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The Role of Probiotics as Effective Therapies for Dysbiosis and GI Dysfunctions

As most are now aware, the human GI tract is host to countless microbes (some estimate 100 trillion bacteria alone) that have a powerful impact on human health. This impact extends well beyond the gut lumen, and has been implicated in nearly every facet of human physiology and metabolism.[1,2,3] In fact, the gut microbiome is now commonly viewed by many as a semi-autonomous symbiotic organ or organ-like system within the GI tract. Currently, our knowledge of the commensal gut microbiota is heavily weighted toward bacterial species, though there is a growing base of knowledge on GI-resident viruses, bacteriophages (viruses that infect bacteria), fungi, and protozoa. Recent technological advances that allow for the recovery, amplification and sequencing of genetic material from the gut have given us exponentially more information than the plating/culturing technologies of the past, allowing for the identification of more than 1,300 different bacterial species† in humans worldwide.[4,5]

However, while our knowledge of the microbiome within the human gut has greatly expanded in just the past few years, there is much we still do not know about this complex ecosystem, especially as it pertains to modifying its structure and metabolic functions to favor a healthy host phenotype. This monograph summarizes several of the clinically relevant aspects of gut microbial balance, focusing on evidence-based strategies to help correct microbial dysbiosis.

Dysbiosis: Microbiome Out of Balance

Most integrative and functional medicine clinicians are familiar with the term gastrointestinal dysbiosis. Essentially, it describes any significant imbalance in the gut microbial ecosystem, especially one that leads to a negative host response. This includes either an overgrowth or depletion of some particular commensal species, family or phylum of bacteria, or a geographic dislocation of one or more species (i.e., colon bacteria colonizing the small intestine). While an infection by a pathogenic microbe such as *Salmonella* is not usually called "dysbiosis," the opportunistic overgrowth of bacteria like *Clostridium difficile* or a yeast like *Candida albicans* is often directly related to an infection or an antibiotic-induced alteration in the gut microbiota (i.e., dysbiosis-induced). Ironically, with the advanced technologies now available to help the clinician analyze specific changes in the gut microbiota, the term "dysbiosis" has almost become too generic for the research setting, and clinicians should be aware that more specific terms might be used to define specific microbiome-host dysfunctions. Still, the notion that a disturbance in the gut microbial ecosystem —dysbiosis— may be a major trigger in a wide range of gastrointestinal and systemic disorders is an important factor often missed by clinicians uninformed by these recent discoveries.

Basic Dietary Principles to Benefit the Microbiome

It is not surprising that the basic dietary principles that promote overall health and reduce the risk for most chronic diseases are also those that promote a healthy gut microbial community.

- Diversity is key; the diet should contain a wide range of foods, especially those derived from plants with different phytochemicals.
- Dietary fiber, resistant starch, and complex carbohydrates should be emphasized.
- Eat food as fresh and unprocessed as possible (safely).
- Eating seasonally (using local foods), may help diversify gut microbiota.
- Individuals should avoid foods they suspect will trigger GI discomfort, allergic reactions or cause noticeable changes in bowel transit time.
- Limit access to foods (i.e., factory-farmed meats) that contain antibiotics.

† There is some debate about the number of species identified within the global human microbiome, based on the definition of a species and the techniques used to identify genetic differences. This number continues to expand as better genetic tools become available and larger populations are sampled.

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Re-Establishing a Healthy Microbiome

One of the main goals for any clinician should be to help all patients, especially those with GI-related dysfunction, maintain a healthy gut microbiome. Within the 4R model taught to many functional medicine clinicians, this has been defined as "re-inoculate," though we think the goal to "re-establish" a healthy microbiome is a more comprehensive way to understand this idea.† Certainly, adding microorganisms (i.e., inoculation) in the form of probiotics or fecal transplantation can be a major therapeutic strategy to help maintain a healthy microbiome, but there are many fundamental strategies that do not involve adding live organisms.

Diet and the Microbiome

An individual's diet (historical and current) is likely the single greatest influence on the gut microbiome, as it serves as both a source of inoculation of microbes and provides the nutrients upon which the resident commensal organisms feed. For example, research looking at diverse dietary patterns has revealed significant differences in the gut microbiota from vegetarians compared to meat-eaters, Western urban children vs. rural African children, and a range of elderly subjects consuming different dietary patterns over time.[6,7] Not surprisingly, healthy dietary patterns like the Mediterranean diet have been shown to be associated with a more diverse and healthy gut microbiota, compared to standard unhealthy Western dietary patterns.[8]

To investigate the adaptability of the microbiota to changes in dietary patterns, researchers at Harvard University analyzed the fecal microbiota of individuals when shifted between an exclusively plant-based diet to an exclusively animal-based diet.[9] They found the microbiota pattern was noticeably shifted shortly after changing diets in a similar and predictable manner in multiple subjects (N = 11). This suggests the microbiota within the gut can adapt to changing nutrient availability and can do so quite rapidly (within days). Clinicians should be aware of this adaptability when they are using dietary interventions while also performing stool microbiota analysis to understand a patient's health. Since some patients delay taking their stool test for weeks after receiving a sample kit, they may have changed (or improved) their diet based on the advice of their clinician long enough to alter the microbiota prior to sampling. Therefore, the analysis may not accurately reflect the "baseline" microbiota.

There are several components of the diet that appear to have the greatest influence on the species diversity and abundance of the gut microbiota (at least as measured by fecal metagenomics).[10] Most of the population research has focused on macronutrient content (especially the diversity and complexity of the carbohydrate components), and phytonutrient diversity, though individuals consuming foods that radically alter bowel transit time or that cause a major inflammatory response will also experience an altered gut microbiota.

† This model of GI healing is defined as the "4R Model" in the 2010 edition of the *Textbook of Functional Medicine*, published by the Institute for Functional Medicine.

Carbohydrates: Overall, carbohydrates are the principal energy source for a majority of the gut microbiota.[11] Individuals who consume a more diverse diet, including high amounts of dietary fiber and complex carbohydrates, typically have a more diverse (and healthy) gut microbiota. An analysis of the data collected by the American Gut Project found that the self-reported number of unique plant species consumed by individuals was more predictive of microbial diversity compared to individuals self-identifying as vegan or omnivore.[12] This relationship is perhaps not surprising given the diverse array of fiber compounds (e.g., pectins, cellulose, hemicelluloses, gums, fructans, etc.) found in various plants. Fiber is a heterogeneous category of dietary compounds that vary greatly in their molecular structures (e.g., polymer chain length, branching, etc.); this heterogeneity in molecular structure gives rise to fiber's diverse properties (e.g., solubility, fermentability, digestibility, etc.) and consequent physiological effects.[13,14,15] The human host has a limited enzymatic capacity to hydrolyze fiber's chemical linkages; however, commensal bacteria within the gut microbiota have a wide variety of enzymes (e.g., glycoside hydrolases, glycosyltransferases, polysaccharide lyases, carbohydrate esterases, etc.) able to use different linkages within fiber molecules as substrates for metabolism and proliferation.[15] Therefore, consuming a diet diverse in plant species leads to an increase in the heterogeneity of fiber compounds available for the commensal gut microbiome to hydrolyze as substrates for growth, in turn supporting a more diverse gut microbiome.

Prebiotics: Certain fibers are termed prebiotics for their ability to stimulate commensal bacterial growth via fermentation.[11] Good food sources of prebiotic fiber include onions, garlic, Jerusalem artichokes, bananas, and chicory root.[11] More specifically, some of the most common prebiotic fibers include inulin, galacto-oligosaccharides (GOS), fructooligosaccharides (FOS), oligofructose, and chicory fiber. Many of these prebiotic fibers are available as dietary supplements allowing them to be consumed in higher amounts than can be achieved through dietary intake alone. Breast milk is a source of prebiotics (i.e., human milk oligosaccharides) which support the proliferation of *Bifidobacteria* in the infant gut.[16]

Prebiotics have been studied for their ability to affect the composition of the gut microbiota. Before the advent of advanced metagenomic analysis of fecal samples, prebiotics were studied for their ability to support the growth of a limited subset of microbes (typically *Lactobacilli* and *Bifidobacteria*); however, more recent technology suggests that prebiotics serve as substrates for other commensal genera and species as well.[17,18] Understanding the effect of prebiotics on the gut microbiota is complex given the vast heterogeneity of chemical structures within the prebiotic category, which gives rise to different effects on the microbiota composition for different prebiotics. Differences in inter-individual response to prebiotic supplementation (responder vs. non-responder) is

also reported between subjects as host-related factors affect response to prebiotic supplementation (e.g., host genetics, baseline microbiota composition, etc.) as well as the dose of prebiotic administered.[15,‡]

Mechanistically, fermentation of prebiotic fibers may affect the gut microbiota composition in several ways. First, depending on the chemical structure, the prebiotic may be a direct substrate for a commensal organism leading to the commensal's proliferation. In the process, several important short-chain fatty acids (SCFAs) such as acetate, propionate and butyrate are produced. These, as well as other organic acids produced by fermentation (e.g., lactate, succinate, etc.) influence the colonic pH, inhibiting the growth of pathogenic bacteria and thus affecting the gut microbial composition. Another indirect mechanism by which prebiotic supplementation may affect the gut microbiota composition is through a phenomenon called community cross feeding; whereby, commensal organisms that cannot directly ferment the administered prebiotic fiber are able to metabolize metabolic end products from other commensals that are able to directly metabolize the administered prebiotic, thus indirectly affecting the community structure.[15] While it is well known that butyrate serves as the principal source of metabolic energy for the colonocytes, the SCFAs produced through fermentation also influence GI epithelial cell integrity, immune function and serve as important signaling molecules for critical metabolic functions throughout the body (e.g., lipid metabolism, glucose homeostasis, appetite regulation).[19,20] Clinical trials using various prebiotic fibers range in dose from around 5 grams to over 25 grams per day. Though the details of these studies are beyond the scope of this overview, wide-ranging health effects have been studied including changes to the gut microbiota composition, metabolic (e.g., blood glucose regulation, lipids, weight loss, etc.), immune, neurological, and gastrointestinal outcomes (e.g., colon transit and IBS).[11]

Products combining prebiotic fibers with probiotic organisms are called "synbiotics." Since the probiotic must remain inert prior to ingestion, there is no particular benefit to delivering both a prebiotic and a probiotic in the same product, with the exception of convenience. If manufacturers are not careful to ensure fibers mixed with probiotics maintain a low water activity, this addition may inadvertently reduce the viability/shelf life of a probiotic by introducing moisture during the manufacturing process.

Proteins: Dietary protein is also very important with respect to the gut microbiota, as it provides the major source of nitrogen for these organisms. However, while fermentation of amino acids is also an important byproduct of colonic bacterial metabolism (e.g., isobutyrate and isovalerate), the availability of certain undigested peptides and amino acids can result in byproducts known as putrefactive short-chain fatty acids that are thought to play a role in inflammatory bowel diseases and colorectal cancer.[21,22] This has spurred the debate around the role of diets skewed toward animal protein and the risk for colorectal cancer, mediated by changes in the microbiota and their metabolites.[23] While there is still much we do not know about these relationships, it appears that the potential negative effects that occur when excessive fermentation of amino acids occurs as a result of a depletion of available fermentable carbohydrates (e.g., resistant starches), further suggests that eating excessive animal protein in the context of low dietary fiber may actually be the underlying culprit.[24]

Fats: The effect of dietary fat intake (amount and type) on changes in the microbiota have been studied, mostly in the context of high-fat Western (or experimental) diets. Generally, these diets show a similar shift in the microbiota as other studies of Western diets (characterized as low in both food diversity and dietary fiber, and high in saturated fat and animal protein). Curiously, high-fat intake is associated with increased circulating levels of bacteria-derived lipopolysaccharides (LPS), presumably from an alteration in gut permeability to LPS.[25] While more research is needed, there is some evidence to suggest this phenomenon is higher for saturated and omega-6 fatty acid intake, while omega-3 fatty acid intake may limit these negative outcomes.[26]

Another way fat intake can modify the gut microbiota is via bile acid availability. When fat intake is increased, so is the amount of bile that avoids enterohepatic recycling and enters the colon, where these bile acids can be metabolized into secondary bile acids by colonic bacteria.[27] The implications of this mechanism may be far-reaching, as recent research has shown that secondary bile acids are absorbed from the colon and have a number of metabolic signaling effects that impact health and disease in a range of tissues (e.g., glycemic control, obesity, hepatic dysfunction, etc.).[28,29] Changes in the gut microbiota are now known to influence the types and amounts of these secondary bile acids, mediating some of the negative consequences related to dysbiosis.

Phytonutrients: A healthy diet should include a wide range of fresh fruits, vegetables, herbs and spices. Not only will this type of diet provide a large amount of dietary fiber to benefit a healthy gut microbiota, it will also provide a diverse array of bioactive plant compounds generally referred to as phytonutrients. These include carotenoids, glucosinolates, and a large family of compounds called polyphenols, many of which have been studied for their health-related benefits following dietary intake (or via supplementation). Since many of these compounds are known to have poor gastrointestinal absorption, their higher concentrations throughout the GI tract allow for microbial metabolism and signaling.

‡ Clinicians should be aware that high levels of prebiotic fibers are not tolerated by every patient, and those beginning to supplement with prebiotics will likely need to adjust to increasing prebiotic doses. Gas, bloating and related GI discomfort often accompany the increased intake of fermentable fiber and can be modulated by reducing the dose or the addition of probiotics. Clinicians should also be aware that many prebiotic fibers may be contraindicated in certain therapeutic diets (e.g., low FODMAP diet).

Ironically, much of the early studies on polyphenolic compounds (e.g., flavonoids, catechins, anthocyanins, isoflavones, lignans, stilbenoids, curcuminoids, tannins, etc.) were related to their potential antimicrobial activities.[30] In fact, while many are still commonly used as antimicrobial agents, today researchers are realizing that many of these dietary plant compounds have a diverse range of specific influences on the microbiome that can help explain their health-promoting outcomes. In addition to these findings, it is now appreciated that many of these polyphenolic compounds would be of little health benefit to the host without first being metabolized by the gut microbiota, either to produce the "active" compound or to alter the compound to improve its bioavailability. Therefore, the relative efficacy of certain plant phytonutrients (either from dietary intervention or supplementation) may greatly depend upon the metabolic activities expressed by an individual's gut microbiota.[31]

There are numerous well-studied examples of microbial metabolism altering the biological effect of dietary phytonutrients. One of the most well studied is the conversion of the soy isoflavone compounds daidzin and genistin into the more absorbable daidzein and genistein, along with the deconjugation of their liver metabolites and the creation of secondary metabolites with specific estrogen-like effects (e.g., equol).[32] Therefore, the ability for these compounds to generate a biological effect in those consuming soy is partly (perhaps mainly) influenced by the availability of certain bacterial species, which are themselves influenced by the diet and genetics of the host.[33,34] This may explain many of the differences between certain epidemiological disease risk in populations that regularly consume soy from early life and intervention trials using concentrated soy isoflavones in populations with little history of soy consumption.

Another example of this back-and-forth relationship between the microbiota and a well-known phytonutrient is found in the alkaloid berberine, known to possess "antimicrobial" activity and used in a variety of ancient medicinal traditions. More recently, berberine has demonstrated profound metabolic benefits, especially in subjects with metabolic syndrome, type 2 diabetes, hypertension and certain dyslipidemias.[35] Along with its well-described cellular signaling effects, berberine has been shown to alter the host gut microbiota to a metabolically favorable profile—perhaps accounting for some of its clinical efficacy.[36,37] Furthermore, it has now been shown that the efficacy of berberine is itself altered by microbial metabolism through the gastrointestinal conversion of ingested berberine (which is poorly absorbable) to dihydroberberine (which has high bioavailability).[38] Therefore, the microbiota may be the target of and the facilitator for the metabolic effects of this alkaloid.

These are merely two examples of perhaps thousands of different phytonutrients that modify, or are modified by, the microbiota. We should caution, then, that while poor bioavailability of certain phytonutrients is often considered to be a detrimental factor leading many drug and supplement companies to modify these compounds to improve their bioavailability (e.g., liposomal technologies), these changes may inadvertently avoid the necessary microbial interaction activating these compounds, while delivering higher levels of an inactive precursor to the serum. If, as is becoming clearer, the microbiome is one of the most active metabolic "organs" in the body, we may need to readjust our understanding of the biological potential of compounds with limited human bioavailability and consider a plant compound's microbial accessibility as equally important.[39]

Fecal Microbiota Transplantation (FMT)

The transfer of microbiota from one person (or animal) to another through fecal transplantation is being explored as a therapeutic strategy to re-establish a healthy microbiome. In humans, this technique primarily involves diluting donor feces in a manner to be infused into the recipient either through a retention enema, colonoscope or nasogastric/nasoduodenal tube.[40] While FMT has been investigated for a range of microbiome-related conditions, the treatment of *Clostridium difficile* infection is by far the most studied and successful indication. Other indications being explored for FMT include inflammatory bowel diseases, functional bowel disorders like IBS, and obesity/type 2 diabetes, though many others have been proposed.[41,42]

The use of FMT may prove to be an extremely helpful remedy for a number of important conditions for which dysbiosis is a major contributing factor. While limited data exists, microbiota changes initiated by FMT may last for an extended period (> 1 year), though this is highly dependent on host behaviors (e.g., diet) after the FMT.[43] We encourage clinicians to investigate the most up-to-date methods, guidelines and regulations in order to ensure the use of this therapy maximizes the intended benefits to their patients.

The Effects of Antibiotics on the Gut Microbiome

The discovery and use of antibiotics is considered one of the great watershed moments in medical history, helping to turn the tide on a host of microbial pathogens that ravaged the world for centuries. Today, however, we are acutely aware of the immense collateral impact the sustained use of antibiotics has had on the host-microbe relationship. Two of those impacts are the rise of antibiotic-resistant strains of pathogenic organisms and the alteration of the human microbiome, both individually and globally.[44,45]

Broad-spectrum antibiotics have been prescribed for decades in the attempt to protect patients from pathogenic organisms. Commonly, these include ampicillin, ciprofloxacin, tetracyclines, clarithromycin, clindomycin and metronidazole. Since they kill a broad-spectrum of potentially pathogenic bacteria, these antibiotics also kill a wide range of commensal organisms in the gut and elsewhere on or in the patient. How destabilizing a particular antibiotic is for an individual person is still debated.

Some studies suggest the "core" species of an adult commensal microbiota are somewhat resilient to antibiotic therapy, or at least recover to pre-antibiotic levels once therapy is discontinued.[46] However, when the microbiota in healthy subjects is analyzed after treatment with a broad-spectrum antibiotic (via fecal sampling or biopsy) there is an immediate reduction in species diversity, which often does not fully mirror the pre-antibiotic sample for up to 12 months after ceasing the antibiotic.[47,48,49,50] Perhaps even more important than the alterations in species diversity caused by antibiotic therapy is the altered function of the microbiome. Using multi-metagenomic analysis (transcripts, proteins and metabolites from gut microbes), research now shows that the metabolic functions can be quickly and significantly altered in the gut microbes that survive the antibiotic therapy and are altered in ways that can detrimentally affect the host.[51]

Probiotics for the Treatment of Dysbiosis

The use of probiotics to help re-establish a healthy gut microbiome is now a well-accepted practice across the globe. Research into various types, combinations and doses of probiotics for nearly every potential outcome is expanding at a rapid pace, making it difficult for clinicians to assess the validity of the products that are available for their use or recommendation. Within the context of this monograph, probiotics describe microorganisms intentionally consumed for an intended health benefit, usually to help re-establish a healthy gut microbiome. We specifically distinguish the term "probiotics" from both foodborne microbes (e.g., from soil on vegetables) and microbes used in the production of foods (for fermentation, etc.).[52] While both can be important influences on an individual's gut microbiota over time, the overwhelming majority of research on probiotics in human health is based on commercially-prepared products derived from concentrated bacterial strains. In some cases, these probiotics are added to fermented foods in order to deliver added benefits to the consumer (e.g., yogurt, kefir).

It is also important to establish that commercial probiotics should not be confused with commensal (or indigenous) organisms and, therefore, the therapeutic use of probiotics should not be strictly viewed as "re-inoculating" or "re-colonizing" the gut. Instead, probiotics should be viewed as highly domesticated varieties of a very limited subset of the "wild" population of microbes living in the human gut. Depending on the strain, probiotics retain some of their "wild-type" characteristics, allowing them to confer benefit alongside the commensal organisms, though they usually lack other characteristics that would permit them to become permanent residents within the host. Probiotics, then, can benefit the host by direct (but transient) effects, and/or by modifying various gut micro-environments that benefit commensal organisms (e.g., limiting pathogenic or pathobiont organisms, altering pH, signaling immune cell function, etc.).

The definition of a probiotic assumes two other features: they are "live" organisms and they have a defined health benefit. While both of these are prominently part of the agreed-upon definition of a probiotic ("live microorganism that, when administered in adequate amounts, confers a health benefit on the host"), they are not as easily defined, and even more difficult to regulate.[53] Indeed, "live" is a relative term when it comes to probiotics, since most strains are prepared in a manner as to suspend their biological activity (i.e., freeze-drying), many do not survive the transit to the intestines, and some are in the form of spores. Also, there is evidence that some limited benefits can be realized with "dead" probiotics.[54] We will use the term "viability" when discussing both the ability of a probiotic to survive and become "live" in the intestines after consumption and also as a measure of product stability and shelf life (measured in the laboratory).

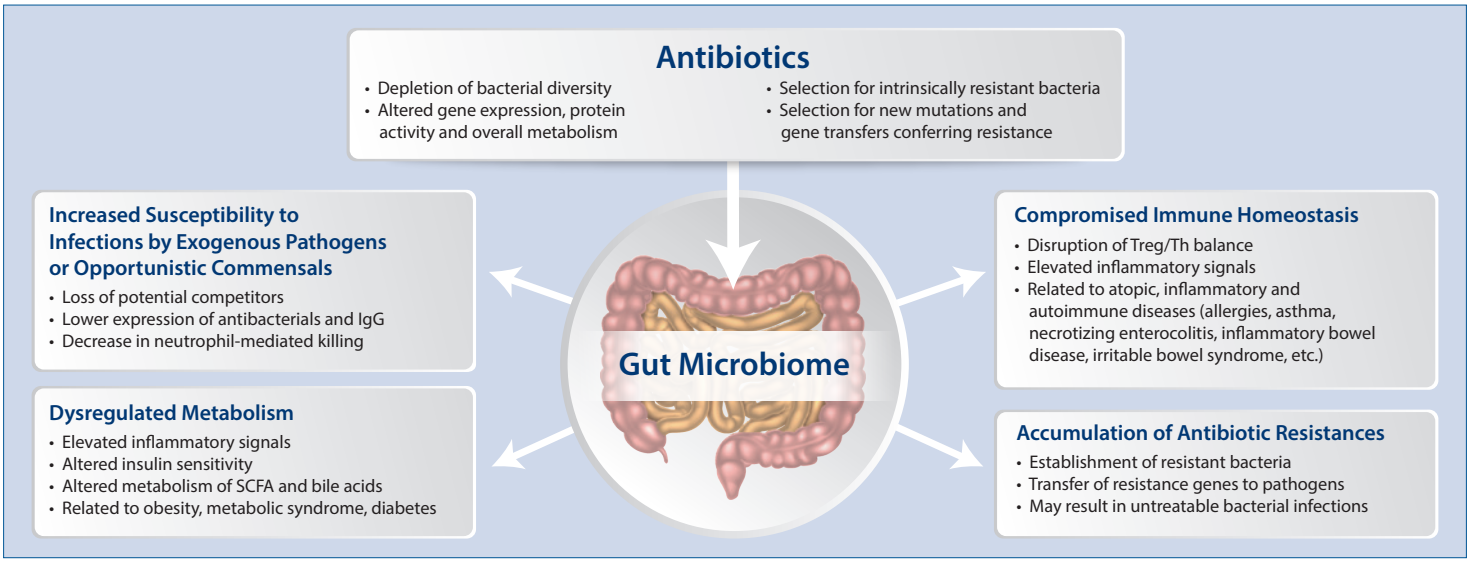

Figure 1: The Devastating Effects of Antibiotics on the Gut Microbiome. This figure shows the basic changes (upper box) in the gut microbiome that occur with the use of broad-spectrum antibiotics; along with the physiological and global ramifications of these changes.

Finally, the level of evidence required before a particular strain can be deemed to "confer a health benefit on the host" is highly variable and unregulated. There are many probiotic strains with dozens of randomized clinical trials, and others with no published data that are still marketed as "probiotics." For instance, many so-called soil-based probiotics claim to contain many different strains of bacteria (usually of unknown quantities), most of which have never been shown to have demonstrable health benefits when administered to humans. There are several types of mechanisms (and outcomes) that can be demonstrated for probiotics, partly dependent on how they are studied. Most probiotic strains share common benefits, while some trials have demonstrated species-specific and even strain-specific benefits.

Common Probiotic Genera

Lactobacillus spp. **Phylum:** Firmicutes **Family:** *Lactobacillaceae*

Lactobacilli are Gram-positive bacilli (rod-shape) that are non-spore-forming, non-motile, facultative anaerobes commonly found in the normal microbiome of the mouth, gastrointestinal tract and female genitourinary tract.[55] Within the GI tract, most *Lactobacilli* are found in the stomach and small intestine where they are metabolically active and have a more unfettered interaction with the immune system. *Lactobacilli* are prototypical lactic acid bacteria (LAB) because lactic acid is their major metabolite of fermentation.[56] Other minor metabolites include acetic and succinic acids.

There are well over 100 different species of *Lactobacilli* that have been isolated and identified from a variety of human, animal, soil, or dairy (hence, lacto) sources. Many of these are used in food preparation/fermentation and as probiotic organisms. Historically, *L. acidophilus* was the most used probiotic species, though many other species and subspecies of this genera have now surpassed *L. acidophilus* in the number of clinical studies performed and biological mechanisms proposed. The *Lactobacillus* genus currently includes more than 240 heterogenous species of bacteria and because of this heterogeneity scientists have recently proposed to separate the *Lactobacillus* genus into new genera based on phylogeny.[57]

Bifidobacterium spp. **Phylum:** Actinobacteria **Family:** *Bifidobacteriaceae*

Bifidobacteria are non-motile, non-spore-forming, Gram-positive, Y-shaped ("bifid"), anaerobic bacteria resident to the gastrointestinal and female genitourinary tract. Within the GI tract, most *Bifidobacteria* are localized in the colon and terminal ileum. *Bifidobacteria* represent up to 25% of the cultivatable fecal bacteria in adults and 80% in infants (Henry Tissier originally isolated *Bifidobacteria* from the feces of breast-fed infants).[58] *Bifidobacteria* are known to ferment a variety of oligosaccharides usually referred to as "prebiotics" and produce a number of short-chain fatty acids, such as butyrate.

Several dozen species of this genera have been isolated and identified, many of which have confirmed probiotic characteristics. Along with *Lactobacilli*, *Bifidobacteria* species are some of the most widely used commercial probiotic strains.

Streptococcus spp. **Phylum:** Firmicutes **Family:** *Streptococcaceae*

Streptococci are spherical, non-motile, Gram-positive bacteria. The term "*Streptococci*" means "twisted berry" and describes the bacteria's characteristic growth pattern in chains or pairs. Like *Lactobacilli*, many members of the genus *Streptococcus* are facultative anaerobes, reside in the upper part of the GI tract, and produce lactic acid as a major metabolic end product (as such, they are termed LAB). Many members of the *Streptococci* genus are pathogenic; however, *Streptococcus salivarius* subsp. *thermophilus* (often called *S. thermophilus*) is generally recognized as safe by the FDA and is an important dairy starter culture (e.g., yogurt).[59] It is also recognized to have probiotic characteristics and is sometimes added to probiotic formulas.

Enterococcus spp. **Phylum:** Firmicutes **Family:** *Enterococcaceae*

Bacteria belonging to the genera *Enterococcus* are Gram-positive, facultative anaerobic, non-spore-forming cocci that occur as single cells, diplococci (pairs), or short chains.[60] *Enterococci* morphology and phenotypic characteristics are similar to *Streptococci*, and until DNA hybridization studies and 16S rRNA sequencing technology became available in the 1980s, *Enterococci* were originally classified as *Streptococci*. Despite their inability to form spores, *Enterococci* are able to survive in diverse environmental conditions, such as extreme temperature ranges, high sodium chloride concentrations and wide pH ranges – a feature not shared among *Streptococci*. *Enterococci* belong to the clade Lactobacillales and as such are lactic-acid-producing bacteria.

At least 37 species of *Enterococci* had been described; however, two are important medically and from a food microbiological perspective: *E. faecalis* and *E. faecium*.[61] These two species are commensal to the GI tract and involved in food fermentation and food spoilage, but also implicated as opportunistic pathogens in many human infections, including urinary tract infections, bacteremia, bacterial endocarditis, diverticulitis, and meningitis. Many *Enterococci* strains have a high level of intrinsic antibiotic resistance and virulence factors, contributing to their pathogenic nature.[62] Both *E. faecium* and *E. faecalis* are used as probiotics in humans and animals. According to Franz et al., the strains used commonly as probiotics, *E. faecium* SF68 (NCIMB10415) and *E. faecalis* Symbioflor 1, have been used for more than 20 years without any reported safety concerns. Still, the safety of currently used *Enterococci*

probiotic strains and potential probiotic strains should be carefully monitored based on the possibility of genetic transfer of virulent traits.[61] In humans, these probiotics have been studied to treat diarrhea, antibiotic-associated diarrhea, and irritable bowel syndrome; to lower cholesterol; and to improve host immunity.[61]

Saccharomyces boulardii **Phylum:** Ascomycota (Fungi) **Family:** *Saccharomycetaceae*

Originally isolated from the litchi fruit (*Litchi chinensis*) in 1923 by Henri Boulard, *Saccharomyces boulardii* differs from other probiotics in that it is a non-pathogenic yeast, not a bacterium.[63] *S. boulardii* is phylogenetically related to, but genetically and metabolically distinct from the baker's yeast used in bread and beer making (*S. cerevisiae*). Although yeast represent less than 0.1% of the resident microbes within the microbiome, yeast cells are about 10 times larger than bacterial cells—a proposed beneficial property of *S. boulardii* is steric hindrance against pathogens in the GI microbiome.[64]

According to Czerucka et al., yeast cells typically reside within the stomach and colon.[64] Additionally, *S. boulardii* is particularly hardy and able to withstand many local stresses within the GI tract, including pH variability, bile salts, enzymes, antibiotics, and organic acids. Therefore, *S. boulardii* has shown great probiotic potential for the treatment and prevention of numerous GI-related conditions such as *Clostridium difficile* infection and antibiotic-associated diarrhea.[65,66] An important consideration when evaluating studies supplementing *S. boulardii* as a probiotic relates to its dosing. Historically, *S. boulardii* has been dosed in mg rather than CFU, though the products used have generally been thought to contain 20 billion CFU/gram of material (i.e., 5 billion CFU/250 mg). Since most of the materials and method sections for these clinical trials do not specify further, it is difficult to confirm the exact dosing used in these trials.

Bacillus spp. **Phylum:** Firmicutes **Family:** *Bacillaceae*

Bacilli are Gram-positive, motile, sporulating, aerobic or facultative anaerobic, rod-shaped bacteria.[56] The ability of vegetative cells to form spores in response to environmental stress (e.g., reduced nutrient availability) sets *Bacilli* apart from many other microbes.[67] The spore coat, rich in peptidoglycan and protein, forms under stressful conditions to protect the dehydrated endospore from UV radiation, extreme heat, solvent exposure, freezing, radiation, hydrogen peroxide and enzymes – a characteristic that greatly increases shelf life and stability of *Bacillus* spore products. Once exposed to the appropriate nutrients, the endospore transitions to a vegetative cell once again and loses its resistance to environmental stress.[68] *Bacillus* species are almost entirely isolated from the soil, water, dust and air.

Bacillus species are ubiquitous in nature and have a wide range of characteristics, secondary metabolites and enzymatic activities. For instance, the *B. subtilis* strain natto is used in the fermentation of soybeans to make the Japanese food natto. Vegetative cells of this strain produce a serine protease, nattokinase, which is used as a dietary supplement for reducing blood clotting via fibrinolysis.[67] In addition *Bacilli* produce many structurally heterogeneous secondary metabolites (e.g., antibiotics, lipopeptides, polypeptides, macrolactones, fatty acids, polyketides, lipoamides, isocoumarins, etc.) that have diverse physiological effects.[70] Some *Bacillus* species (e.g., *B. indicus*, *B. firmus*, etc.) have been shown to produce carotenoids, and these carotenoids have been shown to be bioavailable and bioaccessible to human cells *in vitro*, however, it is not well understood how supplementing carotenoid-producing *Bacillus* bacteria affects the carotenoid status of the human host *in vivo*.[71,72] Notable *Bacillus* species include *B. clausii*, *B. coagulans* (often mislabeled as *Lactobacillus sporogenes*), *B. subtilis*, *B. cereus* (a known human foodborne pathogen, though not all strains are virulent) and *B. anthracis* (a human pathogen).[67,69] In 2008, *B. coagulans* strain GanedenBC30 was the first *Bacillus* strain to be given self-affirmed GRAS approval in the United States.[67] Other *Bacillus* species have been added to dietary supplements sold to consumers as probiotics and used in the food supply.

Spore-forming probiotic organisms are marketed primarily for their shelf-stability, as they are more resistant to manufacturing and shipping stressors that harm the viability of most other probiotic strains. However, since spores are small, light and difficult to destroy, many manufacturers find them challenging to work with, as they persist within the manufacturing environment and require special handling to prevent cross-contamination. Nonetheless, the increased availability of spore-forming organisms for clinical research is expanding their potential clinical utility. Animal and *in vitro* studies have highlighted many mechanisms through which spore-forming *Bacillus* probiotics many enhance host health such as stimulation of the immune system, production of antimicrobial molecules (e.g., bacteriocins, enzymes, etc.), modulation of the composition of the gut microbiota, suppression of pathogens, etc.[74]

Which Strains are Best?

One question that always comes up when discussing probiotics is, "What strain, or strain combination, is the best?" Of course, this begs the follow-up question, "For what purpose?" The use of a probiotic as a preventative measure against potential dysbiosis (as one would use a multivitamin), is quite different than using a probiotic to treat a subject with antibiotic-associated diarrhea or inflammatory bowel disease. Therefore, this question must be answered in a step-wise fashion, owing to the unique nature in which probiotic research has been done over the years.

First, while there are many different strains of probiotic organisms currently available, the majority of these are very similar to each other, especially when compared to the overall diversity

within the gut commensal community. Most commercially available probiotic strains have never been compared head-to-head in human clinical trial settings. Therefore, while some studies may suggest one strain is preferable to another for a particular outcome, this is often simply because that particular strain was used to perform one or more (positive) clinical trial(s), a trial which simply was not done with, or compared to, other similar strains. Therefore, choosing the strain(s) used in positive clinical trials may be "evidence-based," but large gaps in study design and the lack of strain-to-strain comparison often limit definitive strain-specific recommendations.

We believe it is safe to say that there is not one strain or strain combination of probiotics ideally suited for each individual or clinical outcome. For the most part, increasing commensal diversity is likely to benefit most individuals, something that is likely to occur when consuming a product containing a diverse mix of probiotic species or strains, what we call a multi-species or multi-strain approach. Choosing a specific strain or strain combination (or a higher dose) may be warranted when that dose or strain(s) has shown consistent clinical evidence for the specific goal of the therapy. Healthcare providers should stay abreast of the published literature in this area, as new studies (and some new probiotic strains) are likely to influence clinical decision-making for recommending probiotics to patients.

Multi-Strain vs. Single-Strain vs. Rotating Strains

Since there are many purposes for which a probiotic may be recommended, and the published literature includes a wide range of probiotic formulas, strains and doses, many different theories of proper probiotic recommendations have emerged. Two, in particular, are the multi-strain and rotation of single-strain approaches. We advocate for using a multi-strain approach for the vast majority of applications within the clinical setting. First, since a diverse composition of commensal species appears to be one of the most important factors in maintaining a healthy gut microbiome, using a range of different probiotic strains has the best chance of promoting a wider array of commensal organisms. In addition, because of individual differences in strain survival, compatibility and metabolic potential, a multi-strain approach allows for a more diverse benefit in a wider range of individuals. Finally, recall that probiotics represent a part of the transient microbiome, which is normally encountered as a range of organisms ingested in the diet, and through contact with the soil and air. A multi-strain approach is therefore more consistent with this natural encounter with ingested microorganisms.

There are several ways to define a multi-strain approach. In general, we define this as a product with five or more proven probiotic strains containing at least two *Lactobacilli* and two *Bifidobacteria* strains. One method to enhance the potential for diversity when using this multi-strain approach is to choose products containing species (or sub-species) with a range of genetic variability (see phylogenetic relationship of common probiotic strains in Figure 2). By combining more genetically diverse strains, rather than clustering the diversity with closely related subspecies, a greater potential for diversity may be achieved. Inclusion of *Streptococci*, *Saccharomyces boulardii*, *Bacillus* spp., or other genera will expand this diversity even further.

The other popular means of attempting to achieve diversity is to rotate single-strain probiotics; consuming a different strain for a few months and then switching to a different strain and so on. The difficultly in recommending this approach is that there is virtually no information available to evaluate this strategy and, in comparison to the multi-strain approach above, does not mimic the way we encounter transient microbes from our food or environment. Also, since many probiotics require several weeks or months of continuous use to achieve noticeable benefit, the subject might be planning to rotate to a new strain just as tangible benefits may be realized from the current product, and switching may not be in the best interest of the patient. While switching from one product to another may be necessary to find a product more suited for an individual or a therapeutic purpose, rotating single-strain products in an attempt to increase overall microbiota diversity is likely a hold-over from the days when only single-strain products were available, and is accomplished more efficiently by the use of multi-strain products. As a final note, rotating different multi-strain products is not discouraged, provided they are equally diverse, though research investigating this approach is also lacking.

Changes in Commensal Population from Probiotic Intake

Compared to the changes detected in commensal populations that occur after radical dietary changes, bariatric surgery or antibiotic use (or fecal microbial transplants), detectible changes in the commensal microbiota after probiotic consumption are much more limited.[75,76] This should come as little surprise since the strains used as probiotics are generally limited to just a few genera, are almost exclusively transient in nature (because of their species and/or commercial domestication), and are usually given in doses that are difficult to detect in fecal samples (by culture or genetic methods). However, there are several important points to be considered before determining such subtle changes are unimportant.

First, even when no major changes are detectible in commensal microbiota after ingestion of certain probiotics, there is often still a demonstrable improvement in GI or other health outcomes. This suggests that subtle changes in commensal species abundance or function, along with the direct metabolic contributions of the transient probiotic species, can have profound and important health benefits for the host. Second, the dose of most probiotics is likely to alter the immediate microbial balance within the small intestine where there are fewer numbers

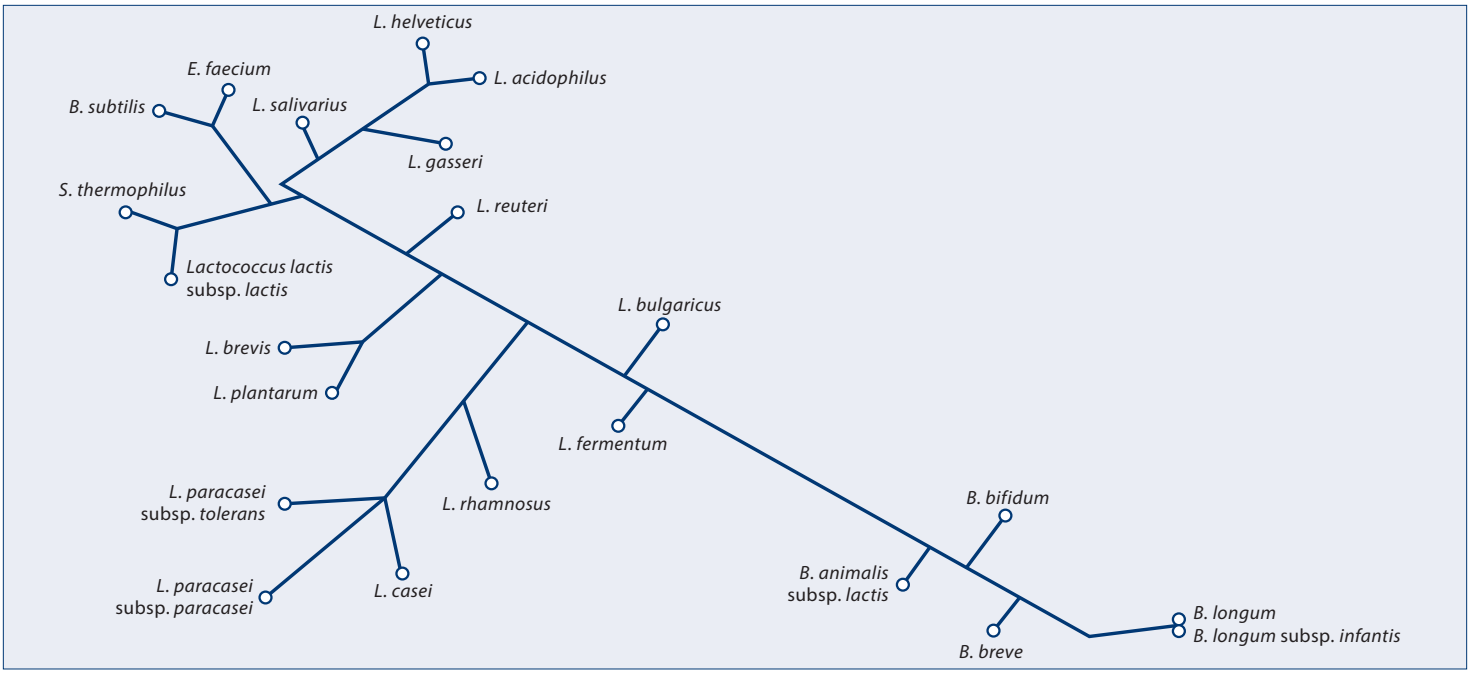

Figure 2: Phylogenetic relationship between common probiotic strains based on unrooted maximum parsimony clustering of partial 16S rRNA gene sequences (courtesy of Danisco/Dupont).

and species of microorganisms, though this population is poorly represented in fecal microbial analysis. This means that probiotics, especially *Lactobacilli* strains, may profoundly alter the balance of microbial species in the small intestine and subsequently benefit the host, without demonstrably altering the total gut microbiota when measured via fecal analysis.[77,78,79]

Finally, it is not generally agreed that radical microbiome alterations would be a desirable outcome of probiotic use, since the potential for monoculture or core microbiome disruption could be more harmful than other "natural" forms of dysbiosis. Recall that probiotic therapy should not be considered a re-inoculation of commensal strains as much as providing a commensal-friendly transient population. Oral doses of commensal organisms in the form of encapsulated frozen feces may have the effect of "re-inoculation" and have been studied for use in subjects with recurring *C. difficile* infections with some success, though these products are not technically "probiotics" and their regulatory status is currently unknown.[80] Therefore, it is more reliable to use patient signs and symptoms (or other biomarkers of disease progression, if they exist), rather than changes in fecal microbiota, to judge the efficacy of probiotic therapies.

Probiotics: Part of the Transient Microbiome

We have already emphasized the idea that the strains of commercially prepared probiotics currently available should be considered temporary, rather than permanent, members of the gut microbiome. While many view the temporary status of probiotics as a negative feature, this is likely due to the lack of appreciation for the importance of the transient microbiome. Perhaps one of the key reasons that probiotic strains are generally safe and applicable for most individuals is related to the fact that they are transient. This feature allows them to function primarily to promote a friendly environment for the variable and core species of the microbiome, which are different in each individual. In addition, while probiotic residence may be temporary, certain effects (e.g., immune modulation, pathogen diminution, etc.) may persist long after their presence in the gut has ceased.

Generally, probiotics that survive the initial transit are considered to persist in the GI tract for one to two weeks after ingestion, though this is strain-, dose- and host-dependent.[81] Most of these studies define persistence as the detection of the consumed strain (or its genetic material) using fecal samples, though biopsies and simulated gastrointestinal environments have also been used with similar results.[82,83] While the relative persistence of every strain has not been confirmed in human subjects, similar persistence has been seen in most strains tested, including *Lactobacilli*, *Bifidobacteria*, *Saccharomyces boulardii* and spore-forming *Bacilli*.[84,85] Obviously, probiotics consumed on a regular basis continually replenish this transient portion of the microbiome and, depending on dose, can significantly alter the patient's health during that time. Finally, recent studies suggest that baseline microbiome communities create a "permissive" or "resistant" environment for certain species of probiotics in certain individuals, making it difficult to predict the persistence of each strain in any given subject.[86]

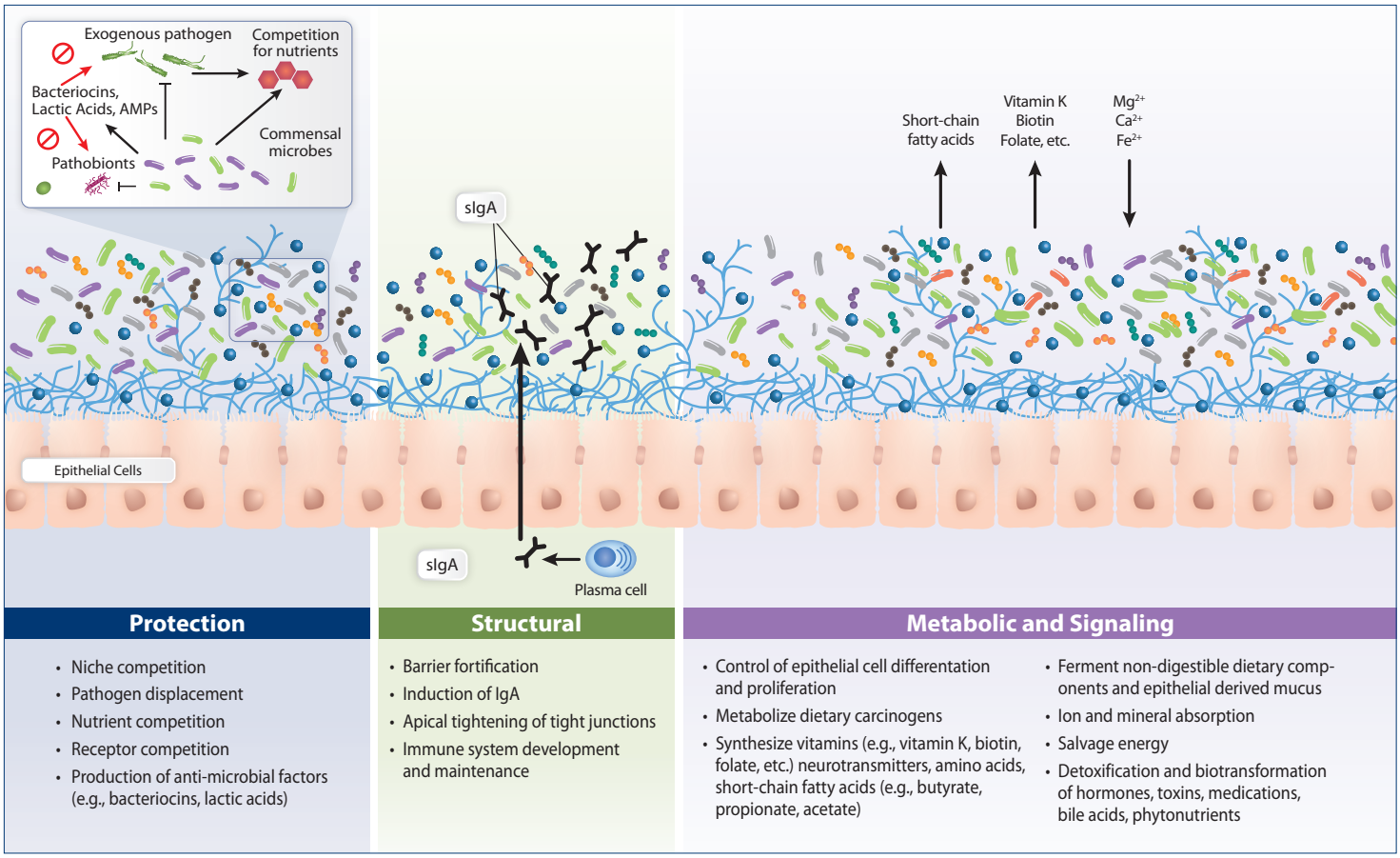

Figure 3: Basic Function of the Commensal Gut Microbiota. From the perspective of the host, this figure illustrates the three basic functional categories performed by the commensal gut microbiota. Some of these activities are also provided by certain probiotic organisms. See text for more details.

What is the Right Dose?

Probiotics can be delivered in relatively low-dose functional foods (primarily yogurts) providing several million up to a few billion colony forming units (CFU); or in modest doses, in the form of dietary supplements of 5 to 25 billion CFU. However, over the past several years, a trend has emerged in which much higher doses of probiotics are being used in both clinical practice and research. Since most probiotic strains or strain combinations have not been clinically researched using dose-response relationships as a primary analytical goal, we review below the limited available evidence on dose-to-dose outcomes and also the types of studies where high-dose probiotic therapies (defined here as > 100 billion CFU/day†) have been successful.

Dose Comparison Studies

Few dose-response or dose-comparison clinical trials (high- vs. low-dose) using probiotics have been performed in human subjects. Two studies, using very different definitions of "high-dose" therapy, have evaluated the dose-effects of probiotics in subjects using antibiotics and their subsequent risk for antibiotic-associated diarrhea (AAD) or *C.difficile*-associated diarrhea (CDAD). In both cases, the higher dose reduced the incidence of AAD and/or CDAD in a statistically and clinically significant way, compared to the lower dose (see details on page 12).

Also, a few dose-response studies have studied bowel transit and stool consistency. One study assessed two different doses of *Bifidobacterium lactis* on whole gut transit time (WGTT, assessed by abdominal X-ray) and GI symptoms in subjects with an average of one to three bowel movements per week.[87] Subjects were given capsules containing placebo, 1.8 billion CFU (low-dose), or 17.2 billion CFU (high-dose) at breakfast for two weeks (capsules opened and eaten with yogurt). After 14 days, both doses of B. *lactis* improved transit time and functional GI symptoms, though the high-dose therapy was slightly better (and more statistically different compared to placebo) than the low-dose therapy. Another study investigated the effect of supplementing healthy young adults with a combination of *L. paracasei* and *B. lactis* at doses ranging from 100 million CFU/day to 100 billion CFU/day in tenfold increments.[88] The authors reported a significant dose-dependent improvement in fecal consistency (looser stools) as the dose increased.

† This definition of "high-dose" is somewhat arbitrary based on the recent trend of available products. Some would argue that therapies delivering > 20 billion CFU should be deemed "high," while others may suggest that "high-dose" therapy is not reached until > 450 billion CFU has been exceeded.

While these data suggest that the dose of certain probiotics may influence certain outcomes in certain patients, it is difficult to generalize beyond these particular strains and the limited outcomes reviewed here. Since the vast majority of clinical trials using probiotics use only one dose (comparing to placebo), often with no explanation as to the reason the particular dose (or strain[s]) were chosen, clinicians should consider altering the suggested dose before concluding that a patient can receive no benefit from the particular probiotic product chosen.

High-Dose Therapies (A Therapeutic Trend)

Over the past decade, there has been an emerging increase in probiotic dosing seen in both clinical research and clinical practice. Not surprisingly, the initial focus of the clinical research on high-dose probiotics has been on specific GI disorders such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and antibiotic-associated diarrhea (AAD). These conditions represent extreme examples of dysbiosis and dysfunction within the mucosal immune system of the gut, a system which is integrally associated with the gut microbiota. Although supplemental probiotics are only transient members of the intestinal microbiota, the introduction of large quantities of probiotics may sufficiently alter this environment, allowing the probiotics to act as potent bio-therapeutic agents in a manner that a lower dose may not.

The majority of these high-dose clinical trials have been performed using various doses of one particular combination of probiotic strains (i.e., VSL#3: containing three strains of *Bifidobacteria*, four strains of *Lactobacilli*, and one strain of *Streptococcus salivarius* spp.). These doses have ranged from 450 billion CFU/day to as high as 3.6 trillion CFU/day, and from as short as four weeks of supplementation to as long as one year. Positive benefits (most statistically significant compared to placebo) were noted in patients with ulcerative colitis, pouchitis, and antibiotic-associated diarrhea. However, as mentioned earlier, only one very high dose was used in these clinical trials (compared to placebo), and it is therefore difficult to determine if lower doses would have accomplished similar results in similar subjects. Since lower doses and different strain combinations have also been demonstrated to have clinically meaningful and statistically significant benefits in similar patients, this suggests that high-dose therapies may not always be necessary to achieve meaningful clinical results.

Proposed Mechanisms of High-Dose Probiotic Therapy

Numerous mechanisms are attributed to a wide dose range of probiotic therapies that mirror the mechanisms of beneficial commensal organisms (see Figure 3). As mentioned earlier, adding significantly more metabolically active cells into the GI microbiome can have significant implications for the balance of commensal organisms, resulting in more pathogen/pathobiont exclusion, nutrient fixation, bacteriocin production, etc. These transient intraluminal effects may account for many of the benefits seen in AAD and CDAD studies when higher doses are used.

Another major focus of this research is the interaction between bacterial organisms in the gut and specific cells within the gut-associated lymphoid tissue (GALT), especially the dendritic cells which act as specialized antigen-presenting cells within the gastric mucosa. These dendritic cells are critical for both the maturation and tolerance of the immune system. A recent study showed that by increasing the number of probiotic organisms interacting with dendritic cells *in vitro* (this study used *Lactobacillus rhamnosus*), a much different genomic response was elicited. When researchers increased the multiplicity of infection (MOI), or the number of *L. rhamnosus* plated with immature human dendritic cells by hundredfold, they induced a sharp increase in gene expression of over 1,700 different genes compared to dendritic cells in the presence of fewer bacteria. Most of the changes in gene expression were for genes that control immune and inflammatory signaling or dendritic cell maturation. In fact, these data showed a progressive dose-response increase in specific dendritic cell surface markers at five different probiotic:dendritic cell ratios. This genomic effect is an exciting new line of research that is likely to lead to a greater understanding of how commensal and probiotic organisms help regulate immune function and attenuate numerous conditions related to the gut. In this case, a progressively higher bacterial concentration triggered a much different response in immune system regulation than a lower concentration, suggesting one broad mechanism by which high-dose probiotic therapies may differ from similar strains at lower doses.

Since this study has not been repeated with other strains or other species (or directly in humans), it is unknown how applicable these findings would be to other probiotic strains, though similar modulations of immune cell functions are likely with many other strains of probiotics. In fact, a study comparing two different doses of *Lactobacillus plantarum* ("low-dose" 500 million CFU and "high-dose" 5 billion CFU) in institutionalized elderly subjects showed significant differences in immune cell activation that differed based on dose.[90] Since many clinicians consider both of these doses "low," it should be noted that high doses are not necessarily needed to achieve meaningful benefits in some subjects, only that high (or very high) doses may achieve different (or additional) benefits.

Final Thoughts on High-Dose Therapy

While the available clinical research on high-dose probiotic therapy is relatively recent, a trend is emerging in clinical practice to begin increasing probiotic doses, primarily for GI-related dysfunctions. More data is needed in order to discern whether specific GI disorders would be better supported using specific probiotic strains or combinations of strains (at different doses). Until such a time, doses of between 200 billion and several trillion

CFU/day of products consisting of mixed probiotic strains should be considered safe for adjunct therapies for patients with IBD, IBS and AAD. This approach should be considered short term (four to eight weeks for functional bowel disorders or until symptoms cease for AAD). Cost may limit the accessibility of this therapy for many patients.

Probiotics for AAD and CDI Prevention and Treatment

If the absence of a healthy commensal environment is a critical intermediate between antibiotic use and the overgrowth and invasive activity of *C. difficile*, then the use of probiotics has the potential to mitigate this activity.[91] And, since both *C. difficile* infections (CDI) and probiotics are both important research trends, it is not surprising that well over 100 clinical trials have been performed investigating the potential benefits of probiotic therapies for antibiotic-associated diarrhea (AAD) and/or *C. difficile*-associated diarrhea (CDAD).

In 2012, *JAMA* published a systematic review and meta-analysis of more than 80 studies using probiotics for the prevention or treatment of AAD.[92] Though these studies were generally small, and some of the specific probiotic strains were poorly documented, the authors concluded the "evidence suggests that probiotics are associated with a reduction in AAD." In fact, in the trials reporting on the number of patients with AAD, the relative risk was reduced by over 40% (RR 0.58). Of the trials used for the analysis, 57 of 82 included *Lactobacillus* alone or in combination (32/82 in combination with a *Bifidobacterium* strain). Sixteen of the studies used *Saccharomyces boulardii* alone, commonly used for AAD and *Clostridium difficile*-related diarrhea.[93] A more recent meta-analysis of 16 studies (of various sizes and quality) shows a significant benefit in most studies of AAD or CDAD.[94]

Overall, a wide range of probiotic strains, strain combinations and doses have been used in clinical research investigating the role of probiotic therapies and AAD/CDI (in the case of CDI, usually with concomitant antibiotic therapy). This heterogeneity of trial design and outcomes has prevented most organizations from making any probiotic recommendations within their AAD and CDI prevention and treatment guidelines (although most have not been updated since 2013), though numerous positive clinical trials have since suggested their overall efficacy and safety.[95] One notable exception to this positive trend was the PLACIDE trial, performed in hospitalized elderly patients in Wales and England. It is one of the largest studies to test probiotics for AAD prevention (N = 2,941).[96] In this study, researchers used a four-strain combination: two strains of *L. acidophilus* (CUL60 and CUL21), along with *B. bifidum* (CUL20) and B. *lactis* (CUL34), at a dose of 60 billion CFU/day (strain ratio not described). The probiotics were taken with food and, when possible, between antibiotic doses for 21 days and subjects were monitored for the occurrence of AAD within eight weeks and CDAD within 12 weeks of recruitment. There were no statistical differences in AAD or CDAD incidence between the treatment or placebo groups in this study. Interestingly, the number of subjects with confirmed AAD or CDAD (~10.6% and 1%, respectively) was much lower than is typically reported in other similar trials. This may suggest that this study design (or population) is less suitable for evaluating the benefits of probiotic prophylaxis of AAD/CDAD, though it reminds us more research is still needed to understand the role of probiotics for these conditions.

Nonetheless, while numerous strains of both *Lactobacilli* and *Bifidobacteria* species (or combinations) have been associated with positive clinical outcomes, one probiotic that does get mentioned in some guidelines (though with limited information) is the yeast *S. boulardii*. In fact, a recent systematic review with meta-analysis confirms the efficacy of *S. boulardii* in reducing AAD in children and adults.[97] A range of doses have been used for this probiotic, mostly described as from 250 mg to 1,000 mg/day (generally, this is equivalent to 5 to 20 billion CFU, though many trials do not specify a concentration). Because yeast strains are generally not affected by antibiotic use and *S. boulardii* has been both safe and effective for AAD and CDAD, we highly recommend the use of this particular probiotic for these conditions (alone or in combination with other probiotic species). Yogurt is unlikely to help therapeutically against either AAD or CDAD, though can be safely consumed throughout the use of other probiotics.[98]

Higher Doses for AAD and CDAD

While there is limited evidence, higher doses of probiotics appear to be more effective at reducing the incidence of AAD or CDAD when compared to lower doses of the same formula. Two studies, using very different definitions of "high-dose" therapy, have evaluated dose effects in subjects using antibiotics and the risk for AAD or CDAD. The largest such study was performed using 503 Chinese subjects who were hospitalized for various diseases requiring antibiotic therapy.[99] Subjects were randomly assigned to one of three therapies: placebo, 4.2 billion CFU (low-dose) or 17.4 billion CFU (high-dose) of an encapsulated product containing equal proportions of *Lactobacillus acidophilus*, *Lactobacillus paracasei*, and two strains of *Bifidobacterium lactis*. Products were consumed during the use of the antibiotic therapy, and continued until seven days after discontinuing the antibiotic (dose taken two hours after antibiotic/breakfast). The incidence of AAD was highest in the placebo group (24.6%) and was statistically lower in the high-dose group (12.5%, P = 0.005), while the low-dose realized a non-statistically significant reduction from placebo (19.6%). Overall, both doses of probiotics were also able to reduce the CDAD incidence compared to placebo (1.8% vs. 4.8%, respectively), though only the higher dose reached statistical significance compared to placebo.

A similar study was previously performed in 255 hospitalized elderly Chinese subjects prescribed antibiotics for various diseases.[100] These subjects were randomly assigned

to one of three therapies: placebo and either one capsule (50 billion CFU) or two capsules (100 billion CFU) of a probiotic containing *Lactobacillus acidophilus* and *Lactobacillus casei* (ratio not specified). Products were consumed during the use of the antibiotic therapy, and continued for five days after discontinuing antibiotic (dose taken two hours after antibiotic/breakfast). Incidence of AAD was highest in the placebo group (44.1%), and statistically lower in both the 50 billion/low-dose (28.2%) and 100 billion/high dose (15.5%) groups. Dramatic reductions in the incidence of CDAD were also reported in both the high-dose and low-dose probiotic therapies (1.2% and 9.2%, respectively) compared to placebo (23.8%). The higher incidence of AAD and CDAD in this trial, compared to the previous study mentioned, were likely due to the higher number of elderly people in this group.

Recommendations for AAD and CDAD

In agreement with our previous general recommendation for the use of probiotics with the use of any antibiotic, we recommend that clinicians consider using a multi-strain probiotic that includes a minimum of 5 billion CFU of *S. boulardii* in addition to other appropriate therapies for AAD or CDAD (e.g., antibiotics, FMT). Due to the safety and likely higher efficacy, clinicians should consider recommending products that deliver a total of at least 50 billion CFU/day of total probiotics (*Lactobacilli* spp. and *Bifidobacteria* spp.) along with the *S. boulardii* dose. This may be achieved by taking multiple capsules of a single product, or by consuming a separate *S. boulardii*-only product in addition to a high-dose multi-strain probiotic. Clinicians should specify to patients that the probiotic dose should be consumed at least two hours after any oral dose of antibiotics.

Probiotics for Inflammatory Bowel Disease

Since a variety of alterations in the gut microbiome are common in subjects with IBD and the microbiota-immune interface is considered a key pathophysiological mediator, it seems quite plausible that probiotic therapy would have a positive outcome on disease progression. However, there is a large heterogeneity of studies that complicate the evaluation of probiotics as therapeutic agents for inducing or maintaining remission in patients with IBD. Recently, several systematic reviews and meta-analyses have been published in the attempt to provide the clinician some clarity as to the potential benefits of certain species or doses of probiotics.[101-106]

In general, there appears to be a substantial difference in the reported efficacy of probiotic therapy between the two forms of IBD, favoring UC. That is, few clinical trials using probiotics have been effective at inducing or extending the maintenance of remission in patients with Crohn's disease, while numerous clinical trials using probiotics have been successful in subjects with ulcerative colitis. It is unknown whether this is a fundamental difference in the immune-microbiome interface between these two conditions, the concentration of lesions in the distal colon in UC versus the many intermittent lesions in CD or simply in the quality of the trials performed. Nonetheless, these differences have been maintained over many years of trials using a variety of probiotic preparations. We will, therefore, review the information for both conditions separately.

Probiotics for Crohn's Disease

As mentioned above, the general consensus is that probiotic therapy has shown little benefit for CD subjects in controlled clinical trials. However, since probiotics are generally considered safe in CD patients, we will briefly review the outcomes of a few trials where positive outcomes were reported to inform the clinician in the event they intend to use probiotics in such patients (see review for all trials).[73] A few small studies performed with the probiotic yeast *Saccharomyces boulardii* have yielded improvements in symptom scores or prolonged relapses in CD patients in remission. In one very early double-blind, placebo-controlled study (1993), twenty subjects with CD suffering from diarrhea and moderate complaints as measured by the BEST Index were treated with *Saccharomyces boulardii* (250 mg/tid, likely 15 billion CFU/day, though the study did not define concentration) for two weeks in addition to conventional treatment.[107] Patients saw a statistical reduction in both bowel movement frequency and disease index score. Ten of the subjects were then randomized to continue the treatment of *S. boulardii* (same dose), while seven were given placebo for an additional seven weeks. After 10 total weeks, the group given *S. boulardii* had further reduced bowel frequencies and disease index scores while both measures rose to near their original baseline values in the placebo group.

A second study (2000) evaluated the benefit of *S. boulardii* as an adjunct therapy with mesalamine in 32 CD patients in clinical remission for an average of 33 weeks.[108] Patients were treated for six months with either 3 g per day of mesalamine (1 g/tid) or 2 g per day of mesalamine (1 g/bid) with 1 g *S. boulardii* (given as two, 500 mg capsules in the morning; the concentration was not listed, but likely 20 billion CFU). Of the sixteen patients on mesalamine alone, six subjects experienced a clinical relapse, while only one subject in the mesalamine plus *S. boulardii* group experienced a relapse ($P = 0.04$). Even in a more recent and larger trial (2013) that failed to show statistical benefits in relapse prevention using *S. boulardii* (1 g/day), the interaction between *S. boulardii* treatment and smoking status was statistically significant. In *post hoc* analysis, nonsmokers given placebo had more relapses (72.0%) than those treated with *S. boulardii* (34.5%). However, in smokers and former smokers, the proportion of relapse was not significantly different. When adjusting for this stratification factor, nonsmokers treated with *S. boulardii* were 82% less likely to relapse than those receiving placebo (OR, 0.18; $P = 0.006$).

The only other species of probiotics to show marginal benefits in CD subjects in a controlled trial is *Bifidobacterium longum* (400 billion CFU/day consumed as a synbiotic with

inulin at 6 g/day).[109] However, a combination of *B. longum*, *B. breve* and *L. casei* (combined with psyllium) showed some improvement in an open-labeled, uncontrolled trial.[110] The high-dose combination product VSL#3, which has been used successfully in subjects with UC or pouchitis, has been tested with only limited reported success in subjects with CD.[111]

Based on the available evidence, there is limited data upon which to make a strong recommendation for the clinical treatment of CD using probiotics. The use of probiotics in such patients is generally recommended to help maintain a commensal-friendly environment, though they should not be relied upon to induce or extend remission. The use of *S. boulardii* at 1 g (20 billion CFU) may be an exception, clinicians should consider the use of this dose as a potential adjunct therapy in subjects with CD, or include *S. boulardii* in a mixed-strain probiotic.

Probiotics for Ulcerative Colitis

Unlike the situation for CD, the evidence supporting the successful use of probiotic therapy for UC is much more promising, though concentrated around a few strains or strain combinations. In three double-blind, placebo-controlled randomized clinical trials, researchers found that a non-pathogenic strain of *E. coli* (Nissle 1917) was equally effective as mesalazine in maintaining remission among UC patients. In one trial involving 120 patients, Kruis et al. reported that patients receiving this probiotic strain (50 billion CFU/day) had a similar relapse-free time (106 ± 5 d) compared to UC patients who were given mesalamine (103 ± 4 d).[112] Similar outcomes were confirmed by Rembacken et al. in a trial involving 116 patients with UC. Relapse rates were 73% for the mesalazine group and 67% for the *E.coli* group (100 billion CFU/day), and the time to relapse was not significantly different between both groups. In another larger clinical trial of 327 patients, researchers also found that *E.coli* (Nissle 1917) was effective and safe in maintaining remission equivalent to the gold standard mesalazine in patients with UC (dose 50 billion CFU/day).[114] The *E.coli* (Nissle 1917) strain, sold elsewhere in enteric-coated capsules as "Mutaflor®," is not currently available in the United States as it was not considered a dietary ingredient by FDA, nor has it been approved as a drug.[115]

The other major probiotic mixture used in subjects with UC is the eight-strain probiotic blend known as VSL#3. This probiotic preparation (containing three strains of *Bifidobacteria*, four strains of *Lactobacilli*, and one strain of *Streptococcus salivarius* ssp.) has been used in at least nine different clinical trials in UC patients, most of them at very high doses. A recent meta-analysis of five of these trials (N = 441) has been published, showing a pooled remission rate of 49%.[116] When pooling together three studies in which subjects were given 3.6 trillion CFU/day of the probiotic blend (in patients also given 5-ASA and/or immunomodulators), they realized a > 50% decrease in disease activity index in 44.6% of subjects taking probiotics (placebo 25.1%, P = 0.008), a response rate of 53.4% (placebo 29.3%, P < 0.001), and a remission rate of 43.8% (placebo 24.8%, P = 0.006). Studies were short, generally eight weeks, and there were no serious adverse effects at these doses.

Compared to these two preparations, there are only a few other probiotic strains or strain combinations that have been tested for benefit in UC subjects such as *S. boulardii*, *L. rhamnosus* GG, *B. breve*, *B. bifidum*, *L. acidophilus*, *B. lactis*, *L. casei*, *L. reuteri*, and *B. longum*, though most of these trials were small pilot trials, uncontrolled, given rectally or open-label studies.[117-120] Unfortunately, systematic studies have not been performed on most strains of probiotics (or combinations of strains) at high doses to compare to VSL#3. This strain combination generally fits with our recommendation of a multi-strain probiotic, though there are no specific studies comparing this particular blend with other similar blends. While no clinical study has been published to compare, it is likely that similar strain combinations given at similar doses would have similar results. Clinicians should choose products, typically provided in pouches or sachets, that allow for very high dosing, as doses of greater than 3 trillion CFU/day may be needed over a two-month period to realize benefits in patients with UC.

In light of these studies, the clinician must always remember that that the efficacy of a probiotic preparation is unlikely to be the same in all patients or in the same patient at different stages of disease. Success of treatment may also be dependent on several variables, such as characteristics of a patient (gender, lifestyle habits, smoking status, age), lesions in IBD (location, extent, type of gross lesion), and risk factors (genetic predisposition, familial history).

Probiotics and IBS

The use of probiotics to improve the microbiome and reduce symptoms in subjects with IBS is a fairly common practice around the world.[121] In fact, so common is the practice that well over 50 clinical trials, using most of the commercially-available *Lactobacilli* and/or *Bifidobacteria* strains, have been performed and published over the past few decades; and at least 10 systematic reviews and meta-analyses have been performed on various subsets of these trials.[122] One might assume that with this much data, there would be a clear understanding of the role of probiotics in IBS subjects. However, since these studies were performed by dozens of different groups around the world, using different strains (single-strain products or mixed-strain products), doses, length of treatments and delivery mechanisms, combined with the complex diagnostic and subtyping issues inherent with IBS, the picture is all but clear.

Therefore, depending on which subset of published trials are collected for the given meta-analysis, a different recommendation (or no specific recommendation) is made.[123-125] What we can say at this time by combining the published literature on this topic and discussion of the use of probiotics for IBS with healthcare providers is this: 1) probiotic therapy is safe in subjects with

IBS, adverse effects are very limited and cease when probiotic is stopped, 2) the same strains or combinations of strains will not work in all subjects with IBS or even in every subtype of IBS, 3) symptom improvement using a particular probiotic may diminish (or improve) over time, especially in subjects with IBS-M, 4) positive benefits have been seen at relatively low doses (< 10 billion CFU/day) as well as very high doses (450 to 900 billion CFU/day), clinicians should be willing to start low and consider high doses.

Probiotics Therapy for SIBO

The supplementation of probiotics can be an important therapy for a number of gastrointestinal disorders involving dysbiosis. However, since SIBO is an overgrowth of bacteria in the small intestine and its symptoms are nominally related to excess fermentation, some clinicians are reluctant to recommend probiotics in subjects with a positive breath test indicating SIBO. Surprisingly, few studies have been designed to help answer this conundrum. Most published studies have been extremely small or uncontrolled (i.e., pilot studies).[126,127] Therefore, there is little available evidence to make specific recommendations for the use of probiotics for SIBO.

Anecdotal evidence suggests that subjects with SIBO should be given low doses of probiotics first (< 5 billion CFU/day) for a week or so to monitor any change of symptoms related to this therapy. This may require opening a capsule that contains a higher dose. Increasing the dose to 20 to 40 billion CFU/day should be done in step-wise fashion as long as no increased symptoms accompany the stepped-up dosing. Our standard recommendation is a mixed-strain probiotic, which is a good place to start in all patients including those with SIBO. If mixed-strain products are associated with worsening symptoms, patients may want to consider using single-strain products to discover if one particular species or strain may be helpful. Once a patient is breath-test negative (or SIBO symptom-free), a mixed-strain probiotic given at 20 to 40 billion CFU/day should be recommended to help maintain a normal microbiome.

Spore-Forming Probiotics as GI Therapeutic Agents

Since the research and use of spore-forming probiotics (primarily single or multiple strains of *Bacilli*) is often different and separate from the more commonly used lyophilized probiotic bacteria (i.e., *Lactobacilli* and *Bifidobacteria*), their research is reported here, and evaluated separately. Although lyophilized bacteria have considerably more published data to evaluate compared to that available on the spore-forming bacteria, the number of clinical trials evaluating spore-forming bacteria is increasing as is their commercial popularity in the market.

GI Persistence of Spore-Forming Probiotic Bacteria and Their Effect on the Commensal GI Microbiota

Similar to non-spore-forming probiotics (e.g., *Lactobacilli*, *Bifidobacteria*, etc.), research suggests there is great interindividual variability in the gastrointestinal persistence of orally administered spore-forming probiotics. For instance, a randomized, open-label, crossover trial studying the GI survival and persistence of four *B. clausii* strains found their spores were able to survive transit through the gastrointestinal tract and undergo germination, outgrowth and multiplication as vegetative cells to varying degrees.[128] Some *B. clausii* strains appeared to have a higher than expected enumeration in some fecal samples relative to the amount administered to subjects, suggesting that these strains were able to germinate in the GI tract. As with most probiotics, *B. clausii* strains show interindividual differences in GI persistence when compared amongst different subjects. The longest surviving strain was found in the fecal samples up to 12 days after administration, while the shortest surviving strain was only found in the fecal samples up to three days after administration.

Limited studies are available researching changes in the human commensal gut microbiota after supplementation with spore-forming probiotics. One randomized, double-blind clinical trial in elderly men and women (65 – 80 years, N = 36) found supplementation with 1 billion CFU/day of *Bacillus coagulans* GBI-30 for 28 days significantly increased levels of the commensal microbe *F. prausnitzii* from baseline compared to placebo (P = 0.03).[129] *F. prausnitzii* has been previously associated with beneficial health outcomes. Not surprisingly, supplementation with *B. coagulans* led to increases in *Bacillus* spp. in fecal samples of subjects (P = 0.007). However, additional studies are needed to better understand the effects spore-forming probiotics may have on commensal microbes in the gut.

Spore-Forming Probiotics for Diarrhea Prevention and Treatment

The majority of human clinical research on spore-forming probiotics has focused on outcomes related to gastrointestinal health. Most studies in this area have researched the effect of acute administration of *Bacillus* probiotics on diarrhea (from various infections, IBS-D, etc.). Most of these studies have suggested *Bacillus* probiotics are safe and well tolerated, but the results have been mixed for their ability to improve diarrheal outcomes (e.g., duration of diarrhea, stool consistency, etc.).[130-135] These studies have been heterogenous across numerous variables in study design (e.g., the patient population, the species of bacteria, the dose and duration of supplementation, etc.), making it difficult to recommend one strain or another for specific outcomes.

One systematic review and meta-analysis focused specifically on studies supplementing *B. clausii* for diarrhea outcomes in children. The authors found *B. clausii* supplementation significantly reduced the duration of diarrhea (mean difference: -9.12 h, P = 0.015) and the duration of

hospitalization (mean difference: -0.85 days, P = 0.017) compared to control when analyzing six trials studying 1,298 children (ages three months to twelve years).[136] There was a trend for decreasing stool frequency after *B. clausii* supplementation compared to control (mean difference: -0.19 diarrheal episodes, P = 0.14). Dosages of *B. clausii* ranged from 1 x 10^9 CFU twice per day to 4 x 10^9 CFU once per day and many studies compared *B. clausii* to oral rehydration solution with or without zinc (only one trial compared *B. clausii* to placebo). Despite these results, this systematic review highlights the lack of robust clinical trials in terms of quality, as only two studies were deemed "good" quality by the authors. Despite these results, other studies in children, have not found an improvement in diarrhea outcomes when supplementing children with *Bacillus* probiotics (i.e., *B. clausii*, *B. coagulans*, etc.).[130,134]

Spore-Forming Probiotics for Irritable Bowel Syndrome (IBS)

A number of clinical trials have been performed evaluating the effect of *Bacillus* probiotics for symptom relief in patients with IBS, primarily *B. coagulans* strains. Overall, it appears *Bacillus* probiotics improve subjective GI symptoms in IBS patients (as defined by ROME II, ROME III and ROME IV criteria) compared to placebo for the adults and children studied.[133] Similar to the IBS studies with lyophilized probiotic bacteria described previously, most of these studies with *Bacilli* are preliminary and have evaluated various subgroups of IBS. Additionally, some studies have evaluated the role of *Bacillus* probiotics given concurrently with other treatment modalities for IBS such as dietary restrictions (i.e., low FODMAP) and pharmaceutical therapies. The low FODMAP diet has been studied for its efficacy in improving GI outcomes in IBS patients. One study compared the addition of supplementing a low FODMAP diet with 10 billion CFU *Bacillus coagulans* spores to a low FODMAP diet without spores in fifty patients meeting the ROME IV criteria for IBS (of all subtypes).[141] In this study, consuming a low FODMAP diet with or without spores led to significant improvements in many GI measures (e.g., abdominal pain intensity and frequency, satisfaction with bowel habits, quality of life, defecation consistency, and patient-reported severity scores). The addition of *B. coagulans* to the low FODMAP diet led to a greater number of patients reporting improvements in severity scores compared to placebo (57% with *B. clausii* compared to 35% in the placebo group (P = 0.001)). In another study, probiotics (*Bacillus subtilis* and *Streptococcus faecium*) were studied alongside a motility stimulating drug (i.e., mosapride) and were found to be effective for relief of IBS symptoms in patients with constipation-predominant IBS.[142]

Spore-Forming Probiotics for GI Discomfort and Constipation

Bacillus probiotics have also been studied for their effect on GI discomfort and constipation in otherwise healthy subjects. One study found significantly improved subjective measures of GI function in healthy adult subjects (N = 60) with undiagnosed GI discomfort following supplementation with 2 billion CFU of a probiotic blend (i.e., *Bacillus coagulans* [SNZ 1969], *Bacillus clausii* [SNZ 1971], and *Bacillus subtilis* [SNZ 1972]) for thirty-five days.[143] Another study found supplementation with *B. coagulans* Unique IS2 (2 billion CFU/day for 28 days) significantly improved the number of spontaneous bowel movements per week compared to placebo during weeks three and four (P < 0.001) in healthy adults with functional constipation according to the ROME III criteria (N = 100); additionally, the spore-forming probiotic improved stool consistency and the feeling of incomplete evacuation compared to placebo as well as decreasing abdominal pain and defecation pain.[140] These studies suggest spore-forming probiotics may improve subjective measures of GI function in those with undiagnosed GI discomfort and may improve bowel movements in those with constipation.

Spore-Forming Probiotics for Non-GI Conditions

Although the focus of this review is on GI-related outcomes, there have been a number of clinical trials exploring the application of these microbes as therapeutic agents for non-GI-related conditions (e.g., immune health, oral health, metabolic health, etc.).[144-148] Outside of GI-related outcomes, the majority of research has been in the area of immune health. One study found supplementation for thirty days with a combination *Bacillus* spore probiotic formula (4 billion spores of *B. indicus* (HU36), *B. subtilis* (HU58), *B. coagulans*, *B. licheniformis*, and *B. clausii*) reduced postprandial serum endotoxin levels by 42% after five hours and triglyceride levels by 24% three hours following a high-fat challenge meal in college-age subjects (N = 25) who previously had elevated endotoxin levels to the same high-fat meal at baseline (labeled as "responders" to the endotoxin challenge at baseline).[149] In comparison, endotoxin levels increased by 36% five hours after the high fat meal and triglycerides decreased by 5% three hours after the meal in the placebo group. A few studies have evaluated *Bacillus clausii* for immune responses in allergic children with recurrent respiratory infections.[150-152] Another study evaluated *B. coagulans* GBI-30 6086 for rheumatoid arthritis outcomes.[153] Limited trials studying probiotics for oral health outcomes suggest *Bacillus* probiotics may reduce salivary mutans *Streptococci* counts similar to studies evaluating species of other non-spore-forming probiotics (e.g., *Lactobacilli*, *Bifidobacteria*, *Streptococci*); however, a study evaluating a toothpaste containing *B. subtilis*, *B. megaterium* and *B. pumulus* spores (5 x 10^7 CFU) for 8 weeks did not show improvements in plaque and gingivitis outcomes compared to placebo.[144,145] Limited studies have evaluated the use of *Bacillus* probiotics for their influence on metabolic outcomes for subjects with diabetes and hypercholesterolemia, and more research is needed in this area.[146,147,148]

Safety of Spore-Forming Probiotic Supplementation

We should note that two *Bacillus* species, *B. anthracis* and *B. cereus* are well-known human pathogens (though not all strains of these species are virulent); however, many animal and *in vitro* safety studies have shown no indication of adverse effects of the *Bacillus* species used as probiotics.[67] Additionally, no adverse effects have been reported in human clinical trials supplementing *Bacillus* probiotic strains.[74] Similar to other probiotics, there may be a safety concern for ingesting *Bacillus* probiotics in immune-compromised subjects.[74]

Summary: Putting the Clinical Data on Spore-Forming Probiotics into Context

Overall, the human clinical data available on spore-forming probiotics is greatly limited compared to that of non-spore-forming probiotic research. Therefore, the data for spore-forming probiotics is even more difficult to discern with respect to comparisons between trial- and strain-specific effects. The limited clinical trials lack consistency in terms of the *Bacillus* probiotic strains tested, the dosages used, the patient populations tested, the duration of supplementation, and outcomes tested. Because of the variability in study design, it is difficult to recommend one strain or strain combination over another.

However, from the available research it does appear that spore-forming probiotics show promise for many GI-related outcomes. These studies have shown spore-forming probiotics improve subjective symptoms for IBS patients, improve functional GI outcomes and outcomes related to constipation and diarrhea. However, this research is still in its preliminary phases. More research is needed to make clear recommendations for spore-forming probiotics. Since spores, by nature, are resistant to manufacturing stressors, this makes these microbes attractive from a product stability standpoint. Overall, the emerging data for spore-forming probiotics is promising for GI-related outcomes.

Probiotic Supplementation in Infants and Children

With the growing acknowledgment of the importance of the GI microbiome for proper metabolic and immune system development, along with the safe use of probiotics in adult populations, there has been an accumulating body of research on the use of probiotics in infants and children. Here, we will briefly summarize data on the use of probiotics for GI-related conditions in infants and children, along with the safety of using probiotics in these populations. There are several recent comprehensive reviews covering more details, including many non-GI indications.[154-157]

Safety

In the general pediatric population (ages zero to 18 years), probiotics have been well-tolerated and are generally regarded as safe with few adverse events; however, extra precautions should be taken in at-risk pediatric populations when supplementing with probiotics.[158] Such at-risk patient populations are: immune-compromised children; premature infants; those with critical illness, structural heart disease, or a central venous catheter; and in those with the potential for translocation of probiotics from the gut lumen to the bloodstream.

A meta-analysis of 57 clinical trials (and eight follow-up studies) including 10,056 infants under the age of two years found supplementation with probiotics and synbiotics to be safe; furthermore, no serious adverse events or safety concerns were found to be associated with the probiotics and prebiotics studied.[159] "Rarely probiotics may cause bacteremia, fungemia and sepsis in immune-compromised, critically ill children."[160] We should note the number of species (and strains) used in the pediatric population is limited, and therefore the number of strains demonstrated to be safe (compared to adult populations) is also limited. However, based on what is known about the isolation of probiotic strains and their overall record of safety in foods and supplements worldwide, we believe that most currently available strains are likely to be safe in the pediatric population (with the noted exceptions listed above).†

Probiotics in Selected Childhood Conditions

Acute Gastroenteritis

The probiotic strains most studied for treatment of acute gastroenteritis (AGE) in pediatric populations include: *L. rhamnosus* GG[161,162] *S. boulardii*[163-165] and *L. reuteri* DSM 17938.[166,167] In fact, a working group from the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) strongly recommends the use of *L. rhamnosus* GG (> 10 billion CFU/day) and *S. boulardii* (250 - 750 mg/day, generally this is equivalent to 5 to 15 billion CFU, though many trials do not specify a concentration) for AGE in children, along with a "weak" recommendation for *L. reuteri* DSM 17938 (10^8 to 4 x 10^8 CFU/day) for the same indication.[168] Most of these probiotics are recommended to be taken for five to seven days. The only probiotic with a negative recommendation based on this report by ESPGHAN was for *Enterococcus faecium* (SF68 strain); the group strongly recommends avoiding this strain due to safety concerns (specifically related to a possible recipient of vancomycin-resistant genes).

† The limited number of strains used in the pediatric population (perhaps due to their selection by researchers or IRB reviewers based on previous safety data) severely limits the strength of strain-specific recommendations. That is, since most strains (or strain combinations) have never been tested for particular outcomes in children, any strain-specific recommendation is made by default, simply using those limited strains with positive clinical evidence.

Antibiotic-Associated Diarrhea

A 2015 Cochrane Review by Goldenberg et al. included 23 randomized controlled studies studying 3,938 children (two weeks to 17 years old) and found significantly reduced incidence of AAD in the probiotic groups (*Lactobacilli* spp., *Bifidobacteria* spp., *Streptococcus* spp., or *Saccharomyces boulardii* alone or in combination) compared to the control groups (RR: 0.46; 95% CI: 0.35 - 0.61).[169] The authors concluded, "*L. rhamnosus* and *S. boulardii* at 5 to 40 billion CFU/day may be appropriate for preventing AAD in children receiving antibiotics;" however, no other recommendations were made about other strains.

Many RCTs and meta-analyses recommend probiotics for the prevention of AAD. Strains with the most evidence include *L. rhamnosus* GG (5 RCTs, N = 445, RR: 0.48; 95% CI: 0.26 - 0.89)[170] and *S. boulardii* (6 RCTs, N = 1653, RR: 0.43, 95% CI 0.3 - 0.6)[171]; both of which are recommended by the ESPGHAN working group guidelines (2016).[172] Mcfarland (2015) notes in a mini review on deciphering meta-analytic results that the best evidence for a probiotic in pediatric AAD is for *S. boulardii* because the favorable pooled effect for *L. rhamnosus* GG is skewed by one large positive trial.[173] As we mentioned previously, the lack of clinical trials (positive or negative) for other strains is not evidence of their lack of efficacy, and the use of other strains or strain combinations in adult AAD suggests other strains are likely beneficial for this outcome.

Clostridium difficile Infection (CDI)

Compared to the literature surrounding probiotics for *C. difficile* infections in adults, information concerning the use of probiotics in children is limited. A 2013 Cochrane review by Goldenberg et al. considered probiotics for prevention of CDI in both adults and children.[174] After pooling 23 trials including 4,213 participants, use of probiotics was found to decrease the risk of CDI by 64% (RR: 0.36; CI: 0.26 - 0.51).

Necrotizing Enterocolitis (NEC)

Numerous reviews have recently been published on the promising benefits of using probiotics in infants with NEC.[175-177] According to Szajewska (2016), preventing NEC is possibly the most promising indication for the use of probiotics in preterm infants.[99] An updated Cochrane review studied 24 RCTs and compared to the control group, preterm neonates in the probiotics group had reduced risks of NEC stage > 2 (20 RCTs, RR: 0.43) and all-cause mortality (17 RCTs, RR: 0.65), but there was no difference between groups in the risk of nosocomial sepsis (19 RCTs, RR: 0.91).[178]

According to those who have studied this relationship in depth, despite the potential benefit of probiotics in NEC, many questions remain unanswered, including the optimal probiotic formulation and the safety and efficacy of using probiotics in very low-birthweight (birthweight < 1,500 g) and extremely low-birthweight infants (birthweight < 1,000 g).[99]

Infantile Colic

In reviewing data related to the use of probiotics and infantile colic, it was unusual to find nearly all studies were performed with a single strain of *L. reuteri*. Four independent RCTs showed *L. reuteri* DSM 17938 (generally dosed 100 million CFU/day) reduced crying times in breastfed infants with infantile colic.[179-183] However, another study involving both breastfed and formula-fed infants did not confirm this benefit, perhaps because it enrolled children predominantly from the emergency department and included children on proton pump inhibitors.[184] Interestingly, in this study, the formula-fed infants after one month of supplementation with *L. reuteri* DSM 17938 showed significantly more fussing time compared to placebo (P = 0.005).

Systematic reviews and meta-analyses have shown benefit for *L. reuteri* in breastfed infants for infantile colic.[185,186] A 2016 systematic review found supplementation with *L. reuteri* in breastfed infants was associated with a 2.3-fold greater chance of having a 50% or greater decrease in crying/fussing time compared to controls (P = 0.01).[187]

In an investigation of the prevention of infantile colic, a 2014 trial by Indrio et al. enrolled 589 Italian neonates in the first week of life and compared the incidence of developing a functional GI disorder in babies receiving *L. reuteri* compared with those receiving placebo.[188] Functional gastrointestinal disorders (FGID) were defined as "inconsolable crying time, regurgitation, and modification of bowel movements." The study showed a significant difference in crying time from 70.9 min/day in the placebo group to 37.7 min/day in the probiotic group; approximately 1.5 less regurgitations/day in treatment group (4.6/day in control and 2.9/day in treatment); and increased stool frequency (3.6/day vs. 4.2 stools/day treatment).

While these studies appear quite promising, it is difficult to know whether these effects are strain- or even species-specific. Since probiotic therapy is safe in most infants, the clinician should consider recommending a probiotic designed for children, perhaps selecting one with this or similar strains of *L. reuteri*.

Other GI-Related Outcomes

Probiotics have been investigated as a therapeutic intervention for a number of other GI-related conditions in infants and children. For the most part, there is simply not enough evidence to make specific recommendations, as clinical trials have had a range of positive and negative outcomes. There is limited evidence to recommend selected probiotics for ulcerative colitis. The European Crohn's and Colitis organization (ECCO) and ESPGHAN consider VSL#3 and *E. coli* (Nissle 1917) as effective treatment for maintenance in patients with UC, but this recommendation is based on limited evidence.[189-192] According to ECCO/ESPGHAN guidelines, there is not enough evidence to suggest probiotics are beneficial for the maintenance or remission of Crohn's disease in children.[193]

For abdominal pain-related functional gastrointestinal disorders (FGID), Korerink et al. performed a systematic

review of all available RCTs in children with FGID and concluded that *L. rhamnosus* GG, *L. reuteri* DSM 17938 and VSL#3 strains are possibly effective in decreasing pain-related FGID with a pooled risk ratio of 1.5.[194] The studies, however, were relatively heterogeneous and therefore prevented firm conclusions on efficacy.[99] There is also limited evidence to make specific recommendations for either functional constipation or irritable bowel syndrome, though probiotics are likely to be safe in such populations and some small clinical trials have shown benefit.[195-198]

Probiotics for Non-GI Pediatric Conditions

There are many possible therapeutic benefits related to probiotic therapy in children for non-GI-related conditions such as atopic/allergic treatment and prevention, immune-related outcomes, upper respiratory infections, mood-related outcomes and even metabolic-related outcomes. While these topics are outside the scope of this monograph, clinicians should be aware of the broader potential for non-GI-related outcomes for the use of probiotics in children (and adults).

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This Monograph contains several excerpts from our Book: ***Functional Strategies for the Management of Gastrointestinal Disorders: Principles and Protocols for Healthcare Professionals*** (Point Institute, 2016). The book includes much more information on the preparation and proper use of commercial probiotics, in addition to more background information on the microbiome and related GI dysfunctions.

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