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Targeted Approaches for In Situ Gut Microbiome Manipulation

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Abstract

The 2019 Dudrick Research Symposium, entitled "Targeted Approaches for In Situ Gut Microbiome Manipulation," was held on March 25, 2019, at the American Society for Parenteral and Enteral Nutrition (ASPEN) 2019 Nutrition Science & Practice Conference in Phoenix, AZ. The Dudrick Symposium honors the many pivotal and innovative contributions to the development and advancement of parenteral nutrition (PN) made by Dr Stanley J. Dudrick, physician scientist, academic leader, and a founding member of ASPEN. As the 2018 recipient of the Dudrick award, Dr Gail Cresci organized and chaired the symposium. The symposium addressed the evolving field of nutrition manipulation of the gut microbiome as a means to mitigate disease and support health. Presentations focused on (1) the role of prebiotics as a means to beneficially support gut microbiome composition and function and health; (2) designer synbiotics targeted to support metabolic by-products altered by ethanol exposure and microbial effectors that manipulate host metabolic outcomes; and, lastly, (3) types of intervention designs used to study diet–gut microbiome interactions in humans and a review of findings from recent interventions, which tested the effects of diet on the microbiome and the microbiome's effect on dietary exposures. New molecular techniques and multiomic approaches have improved knowledge of the structure and functional activity of the gut microbiome; however, challenges remain in establishing causal relationships between changes in the gut microbial–community structure and function and health outcomes in humans. (*JPEN J Parenter Enteral Nutr.* 2020;44:581–588)

Keywords

alcohol; diet; gut microbiome; gut microbiota; human studies; prebiotics; probiotics; synbiotics

Introduction

The 2019 Dudrick Research Symposium, entitled "Targeted Approaches for In Situ Gut Microbiome Manipulation," was held on March 25, 2019, at the American Society for Parenteral and Enteral Nutrition (ASPEN) 2019 Nutrition Science & Practice Conference in Phoenix, AZ. The Dudrick Symposium honors the many pivotal and innovative contributions to the development and advancement of parenteral nutrition (PN) made by Dr Stanley J. Dudrick, physician scientist, academic leader, and a founding member of ASPEN. As the 2019 recipient of the Dudrick award, Dr Gail Cresci chaired the symposium. The symposium, in addition to providing a broad overview

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of microbiome and nutrition research, addressed the use of prebiotics, probiotics, and synbiotics to maintain or restore the gut microbiome toward improving host health and outlined experimental approaches to studying microbiome-diet interactions in humans.

A microbial ecosystem, composed of bacteria, archaea, fungi, yeasts, and viruses, resides within and is distributed throughout the entire human intestinal tract, with the colon being the most densely populated.[1] Over the past several decades, culture-independent, high-throughput molecular biological techniques have greatly improved our capacity to study and understand the gut microbiota.[2] Techniques based on sequencing the 16S ribosomal RNA (rRNA) gene allow for characterization of the relative abundance of different bacterial taxa in gut microbial communities. Metagenomics and metatranscriptomics, based on shotgun sequencing of DNA and RNA, respectively, capture functional capacity of the microbes. The application of other high-dimensional omics approaches, such as proteomics and metabolomics, has also expanded knowledge of the functional output of microbial metabolism.[3]

A strong symbiotic relationship exists between the gut microbiota and the host. The gut microbiome plays an important role in gut immune-system development; food digestion; metabolic by-product production, such as essential vitamins and organic acids; and colonization resistance from pathogenic organisms.[4] Although a typical healthy adult has trillions of microbes, there is noted intraindividual and interindividual gut microbiota variability at the genus/species/strain level, but how this affects functionality has not been fully defined.[1] Intestinal microbial structure and function are dynamic during infancy, but with weaning and usually by 3 years of age, a child's gut microbiome composition resembles that of an adult.[5] The gut microbial community is relatively stable during adulthood, but numerous factors, including sex, adiposity, diet, physical activity, and medication use (antibiotic use in particular), contribute to differences in community structure.[1,6]

Through their huge numbers and intense metabolic activity, gut microbes are known to play a role in human health status. There is now strong evidence implicating the indigenous gut microbiome in many gastrointestinal diseases, including gastroenteritis, inflammatory-bowel diseases (IBDs), irritable-bowel syndrome (IBS), and some digestive cancers. Moreover, the microbiota may play a role in other systemic conditions not directly associated with the gut, such as cardiometabolic health, cognitive function, and mineral bioavailability. The microbiome contains components (microbes and their products) that are beneficial for health, as well as those that are pathogenic.[7,8] Although there is much excitement in the field of the gut microbiome as a therapeutic option for many conditions, this is a challenging field, and it is still in its infancy.

Prebiotics and Gut Health: Friends in a Low Place (Glenn Gibson, PhD)

The gut microbiome represents a rational target for the prevention and treatment of gut-mediated disorders. Use of probiotics, prebiotics, and synbiotics is a way to selectively target beneficial microbes at the expense of those seen to be detrimental.[9,10] Probiotics are live microorganisms that, when included in the diet in adequate amounts, confer a health benefit. Probiotics are most commonly administered in dairy foods and beverages, or dried versions are provided as pills, capsules, or mixtures that can be added to foods. Lactobacilli and bifidobacteria are the most frequently used probiotics. Both groups have been shown to stimulate immune processes and inhibit pathogens.[11] Prebiotics are ingredients selectively utilized by populations of host microorganisms that confer a health benefit. For gut effects, a prebiotic must escape mammalian digestion and be utilized selectively by a restricted group of microorganisms that have been clearly identified to have health promoting properties, usually in the colon. Synbiotics are physical combinations of probiotics and prebiotics that together confer a health benefit.

Prebiotic substances may be present in isolated form or in foods, but to meet the criterion of being "utilized selectively," they must affect a limited range of microbes, rather than substantial portions of the microbiome. The most commonly studied prebiotics are soluble fibers, inulin, fructooligosaccharides and galactooligosaccharides (GOSs), and, more recently, human milk oligosaccharides. To date, prebiotic substances with the most well-documented health effects are forms of dietary fiber; however, the current definition, presented above, allows for a broader range of substances targeting different host niches to be considered prebiotics, given appropriate scientific support.[9]

Galactooligosaccharides as Prebiotics

GOSs are prebiotics that have been associated with increased abundance of and fermentation by-products of *Bifidobacterium* spp and can be synthesized by enzymatic catalysis of lactose using a β-galactosidase enzyme(s). The amount and nature of the GOS mixture synthesized is dependent upon the source of the enzyme as well as reaction conditions. One type of GOS (called B-GOS) has been synthesized using β-galactosidase from *Bifidobacterium bifidum* NCIMB 41171. This was initially assessed for its prebiotic effect in vitro and in pigs.[12] Subsequently, studies of prebiotic effects of B-GOS have also been extended to humans.[13]

We have conducted a series of trials to test whether B-GOS could influence the gut microbiome and health outcomes in specific human populations. Aging is associated with a lower bacterial diversity; lower abundance of

important commensals, such as bacteroides, bifidobacterial, and lactobacilli; and increased gut permeability and inflammation.[14] A 10-week, placebo-controlled, randomized, crossover trial testing B-GOS (5.5 g/d) in older, free-living volunteers (64–79 years) showed that B-GOS significantly increased numbers of bifidobacteria, at the expense of less beneficial bacteria. This microbial change was associated with positive effects on certain markers of the immune response, including anti-inflammatory mediators.[15]

Several studies from our group also provide evidence regarding the effects of B-GOS supplementation on gastrointestinal symptoms. In a single-center, parallel, crossover, controlled clinical trial, 44 patients with IBS were stratified and block-randomized within each IBS symptom subgroup to receive 4-week treatments (maltodextrin control and 3.5 and 7 g/d B-GOS). B-GOS changed the colonic microbiota by enhancing the proportion of bifidobacteria in a dose-responsive manner and reducing the patients' symptoms.[16] Another placebo-controlled trial in 159 healthy travelers showed that the group taking B-GOS (5.5 g/d; B-GOS) as a preventative measure before traveling experienced statistically significantly reduced incidence and duration of traveler's diarrhea.[17]

Associations between the gut microbiome and health effects beyond the gut have also been reported. The gut microbiota varies in composition and metabolic activity between lean and obese individuals.[18] With the goal to examine the effects of B-GOS on the fecal microbiota and on markers of metabolic syndrome and immune function, we conducted a double-blind, randomized, placebo-controlled, crossover study in 45 overweight adults. Supplementation of B-GOS (5.5 g/d) compared with a maltodextrin control for 12 weeks increased the number of fecal bifidobacteria, increased fecal secretory Immunoglobulin A (IgA), and decreased fecal calprotectin, plasma C-reactive protein, insulin, total cholesterol (TC), Triglyceride (TG), and the TC:High density lipoprotein (HDL) cholesterol ratio. Thus, the intervention, coupled with changes in microbiota, improved immune function and markers of the metabolic syndrome.[19] Children with autism spectrum disorders (ASDs) often experience gastrointestinal distress and exclusion diets (eg, gluten, casein exclusion), and prebiotic/probiotic supplementation are dietary approaches implemented to alleviate this. Our group conducted a randomized, double-blind, placebo-controlled, parallel-designed trial in 26 children with ASD, which provided 80% statistical power.[20] Children were grouped based on whether they followed an exclusion diet or not (casein, gluten) and then randomized within these groupings to receive GOS supplementation (n = 13; 1.8 g/d: 80% GOS) or a placebo (n = 13; maltodextrin control) for 10 weeks. Children on the exclusion diet had significantly lower scores of abdominal pain and bowel movement and lower abundance of *Bifidobacterium* spp and Veillonellaceae family but higher presence of *Faecalibacterium prausnitzii* (FP) and *Bacteroides* spp, as analyzed by 16S rRNA sequencing.[20] B-GOS supplementation was associated with improved antisocial behavior, increases in Lachnospiraceae family, and significant changes in fecal and urinary metabolites, such as short-chain fatty acids (SCFAs), amino acids, and other microbially derived compounds.

Summary

Prebiotics, such as GOSs, exert specific effects upon the gut microbiome, often increasing the abundance of bifidobacteria. In human trials, these microbial changes have been accompanied by a variety of health effects, including amelioration of acute and chronic gut disorders, as well as conditions traditionally considered less directly associated with the gut (eg, metabolic syndrome and ASD). To the extent that a clinical outcome is associated with a specific microbial mechanism of action, prebiotics can be chosen to selectively target relevant microbial activity. It is important not to overgeneralize conclusions about prebiotics. Results are typically specific to formulations, doses, target populations, and clinical endpoints.[21] New developments in metabolomic approaches will assist with understanding functionality of the microbiome in response to prebiotics as well as with respect to long-term health effects.

Targeted Synbiotic Supplementation: Arsenal Against Gut Microbiome Attacks (Gail A. M. Cresci, PhD, RD, LD, CNSC)

Gut Microbiota Fermentation By-products

In response to a diet rich in fermentable soluble fibers, the gut microbiota yields metabolic by-products, such as the SCFAs acetate, propionate, and butyrate at a constant molar ratio.[22] Butyrate is known to have many important biological roles, including aiding in water and electrolyte absorption, serving as a main fuel source for the colonocyte, altering gene expression because of its histone deacetylase activity, and supporting gut integrity and immune function.[22,23] Low colonic levels of butyrate are associated with apoptosis, inflammation, and mucosal atrophy.[24] Therapies with SCFAs and butyrate enemas show improvement in the clinical and pathological status in patients with IBD.[25,26]

Nutrition Manipulation of Gut Microbiota

Orally administered butyrate is completely absorbed in the small intestine. However, the prodrug tributyrin, a structured lipid with 3 butyrate molecules esterified to a glycerol backbone, is digested by pancreatic lipase and absorbed slowly, thus able to increase butyrate concentrations in the proximal colon.[22] A more physiological approach to boost luminal butyrate is to stimulate butyrate production

in the colon via dietary manipulations, which rely on bacterial fermentation of dietary fermentable soluble fibers. Fermentation of certain dietary fibers (eg, resistant starch, oligosaccharides) yield higher proportions of butyrate. The chemical composition and physiochemical properties of the dietary fibers influence the amount and composition of SCFAs produced during fermentation. Therefore, it may be possible to manipulate dietary fiber sources or prebiotics to achieve desired amounts of butyrate production in the colon.[22,27]

Mechanisms of action of probiotics are often strain-specific. Ideally, a probiotic is isolated from a human, able to survive the proximal intestinal tract, colonize the colon, and exert a beneficial effect.[10] FP is an anaerobic commensal butyrate-producing bacterium and a dominant member of the *Clostridium leptum* subgroup of the phyla Firmicutes known to have anti-inflammatory properties.[28] Widely distributed in the intestine, FP accounts for approximately 2%–15% of the total gut bacterial load in healthy adults.[28] Supporting gut health, FP is involved with providing nutrients to the colonocyte, maintaining immune homeostasis and the gut barrier.[29] FP abundance is significantly decreased in several disease states, including Crohn's disease, ulcerative colitis, and colorectal cancer, making it a potential biomarker for risk of various intestinal disorders.[29]

Alcohol Impact on Gut Microbiome–Liver Injury

Alcohol use disorder, estimated to affect roughly 18 million people in the United States, is a major cause of preventable morbidity and mortality worldwide.[30] Alcoholic liver disease encompasses a spectrum of progressive disorders from alcoholic steatosis, fibrosis, cirrhosis, and hepatocellular carcinoma, with alcoholic hepatitis often occurring between these stages.[31] Although nearly all heavy drinkers develop hepatic steatosis, only a small number progress to advanced liver diseases. Reported to contribute to nearly 18,000 deaths in 2013, and despite extensive research, alcoholic liver disease pathogenesis is poorly understood, with few advances in disease management in recent years.[30]

Growing understanding of the gut-liver axis is strengthening our appreciation for the bidirectional communication and link with the gut microbiota and extraintestinal organs through bile, intestinal secretions, and the immune system. The gastrointestinal tract–epithelial barrier provides a physical wall against microbial translocation, as well as a biochemical one characterized by antimicrobial proteins. In addition to processing by-products of digestion and absorption, the liver must respond to its exposure to gut-derived factors, such as bacteria and bacterial components (eg, lipopolysaccharide [LPS]) and bacterial metabolites. Ethanol disrupts the epithelial barrier, an effect that is associated with liver injury both in humans after excessive alcohol consumption and in rodent models of ethanol exposure.[32] Gut-derived LPS exposure of Kupffer cells, hepatic resident macrophages, can activate toll-like receptor-4–dependent production of proinflammatory mediators and propagate liver injury.[31]

Mitigating Alcohol-Induced Gut-Liver Injury via Microbiome Manipulation

Chronic alcohol consumption is a dietary factor associated with compromised gut microbial diversity and function (gut dysbiosis). As a consequence, changes in the gut metabolome are noted with chronic alcohol consumption in humans[33-35] and rodents.[36-38] In particular, depletion of the SCFAs propionate and butyrate and elevation of acetate occur resulting in nonphysiologic SCFA ratios. Prebiotics and probiotics have been studied in both humans and animal models of liver injury, including alcohol-induced liver injury. Studies investigating the role of probiotics in animal models show protective effects against alcohol in the intestine and liver through multiple mechanisms, including beneficially modifying gut microbiota, reducing reactive oxygen species, and enhancing the mucus layer, antimicrobial peptides, tight-junction proteins, and activated hepatic 5′ adenosine monophosphate-activated protein kinase (AMPK).[39] Recently, our group tested the effects of oral supplementation with tributyrin as a means to protect against alcohol-induced gut-liver injury. In studying mouse models of both acute and chronic alcohol exposure, we found that oral supplementation with tributyrin mitigates gut-barrier disruption, liver inflammation, steatosis, and injury.[40,41] Acute binge-alcohol exposure is known to suppress immune responses, and we found that tributyrin supplementation protects against dampened immune responses and vasculature in the intestine, which correlates with preservation of epithelial-junctional proteins and decreased liver injury.[42]

A Targeted Synbiotic Approach for Alcohol-Induced Gut-Liver Injury

A variety of probiotic strains have been used for alcohol-related studies, most of which are of the *Lactobacillus* genus, with *Lactobacillus rhamnosus GG* being most commonly investigated. *Lactobacillus*, of the Firmicutes phylum, is a major part of the lactic acid bacteria group and are gram-positive, facultative anaerobic or microaerophilic, rod-shaped, non–spore-forming bacteria. Because of the beneficial effects we found with tributyrin supplementation on alcohol-induced gut injury, our group recently designed a synbiotic (prebiotic plus probiotic) aimed at physiologically targeting luminal butyrate. The synbiotic consisted of the butyrate-producing and anti-inflammatory commensal

bacteria, FP, and a resistant starch known to yield butyrate upon fermentation (potato starch). Mice exposed to an ethanol-feeding protocol that replicates alcoholic hepatitis in humans (chronic ethanol, 5% for 10 days plus binge ethanol [day 11, 5 g/kg]) were orally supplemented with saline (control) or the synbiotic daily. The mice treated with the synbiotic were protected against ethanol-induced disruptions in tight-junction proteins in the proximal colon, as well as hepatic inflammation and oxidative stress, and resultant hepatic injury and steatosis.[43] Interestingly, the synbiotic influenced luminal SCFAs and expression of SCFA transporters in the proximal colon. These data corroborate our prior work in which we found decreased expression of butyrate transporters and receptors in the proximal colon in conditions of altered gut microbiota, such as germ-free mice[44] and mice treated with broad-spectrum antibiotics.[45]

Summary

Growing research supports the model of gut microbiota-liver axis as central to health, and when disrupted, this relationship adds to the propagation of gut-liver disease and related complications. As alcohol consumption will likely remain a common indulgence, the gut microbiota should be considered when investigating pathological effects of alcohol. Investigating the complex interactions between the host and gut microbiota and incorporating this relationship with investigations of alcoholic-induced organ injury will facilitate new research directives and potential therapeutic options for alcoholic use disorder.

Using Dietary Interventions to Evaluate Diet-Gut Microbiome Interactions in Humans: Whose Diet Is It? (Johanna W. Lampe, PhD, RD)

Food choices and diet composition contribute to shaping gut microbial–community structure (ie, the types of microbes present) and functional activity (ie, microbial gene expression). Both observational studies and controlled dietary interventions show that major shifts in macronutrient content of the diet result in differences in the gut microbial community.[46-49] Some phytochemicals, produced by plants as antimicrobial agents, may also play a role in shaping the gut microbial community.[7] Gut bacteria metabolize both organic and inorganic constituents of diet that are indigestible by human enzymes or that escape digestion in the upper gastrointestinal tract. These end products can (1) supply energy to host cells; (2) act as signaling molecules in host pathways; and (3) be genotoxic or beneficial to host cells.[50] In humans, circulating levels of bacterial metabolites often vary widely among individuals in response to a standard dose of a nutrient or other dietary component, suggesting large interindividual variation in gut microbial capacity to metabolize the parent compounds.[51] This can lead to differential exposure to dietary constituents and thus, potentially variable health outcomes.

Longitudinal and experimental studies are critical to begin to establish causality in human microbiome studies. A variety of study designs lend themselves to testing, experimentally, the effect of diet on gut microbial structure and function and the effect of the gut microbiome in response to diet. These include controlled-feeding studies, behavioral dietary interventions, and placebo-controlled dietary-supplement interventions.

Use of Controlled-Feeding Studies to Test Diet–Microbiome Interactions

Controlled-feeding studies play an important role in nutrition research.[52] They are useful for (1) controlling exposure to the dietary component of interest, as well as the background diet, (2) testing dose response, and (3) comparing response to different dietary patterns. Because feeding studies involve providing all the study food to participants during the intervention periods, they are costly and can be a burden to participants. Consequently, they are typically conducted for a short duration (ie, weeks to a month), and outcomes are limited to intermediate biomarkers of risk. Gut microbial gene expression responds rapidly to changes in diet,[7] such that these study designs lend themselves well to testing the capacity of a microbial community to metabolize particular dietary constituents. A powerful approach in controlled-feeding studies is the use of crossover designs, in which participants are given many or preferably all treatments in a randomized, assigned sequence. This allows for measuring treatment effects within individuals, rather than between individuals, and can improve statistical power. Controlling diet and using crossover study designs can also help to identify the effects of host or microbial genetic differences on metabolism of dietary constituents and health outcomes.[53,54]

Use of Behavioral Interventions to Test Long-term Microbiome Changes

Measurable shifts in the abundance or the activity of taxa present in the gut microbial community can be detected in response to short-term interventions, but substantial changes, such as the introduction and maintenance of new species or consortia previously not part of the community, may take months or years. Behavioral interventions, in which participants are counseled to make dietary changes, allow for longer-term interventions and individualization to improve and meet adherence goals. Such study designs are useful for testing for structural changes in the gut microbial community. Haro et al[55] evaluated the effects of Mediterranean and low-fat diets on bacterial community at baseline

and after 2 years of dietary intervention, administered as group education sessions. Their results showed that chronic consumption of the 2 diet patterns partially restored microbial dysbiosis in obese patients with metabolic syndrome. Incorporation of microbiome measures into these types of interventions are likely to yield important insights into microbial modulation of risk of chronic disease.

Use of Placebo-Controlled Dietary-Supplement Interventions in Microbiome Research

Randomized, placebo-controlled, dietary-supplement interventions also allow for rigorous testing of effects of defined dietary constituents on the gut microbiome. In addition, they provide an opportunity to test effects of measurable, differential microbial metabolism on host biologic response to specific dietary components. In a recently completed flaxseed lignan extract intervention in healthy men and women, we showed that interindividual differences in gut microbial metabolism of plant lignans to enterolactone were associated with differences in gene expression in colonic epithelium.[56] Several anti-inflammatory pathways were suppressed in low- compared with high-enterolactone producers. Similarly, harboring gut bacteria capable of converting the soy isoflavone daidzein to equol varies across individuals[57] and has been shown to result in differential lymphocyte gene expression in response to a soy intervention.[58] In acute feeding of an isoflavone supplement, arterial stiffness improved in equol producers compared with nonproducers, suggesting that the equol producer phenotype contributes to vascular benefits of equol.[59]

Summary

Overall, experimental studies of diet in humans provide a rigorous test of (1) the effect of diet on the gut microbiome; (2) the modifying effect of gut microbiome on host biologic response to diet; and (3) the level of variation in gut microbial metabolism of dietary constituents. The final experimental design chosen depends, in large part, on the specific research questions being addressed.

Overall Conclusion

Ongoing investigations are revealing the importance of the gut microbiome in human health and the strong role that diet plays on the action of the microbiome. A major challenge in the field is establishing causal relationships between changes in the gut microbial–community structure and function and health outcomes in humans.[60] New molecular techniques and multiomic approaches have allowed for in-depth description of the structure and functional activity of the gut microbiome. Parallel approaches using animal models and experimental studies in humans, in which the gut microbiome can be manipulated, as well as large-scale cross-sectional and prospective studies, are needed to provide multiple lines of evidence. In all cases, robust study designs with appropriately described and defined characterization of diet and treatments are critical for moving this field forward and to identifying prebiotic and dietary interventions to prevent and treat disease.

Statement of Authorship

G. A. M. Cresci, G. Gibson, and J. W. Lampe equally contributed to the conception and design of the research; G. A. M. Cresci, G. Gibson, and J. W. Lampe drafted of the manuscript. All authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

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