, claudins) and enhancing the tight junction transepithelial electrical resistance.66

Some probiotics express pathogen-associated molecular patterns capable of engaging with the same pattern-recognition receptors used by pathogens; therefore, probiotics have the means to protect the mucosal surface by competitive exclusion.66 Some probiotics have exhibited the ability to stimulate the innate and acquired immune system via induction of regulatory T-cell function.67 The secretion of anti-inflammatory (eg, interleukin-10) and proinflammatory (eg, interleukin-12) cytokines by immune cells can be affected in a strain-specific manner by some probiotic strains.59 Some probiotics have also been able to activate specific gut opioid and cannabinoid receptors, thus having the potential to modulate visceral pain.68

Probiotic Sources

A challenge to clinicians is finding a commercially available probiotic product that has scientific evidence of its benefit to patients. Many probiotic strains reported in the literature are not commercially available products, limiting the clinician's ability to readily translate scientific evidence into clinical practice. Additionally, most manufacturers do not disclose the strain specificity or quantity of viable organisms (CFU) for products, thus further confounding the clinical application. Table 4-3 lists a select number of commercially available probiotic products.

TABLE 4-3 Select Commercially Available Probiotics

Strain(s)	Initial Product	Supplier	Year Introduced
Lactobacillus casein strain Shirota	Yakult	Yakult	1935
Streptococcus thermophiles *Lactobacillus bulgaricus* *Lactobacillus acidophilus* *Bifidus*	Yogurt	Stoneyfield	1973
Bifidobacterium longum BBS36	Bifidus milk	Moringa Milk Products	1977
Bifidobacterium breve strain Yakult	Mil-Mil (Bifiene)	Yakult	1978
Bifidobacterium lactis BB-12	Yogurt	Chr. Hansen	1988
Lactobacillus rhamnosus GG	Gefilus	Valio	1990
Lactobacillus casei DN-114-001 *(Lactobacillus casei Immunitas)*	Actimel (DanActive)	Danone	1994
Lactobacillus johnsonii La-1	LC1	Nestlé	1994
Lactobacillus plantarum 299v	ProViva	Probi	1994
Bifidobacterium animalis DN-173-010 *(Bifidus regularis)*	BIO (Activia)	Danone	1995
Lactobacillus gasseri LG21	LG21	Meiji Milk Products	2000
Bifidobacterium lactis HN-019	Supplement	Danisco	2001
Lactobacillus casei KW2110	Yogurt	Kirin Holdings	2003
Lactobacillus casei F19	Cultura	Arla Foods	2004
Streptococcus thermophilus *Bifidobacterium breve* *Bifidobacterium lactis* *Bifidobacterium infantis* *Lactobacillus acidophilus* *Lactobacillus plantarum* *Lactobacillus para casei* *Lactobacillus helveticus*	VSL#3	Sigma-Tau HealthScience	2000
Escherichia coli Nissle 1917[a]	Mutaflor	Ardeypharm GmbH	1971
Bacillus clausii[a]	Enterogermina	Sanofi Aventis	1999

[a]Not manufactured in the United States.

Prebiotics

The primary food sources for commensal microbiota are undigested polysaccharides and sloughed proteins. A prebiotic is defined as “a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the GI microbiota, that confer benefits upon host well-being and health.”69 An ingredient must meet necessary criteria to be considered a prebiotic (Table 4-4).70 To selectively stimulate the growth and activity of the gut microbiota in a beneficial manner, a prebiotic must be resistant to the host’s gastric acidity and digestive enzymes and must be fermented in the distal intestine (ileum and colon) by the commensal anaerobic gut microbiota.70 Demonstration of prebiotic activity of an ingredient is difficult; therefore, an ingredient may have prebiotic effects but not yet be called a prebiotic because there is insufficient evidence to prove the ingredient meets the necessary criteria.

TABLE 4-4 Prebiotic Characteristics

Three necessary criteria of ingredients:

- Must be resistant to gastric acidity, to hydrolysis by mammalian enzymes, and to GI absorption
- Must be fermented in the GI tract by gut microbiota
- Must be selective in the stimulation of the gut microbiota growth and/or activity that contribute to health and well-being
- Not available to all bacterial species in gut microbiome

Simple, naturally occurring or synthetic sugars:

- Lactobacilli and bifidobacteria are considered indicator organisms:

– Inulin (chicory, leeks, onion, garlic, artichoke, asparagus): DP 10–60

– Inulin-type fructans (oligofructose or fructooligosaccharide): DPmax 10 via partial hydrolysis of inulin or synthetically from monomers

– Transgalactooligosaccharides

– Enzymatic synthesis based on lactose

– Lactulose

DP, degree of polymerization; GI, gastrointestinal.Source: Data are from reference 70.

Prebiotics are simple, naturally occurring or synthetic sugars that vary with their degree of polymerization. A prebiotic is not available to all bacterial species that inhabit GI ecosystems. Lactobacillus and Bifidobacterium are considered indicator organisms because currently known prebiotics stimulate the composition or activity of these organisms, but not necessarily others, in the gut microbiota.

Upon reaching the distal intestine (ileum and colon), prebiotics are fermented by endogenous anaerobic bacteria and SCFAs; gases (carbon dioxide, hydrogen, and methane) are produced; endogenous bacterial cell mass is increased; and endogenous bacterial enzyme activities are altered. This process decreases intraluminal pH, thereby favoring the predominance of beneficial bacteria (eg, bifidobacteria, lactobacilli, and nonpathogenic Escherichia coli) and decreasing Bacteroidaceae.71 As with a high-fiber diet, ingestion of prebiotics (5 to 20 g/d) can produce unwanted symptoms such as flatulence, bloating, abdominal pain, eructation, and borborygmi,72 with a wide individual variation found. The stoichiometry of fermentation differs for carbohydrates of differing chain lengths and monosaccharide composition, degree of polymerization, and branching, with slower fermentation associated with longer chain length. Prebiotics can also affect bowel function, resulting in a laxative effect through stimulation of microbial growth and increased bacterial cell mass, leading to stimulation of peristalsis by increased bowel content.72 However, the effect on bowel function is small, and well-controlled studies are needed to measure an effect. The type of prebiotic influences fecal stool weight with inulin and

fructooligosaccharides (FOS) increasing stool weight by 1.3 to 2 g, and wheat bran, fruits, and vegetables increasing stool weight by 5.4 to 4.7 g.72 Prebiotic inulin-type fructans significantly increase the absorption of both calcium and magnesium in growing animals and in adolescents when added to the diet.73 In pubertal adolescents, a mixed short- and long-degree of polymerization inulin-type fructan product (8 g/d) provided daily for 8 weeks significantly increased calcium absorption and enhanced bone mineralization. These effects may be modulated by genetic factors, including specific vitamin D–receptor gene polymorphisms.73

Prebiotics are fermented by endogenous commensal gut microbiota producing SCFAs, predominantly acetate, propionate, and butyrate.74 Prebiotics do not all yield equal amounts and the same types of SCFAs.74 SCFAs are known to serve several biological functions, including ion transport (eg, sodium, bicarbonate); modulation of intracellular pH and cell volume; provision of a metabolic cellular fuel source; and regulation of cellular proliferation, differentiation, and gene expression.75 Butyrate in particular contributes to differentiation of epithelial cells, enhances water and electrolyte absorption, modulates immune function, exerts anti-inflammatory effects, and possesses tumor-suppressor effects in the colon through its role as an inhibitor of histone deacetylases and as a ligand for butyrate transporters and receptors.76,77

Synbiotics

A synbiotic is a physical combination of prebiotics and probiotics. Often, candidates for probiotic therapy have inadequate dietary intake of prebiotics and/or their commensal microbiome is altered. Therefore, providing a prebiotic or a probiotic alone may not produce a beneficial effect. A synbiotic product may have the advantage of providing substrate (prebiotic) for a potentially beneficial probiotic, which in turn benefits the host (patient).78 Additionally, a synbiotic might maximize the viability and beneficial function of the probiotic before utilization and later as a therapeutic agent in the host GI tract.79

Prebiotic and Probiotic Applications

Potential Candidates for Prebiotic or Probiotic Use

Prebiotics and probiotics may be beneficial for individuals with poor diets, older adults, patients experiencing stress, and those who are taking medications that alter gut microbial balance. Potential health benefits vary depending on the type, timing, and dosing of the probiotic and/or prebiotic.

Type, Timing, and Dosing

When considering probiotic or prebiotic supplementation, the clinician must investigate the optimal strain for the clinical condition of interest and identify supportive research with evidence for justified and cost-effective usage. This research can be challenging because the literature covers a variety of probiotic strains and doses that are not commercially available. Additionally, diverse patient populations, study designs, and small sample sizes have limited the ability of analysts to make evidence-based recommendations. Further confusion arises because of uncertainty about how probiotic strains

behave in various metabolic environments and clinical conditions, the synergy with strain combinations, or when probiotic strains are combined with various prebiotics.80 Nevertheless, the clinician must adhere to the precept that a probiotic, identified by strain, should only be provided to a patient with a clinical condition in which the probiotic strain has research evidence to support its use. As has been noted previously, probiotics are live viable organisms and may behave differently in various metabolic and environmental milieus. Therefore, providing a probiotic strain without careful consideration of the evidence could potentially lead to adverse effects.

It is speculated based on current literature that the optimal dose for probiotic and prebiotic effect, although condition-specific, is approximately 109 to 1012 CFU/d for a probiotic and 5 to 20 g/d for a prebiotic. Dosing schedules vary from 1 or 2 times daily to 3 or 4 times per week. Probiotic colonization is transient; therefore, continued dosing may be necessary to maintain a treatment effect for some conditions (eg, C. difficile remission).59

Safety Issues

Common, safe probiotic species include many bifidobacteria (eg, B. bifidum, B. longum, and B. infantis) as well as many lactic acid bacteria (LAB). Used in foods for many years, LAB are considered harmless. They fall under the Medical Food and Supplement Act and are afforded GRAS status. LAB that grow as adventitious microflora or are added to foods as cultures do not pose a health risk to healthy humans. Several strains of LAB species have been isolated from the human GI tract and administered to humans without reported adverse effects. These include Lactobacillus reuteri, L. plantarum, and L. casei (subspecies rhamnosus). However, although probiotics are normal commensals of mammalian microbiota and are generally thought to be without pathological potential, individual patients may be susceptible to opportunistic infections via normal microbiota. In case reports, adverse effects seen when probiotics were administered have included sepsis (bacteremia, fungemia), bowel ischemia, and mortality, most of which occurred in patients who had underlying medical conditions.81–84 Other potential theoretical risks are deleterious metabolic activities related to altered polysaccharide fermentation, lipid metabolism, and glucose homeostasis; immune deviation or excessive immune stimulation; and microbial resistance.81

Contraindications

Whereas prebiotic use does not seem to be contraindicated except for patients who are hemodynamically unstable, probiotic use in certain patient populations may not be safe. Studies investigating probiotic benefits typically include strict exclusion criteria that are often not considered by practitioners when using a probiotic in clinical practice. In a review of 92 cases of invasive Saccharomyces infections, treatment with antibiotic therapy and existing intravenous catheters were identified as the most frequent predisposing factors.83 Lactobacillus has been isolated in the blood of patients with short-gut syndrome, leukemia, mitral regurgitation, and cardiac surgery.81 Risk factors for developing infectious complications associated with the use of probiotics include the presence of a central venous catheter, immunocompromised status, administration of the probiotic directly into the

small intestine, concomitant administration of broad-spectrum antibiotics in which the probiotic is resistant, cardiac valvular disease, and intestinal barrier compromise.81,84

Restoring the Gut Microbiota: What Is the Evidence?

Many clinical conditions can alter the gut microbiota, making attempts to correct imbalances attractive. Interventions aimed at preventing or correcting gut dysbiosis have been investigated in a variety of clinical conditions, including diarrhea, allergy, IBD, critical illness, and surgery.

Antibiotic-Associated Diarrhea

Antibiotic-associated diarrhea (AAD) is a common adverse effect of antibiotic therapy. With AAD, the pathogenic bacteria in the gut microbiota outnumber the beneficial by 10 to 1.85 Disturbing the normal gut microbiota is believed to predispose the host to pathogenic bacterial colonization.86 Approximately 25% to 30% of cases of AAD in hospitalized patients involve Clostridium difficile.87 C. difficile infection can be debilitating and has a high recurrence rate; it can cause pseudomembranous lesions in the colonic mucosa, causing severe inflammation.85 Fluoroquinolones are the antibiotics most likely to cause AAD/C. difficile–associated disease (CDAD). AAD typically begins 4 to 9 days following antibiotic cessation, but it can occur up to 8 weeks later. Other risk factors for AAD/CDAD include severe illness, advanced age, presence of a nasogastric tube, provision of medications to raise gastric pH, GI surgery or manipulation, immunocom-promised status, and extended hospital stay.85 The most effective treatment of AAD/CDAD is to stop use of the inciting antibiotic. C. difficile identified by a positive stool culture is typically treated with metronidazole initially, with correction of fluid and electrolyte imbalance as needed. The treatment is repeated in patients that fail initial treatment; if they relapse again, they are then treated with oral vancomycin. Relapses may occur within 1 to 2 weeks of discontinuing antibiotic treatment. Relapse is believed to be caused by the survival of C. difficile spores that later germinate producing vegetative forms and critical illness.85

In theory, providing probiotics in patients receiving antibiotics to prevent gut dysbiosis and recolonize the gut colony with commensal bacteria makes sense. In the past few decades, several strain-specific organisms have been researched in the prevention and treatment of AAD/CDAD. These include Bifidobacterium, Lactobacillus paracasei subspecies, including L. GG (LGG), L. casei, L. plantarum 299v, Enterococcus faecium, Saccharomyces boulardii, S. cerevisiae, Bacillus clausii, Clostridium butyricum, and L. acidophilus.85 Details on individual studies are presented elsewhere.85 Probiotic therapy recommendations to prevent and/or treat AAD/CDAD are elusive because of the heterogeneity in choices of probiotics, lack of criteria for diagnosing CDAD, variation in the definition of diarrhea, and variations in probiotic dosing, timing, and duration. However, in a meta-analysis of 2810 patients, probiotic provision along with antibiotic therapy showed promise toward alleviating AAD, but not CDAD.88 Saccharomyces boulardii, LGG, and probiotic mixtures seemed to exhibit the best protection from AAD. S. boulardii significantly reduced C. difficile recurrence in patients who were also receiving high-dose oral vancomycin (Practice Scenario 4-1).85,88,89

Practice Scenario 4-1

Question: When would the use of a probiotic be appropriate for a tube-fed patient with new onset of diarrhea?

Scenario: A 72-year-old woman residing in a long-term care facility for 3 weeks after being discharged from an acute care facility following a stroke is receiving enteral nutrition via a percutaneous endoscopic gastrostomy and develops diarrhea. Her tube feeding is a fiber-containing standard isotonic formula. She has been receiving her goal volume for 2 weeks with good tolerance until 2 days ago, when she was noted to have excessive, watery, foul-smelling diarrhea. In review of her medical record, she finished a 10-day course of antibiotics 5 days prior to this onset of diarrhea.

Intervention: The nutrition support clinician is consulted to change the enteral formula because it is suspected of causing this patient to have diarrhea. The clinician reviews the medical record and notes the previously mentioned information. She rules out osmotic diarrhea from feeds or medications. Noting that the onset of diarrhea was within 7 days of stopping a fluoroquinolone antibiotic, the clinician suspects antibiotic-associated diarrhea/Clostridium difficile–associated diarrhea (AAD/CDAD). She recommends that a stool culture be obtained and analyzed for C. difficile. It comes back positive for C. difficile. Metronidazole is ordered for 10 days at 500 mg 3 times per day. The clinician also recommends providing Saccharomyces boulardii throughout the course of metronidazole and for 14 days after its cessation.

Answer: Often, enteral formula is blamed as causing diarrhea in patients. In this case, the patient was receiving an isotonic formula in the stomach and had been tolerating it for several weeks. Medications provided for enterally fed stable patients are often liquid but may contain sorbitol or sugar, which can induce an osmotic load in the gut and cause gastrointestinal intolerance and diarrhea, but this patient was not receiving these types of medications. Antibiotics, particularly fluoroquinolones, are most likely to start the downward spiral of AAD/CDAD.85 The onset of this type of diarrhea typically will occur 4 to 9 days following the cessation of antibiotics.85 This patient's course of antibiotics was completed 7 days before the onset of diarrhea, so the clinician reasonably suspected that she might be experiencing AAD/CDAD and had a stool culture ordered to confirm the diagnosis.

Rationale: This patient was positive for C. difficile in her stool sample. In addition to treatment with metronidazole, certain probiotics have been shown to be effective in decreasing the duration and symptoms of AAD/CDAD as well as reducing the recurrence of CDAD, with S. boulardii having the best outcomes against C. difficile.88,89 Providing the probiotic during the course of antibiotics and then for 14 more days helps to maintain and re-establish commensal microbiota that are altered negatively during the antibiotic therapy.

FMT is the process by which a homogenized stool sample from a healthy donor is administered into the GI tract of an individual. Some refer to FMT as the "optimal probiotic." FMT has been shown to eliminate recurrent and refractory C. difficile infection and re-establish a healthy colony in certain patients.90 Because of FMT's cure rate for refractory C. difficile infection, researchers are now exploring its potential to treat other causes of gut dysbiosis.

Critical Illness

The modulating effects of prebiotic, probiotic, and synbiotic supplementation on infections and infectious complications have been extensively evaluated in animal studies and clinical trials. Like the studies of AAD, the clinical trials focused on critical illness are heterogeneous; they vary not only in patient populations and underlying diseases but also regarding probiotic strains and combinations, dosage, duration, delivery method, supplemental feeding, concomitant therapies, and outcome measurements. Multiple systematic reviews and meta-analyses have been performed to determine probiotic, prebiotic, and synbiotic efficacy in critically ill patients. Because of the study heterogeneity and varying primary study outcomes, the results of these reviews vary according to which studies were included in the analysis.

For example, a systematic review of adult patients in the ICU found no evidence to support use of prebiotics, probiotics, or synbiotics in adult patients in the ICU.91 In this analysis, randomized controlled trials including 8 studies of approximately 1000 total patients were reviewed for evidence that these supplements can affect rates of nosocomial infection, length of ICU stay, hospital mortality, or incidence of pneumonia. The supplements used in the studies varied and included Lactobacillus plantarum 299v (2 patients), synbiotic (6 patients), Synbiotic 2000 (lactobacilli, pediacocci, Leuconostoc plus prebiotics; 2 patients), and L. plantarum plus fiber (1 patient). Other trials used different combinations and doses of both prebiotics and probiotics. Patient populations included liver transplant (1 patient), abdominal surgery (2 patients), and general ICU (5 patients).

A recent (2016) systematic review and meta-analysis evaluated 30 randomized controlled trials (N = 2972 patients) that analyzed clinical outcomes associated with providing a probiotic or a synbiotic.92 The primary outcome was overall number of new infections, and secondary outcomes included mortality, ICU and hospital length of stay, and diarrhea. Subgroup analyses were performed to determine a role of other key factors, such as probiotic specificity and patient mortality risk, on the effect of probiotics on outcomes. Although probiotics did not affect mortality, length of stay, or diarrhea, probiotic use was associated with a reduction in infections (relative risk [RR] = 0.80; 95% confidence interval [CI], 0.68–0.95; P = 0.009). Additionally, the incidence of ventilator-associated pneumonia was reduced (RR = 0.74; 95% CI, 0.61–0.90; P = 0.002).

The 2016 guidelines for nutrition support in critically ill patients by the American Society for Parenteral and Enteral Nutrition (ASPEN) and the Society of Critical Care Medicine (SCCM) state that the use of identified probiotics species and strains seems to be safe in general ICU patients, but they should be used only for the select medical and surgical patient populations for which randomized controlled trials have documented safety and outcome benefit (eg, ventilator-associated pneumonia, liver transplantation).93 Currently, no recommendation is available for the routine use of probiotics across the general population of ICU patients.93 For patients with severe acute pancreatitis receiving early enteral feeding, a probiotic is suggested; however, no specific dose or strain is known.93

Necrotizing Enterocolitis

Probiotic supplementation in preterm infants has significantly reduced risk for stage II or higher necrotizing enterocolitis (NEC) and all-cause mortality; decreased time to achieve full feeds; reduced hospital length of stay, feeding intolerance, duration of indirect hyperbilirubinemia; and increased weight and growth velocity.94 One meta-analysis found that the combination of Lactobacillus acidophilus and Bifidobacterium bifidum was associated with a lower incidence of NEC in preterm infants compared with other strains used individually or as combinations of strains.94 However, the evidence for optimal individual strains or combination of strains and dosage of probiotics is not consistent enough to make specific recommendations about which types of probiotic supplementation provide a benefit to neonates at risk for GI complications. There were no noted adverse events related to outcomes associated with probiotic supplementation in the reviewed trials.94 Randomized controlled studies are now investigating the use of synbiotics to reduce the incidence and severity of NEC. Further studies to indicate specific probiotic strains and doses for prevention of NEC are warranted.

Inflammatory Bowel Disease

Ulcerative Colitis and Pouchitis

Currently, investigations aim to identify the effectiveness of individual probiotic strains and doses in patients with IBD. Derikx and colleagues49 determined that Escherichia coli Nissle 1917 and VSL#3 were used in most trials to test the efficacy of probiotics to influence time to remission and recurrence rates in patients with active UC were (Table 4-3). The review concluded that E. coli Nissle 1917 (5–50 × 109 CFU/d) was equally as effective as traditional therapies; however, results do not indicate E. coli Nissle 1917 as a viable stand-alone therapy. In UC patients, VSL#3 (3.6–9 × 1012 CFU/d) was effective in reducing time to remission and recurrence of remission.49 Other probiotic trials include Lactobacillus and Bifidobacterium strains. Endoscopic improvement was seen in one trial utilizing B. breve (3 × 109 CFU/d) in conjunction with the prebiotic galactooligosaccharide (5.5 g/d).49 Although results of these studies have shown promise, larger randomized placebo-controlled studies are needed.49

Crohn's Disease

In theory, prebiotics and probiotics should influence the composition and function of gut microbiota and provide a therapeutic effect in patients with Crohn's disease; however, evidence for the effectiveness of prebiotics and probiotics in patients with this condition remains inconclusive. The use of inulin has shown some potential for prebiotic effects in small trials, but larger, well-powered studies have not exhibited significant benefits for the use of prebiotics in active Crohn's disease. To date, probiotics and prebiotics that have been investigated have not shown significant benefits in patients regarding induction or maintenance of remission or in preventing recurrence of active Crohn's disease.95

Pouchitis

Probiotic supplementation in patients with an IPAA creation has aimed to modify the conduit microflora in an attempt to treat the active pouchitis, prevent recurrence, or, even more importantly, prevent the

initial onset (see Practice Scenario 4-2). Administration of prophylactic probiotic supplementation (VSL#3) in postoperative restorative proctocolectomy patients has been evaluated for effectiveness in preventing initial onset of acute pouchitis.52 The rate of pouchitis-free survival for at least 1 year was 90% in patients who received VSL#3 directly after surgery compared with 60% in control groups ($P < 0.05$). Compared with placebos, VSL#3 was effective in reducing patient's time to remission in episodes of active acute pouchitis and in delaying remission when taken in rotation with antibiotic regimes.52

Practice Scenario 4-2

Question: How would you manage a patient with ulcerative colitis and a newly created ileal pouch who is receiving parenteral nutrition (PN)?

Scenario: A 33-year-old man is admitted for abdominal pain and bloody stools negative for Clostridium difficile. His past medical history includes underlying ulcerative colitis since he was 8 years old, which has required multiple bowel resections and corticosteroids use over the course of several years. Prior to this admission, the patient had oily and foamy chronic diarrhea with a distinct foul odor and was diagnosed with small intestinal bacterial overgrowth (SIBO) and treated with antibiotic cycling. Following a thorough evaluation, the patient undergoes a restorative proctocolectomy with creation of an ileal-pouch anal anastomosis (IPAA) with a loop ileostomy and a remaining small bowel length of greater than 200 cm. The surgeon orders a consult for PN because of the IPAA creation and high ostomy output. The plan is to discharge the patient home with PN for 3 months and then schedule stomal closure and resume use of the ileal pouch in place of the previous rectum. Given the patient's complicated medical history and the initiation of PN, what are the most appropriate interventions for the clinician to recommend in this situation?

Intervention: Begin daily VSL#3 immediately after surgery. Provide oral rehydration solution and a diet high in starch and low in simple sugars once the oral diet is advanced.

Answer: Typically, corticosteroids and/or antibiotics are indicated for treatment of inflammation in this situation, whereas measures for prevention of future onset of inflammation are not necessarily implemented. PN is indicated because of the patient's high outputs, malabsorption, and poor nutrition status, but PN will further compromise the diversity of the gut microbiota. The patient's history of SIBO, altered gut immune function, and new ileal pouch make it appropriate to begin VSL#3 daily immediately after surgery, with a plan to continue the same probiotic daily at home. Additionally, the patient should receive education regarding oral rehydration solutions to prevent dehydration as well as dietary choices to prevent increased ostomy output while optimizing nutrition status when weaned from PN.

Rationale: The patient is at risk for onset of acute pouchitis and SIBO. The effectiveness of VSL#3 in preventing the onset of acute pouchitis in this patient population has been demonstrated. The patient's past and current medical and nutrition status warrant VSL#3 administration immediately after surgery and on discharge as a primary intervention to prevent the onset of acute pouchitis and preserve gut

integrity. The benefits of this daily regimen outweigh the costs of potential complications and readmission.

Clinical Evidence with Prebiotic Provision

Multiple clinical issues complicate attempts to confirm the efficacy of prebiotic use in clinical practice. The gut microbiome is complex, and multiple physiological processes may play a role in treatment effect. These processes include changes in pH, microbe-microbe interactions, host-microbe interactions, antibiotic activities, and metabolic activities. Knowledge of an individual's presupplemented microbiota pattern and prebiotic dietary consumption is necessary to detect a change with supplementation. In people who normally consume a high-fiber diet, studies noted little effect with additional (prebiotic) supplementation.71,74,75 Because data on the state of the gut microbiota and prebiotic intake before supplementation are challenging to collect, most study is in vitro. For example, batch culture studies use fecal slurries incubated with various polysaccharides for defined time periods after which an output reading is done to determine which bacteria genus is most affected.70 Other models include continuous culture systems inoculated with fecal slurries to resemble the proximal colon. The best in vivo model uses germ-free rodents inoculated with various prebiotics and/or probiotics to evaluate pattern changes in the gut microbiota. In summary, challenges with human studies include variance in the host's prior diet composition with prebiotics and identifying whether a treatment effect was related to the prebiotic or changes in host-microbe interactions, metabolic milieu, or microbe-microbe interactions.70

Prebiotic Therapy and Enteral Nutrition

Diarrhea is a common occurrence in patients receiving enteral nutrition (EN) and can be attributed to many factors, including metabolic stress, hyperosmolar formulas or medications, antibiotics, enteropathogenic colonization, and variable colonic responses to enteral feeding. EN is associated with alterations in the gut microbiota, which can lead to decreased production of SCFAs. SCFAs are involved with water and electrolyte absorption.74

The data on the use of prebiotics to prevent diarrhea during EN are limited. Available studies report fiber-containing formulas with additional but unspecified quantities of FOS and a compound with uncertain prebiotic characteristics provide a positive effect on diarrhea.96 A 2015 meta-analysis of 8 studies did not find an effect of prebiotics—including soy polysaccharide, partially hydrolyzed guar gum psyllium, oat, soy fiber, FOS, inulin, banana flakes, and galactomannan—on diarrhea incidence.96 However, a review of trials testing guar gum or partially hydrolyzed guar gum noted a decrease in the incidence of diarrhea in patients receiving EN.97 Overall, results in that review found that fiber had a positive effect on the incidence of diarrhea in patients receiving EN (odds ratio [OR] = 0.47; 95% CI, 0.29–0.77; P = 0.02), particularly in stable patients (OR = 0.31; 95% CI, 0.19–0.51; P <0.01), but not in critically ill patients (OR = 0.89; 95% CI, 0.41–1.92; P = 0.77). Despite its manipulative effect on bifidobacteria concentrations and SCFA in healthy humans, prebiotic supplementation in EN does not consistently decrease the incidence of diarrhea.

Despite the difficulty in assessing true prebiotic effects in humans, the ASPEN/SCCM 2016 guidelines for nutrition support in critically ill patients suggest that a fermentable soluble fiber additive (eg, FOS,

inulin) be considered for routine use in all hemodynamically stable medical ICU and surgical ICU patients receiving a standard enteral formulation. The guidelines suggest 10 to 20 g of a fermentable soluble fiber supplement be given in divided doses over 24 hours as adjunctive therapy if there is evidence of diarrhea.