

Gut Microbiome: What We Do and Don't Know

Gail A. Cresci, PhD, RD, LD, CNSC¹; and Emmy Bawden, RD²

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Abstract

Within the last decade, research regarding the human gut microbiome has exploded. While the gastrointestinal tract was once regarded simply as a digestive organ, new technologies have led the science world to wonder about the impact that the gut microbiota may have on human health and disease. The gut microbiome is now becoming known for its role in metabolism, immune defense, and behavior. From in utero variations to those that rapidly occur post partum, our gut microbiome changes with age, environment, stress, diet, and health status as well as medication exposure. This article reviews what is currently known regarding various influences on the gut microbiome and is meant to encourage the reader to further explore the unknown. (*Nutr Clin Pract.* 2015;30:734-746)

Keywords

microbiota; immunity; diet; antibiotics; life cycle stages; chronic disease; health

Interest in and knowledge of the gut microbiome have increased exponentially in the past decade. An Internet search using key words such as “gut microbiome” or “gut microbiota” generates between 1 and 2.2 million results. Once overlooked, this component of the gastrointestinal (GI) tract is now gaining appreciation for its importance in optimal health. The food industry has taken this to full heights; a plethora of “probiotic” or “fermented” food and supplement products populate store shelves, and advertisements for them can be found in magazines, on the Internet, and on television. Never before has the discussion of one’s bowel habits and GI symptoms been more mainstream. Interestingly, this rapid growth in interest has left both consumers and many clinicians confused, because the data, although increasing, still leave many unanswered questions.

In the early 1900s, Eli Metchnikoff, a Russian scientist of the Pasteur Institute in Paris, associated longevity of rural Bulgarians to their consumption of fermented milk products. He postulated that the lactic acid bacteria in the fermented milk products ingested by these peasants, who were living in poverty in a harsh climate, provided an antiaging effect that contributed to their greatly outliving wealthier Europeans. He named the organism *Lactobacillus bulgaricus*. As Metchnikoff researched, he hypothesized that seeding the gut with healthy bacteria by drinking fermented milk products could fight off harmful bacteria and prolong life. He was the first scientist to suggest that it was possible to modify the gut microbiome by replacing bad bacteria with good bacteria, and he earned a Nobel Prize in 1908 for his work in immunity. The discovery of penicillin in 1928 by Sir Alexander Fleming, a Scottish biologist, turned the attention of researchers away from using bacteria to assist with healing to the practice of using soil fungi derivatives to kill bacteria. During the golden age of antibiotic discovery from 1940 to 1960, most antibiotics were originally isolated by screening soil-derived actinomycetes.¹ As returns diminished on this discovery platform, new discoveries were based on synthetic compounds.

However, the target-focused screening process of large libraries also failed, partly due to the inability of these synthetic compounds to penetrate the bacterial envelope.¹ Unfortunately, the deceleration of antibiotic discovery was accompanied by the spread of resistant bacteria and a major public health threat of untreatable infections. With advances in microbiology and molecular biology techniques, the culture is poised to revisit past successes with bacterial therapy for gut health and immunity in order to develop future treatments.

One of several international efforts, the Human Microbiome Project (HMP), uses high throughput multi-omics analyses to identify and study the microbiome in human health.^{2,3} Funded by the National Institutes of Health common fund in 2008, the HMP has thus far resulted in the isolation and sequencing of >1300 reference strains from the human body.^{2,3} The HMP Consortium has reported the structure and function of the human microbiome in 300 healthy adults (18–40 years old) at 18 body sites from a single time point. This has led to an unprecedented amount of data about the complexity of the human microbiome, allowing for a baseline for further research into the impacts of the microbiome on health and disease. As a precursor to the HMP, the Human Gut Microbiome Project

From ¹Department of Gastroenterology/Hepatology, Cleveland Clinic, Cleveland, Ohio; and ²Center for Human Nutrition, Cleveland Clinic, Cleveland, Ohio.

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Corresponding Author:

Gail A. Cresci, PhD, RD, LD, CNSC, Department of Gastroenterology/Hepatology, Cleveland Clinic, Cleveland, OH 44195, USA.
Email: crescig@ccf.org

widened our appreciation for the bacterial ecosystem that resides within the human intestinal tract. This system is comprised of microorganisms such as bacteria, archaea, fungi, and viruses that are distributed throughout the entire GI tract.⁴ Ongoing investigations are revealing the importance of the gut microorganisms in exerting beneficial effects on human health.

The typical healthy person is inhabited with trillions of microbes (Figure 1). But to confuse matters, 2 healthy people may have very different microbiomes. Analysis of HMP samples with lifestyle and history questionnaires has revealed associations between life-history characteristics and microbiome composition.⁵ Ding and Schloss⁵ analyzed a 16S ribosomal RNA (rRNA) gene sequence data set from the HMP Consortium. Because a significant difficulty in analyzing microbiome data has involved the considerable intrapersonal and interpersonal variation in the composition of the human microbiome, this study took the approach of clustering samples into bins based on their taxonomic similarity. Using bacterial community structures collected from 18 body sites and up to 3 time points, investigators applied community typing analysis to better understand the factors that affect the structure of the microbiome and contribute to human health. Using Dirichlet multinomial mixture models to partition the data into community types for each body site, their analysis of simulated data and the HMP data suggested that the community types represent clusters of relative abundance profiles. Three important observations were made with this approach. First, there were strong associations between whether someone was breastfed, his or her gender and education level, and his or her community types at several body sites. Second, the community types of the oral cavity and gut microbiome were predictive of each other even though the specific taxonomic compositions were different. Third, the community types were least stable in the oral cavity and most stable in the vagina and gut communities over the course of the sampling period. Therefore, the authors concluded that despite substantial intrapersonal and interpersonal variation in the human microbiome, when this variation is partitioned into community types, the types become predictive of each other and are likely the result of life-history characteristics.⁵ The gut microbiome is influenced by multiple factors including mode of infant delivery and feeding, the aging process, diet composition, geography, medications, and stress (Figure 2). The following sections provide a brief overview of current knowledge regarding variances in the gut microbiota related to these factors.

Our First Colonization

In Utero

It was previously believed that the GI tract was sterile in utero and that first colonization and initiation of the gut microbiome occurred during the birthing process.⁶ However, recent studies have challenged this belief by demonstrating

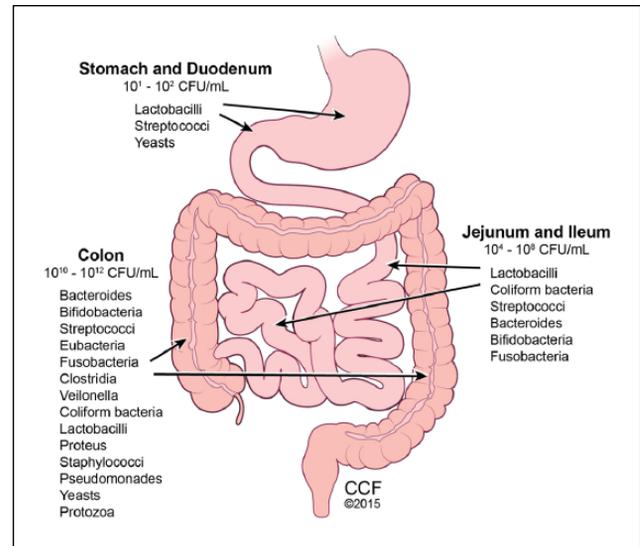


Figure 1. The human gut microbiota. Illustration by David Schumick, BS, CMI. Reprinted with the permission of the Cleveland Clinic Center for Medical Art & Photography © 2015. All rights reserved. CCF, Cleveland Clinic Foundation; CFU, colony-forming units.

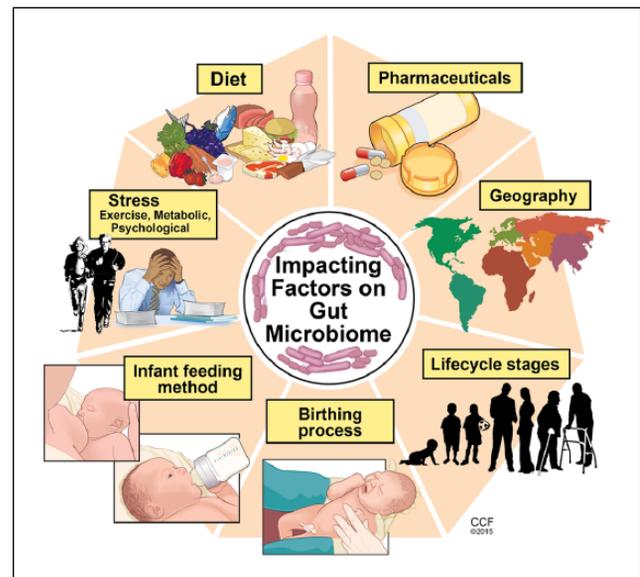


Figure 2. Factors affecting gut microbiome. Illustration by David Schumick, BS, CMI. Reprinted with the permission of the Cleveland Clinic Center for Medical Art & Photography © 2015. All rights reserved. CCF, Cleveland Clinic Foundation.

the presence of microorganisms in the placenta, amniotic fluid, and umbilical cord.⁷⁻⁹ This work is still preliminary but deserves further study as it highlights the importance of maternal gut microbiota health. It is hypothesized that by swallowing amniotic fluid and its bacteria in utero, the fetus begins to colonize the developing GI tract.¹⁰ The meconium,

the first infant stool sample, has also been shown to contain microorganisms.¹¹ Notably, the meconium from preterm infants shows a different microbiota composition from that seen in a sample acquired after the first week of life, demonstrating how normal gut colonization takes place throughout the course of fetal development.¹⁰ Absence of this progressive colonization could place preterm infants at risk for various GI infections, including necrotizing enterocolitis (NEC).¹⁰ Multiple studies have evaluated probiotic supplementation as a means to mimic the natural inoculation of the gut and thus influence the incidence and severity of NEC. A recent Cochrane review, which included 24 randomized controlled trials (n = 5529), assessed the role of probiotic supplementation in preterm neonates. The review confirmed previous findings that probiotic supplementation significantly reduces the risk of stage II or higher NEC and all-cause mortality in this patient population.¹² As probiotics are known to have potentially beneficial effects on gut function and maturity, Athalye-Jape et al¹³ conducted a meta-analysis that included 25 randomized controlled trials (n = 5895) to determine whether probiotic supplementation influenced the time to establish first full enteral feeds in preterm neonates. Overall, the subjects supplemented with probiotics took less time to achieve full feeds (mean difference: -1.54 days; 95% CI, -2.75 to -0.32 days; $P = .01$; $I^2 = 93\%$). The type and/or number of probiotic strains (eg, *Bifidobacterium* or non-*Bifidobacterium*) did not influence this improvement. Additionally, probiotic supplementation reduced hospital length of stay, feeding intolerance, and duration of indirect hyperbilirubinemia and increased weight gain and growth velocity. None of the trials reported adverse effects of probiotics on outcomes.

Delivery Mode

The birthing process exposes newborns to a wide range of microorganisms that also contribute to the colonization of the gut microbiome. The mode of delivery affects the composition of the infant's gut microbiota, and interestingly, the gut microbiota of the newborn will closely resemble the microbiota encountered during birth. During a vaginal birth, the infant is inoculated with vaginal microbiota, which differs from skin microbiota encountered during a caesarian section.¹⁴ At the phylum level, studies have shown that infants delivered vaginally have a larger population of Bacteroidetes than Firmicutes when compared with infants delivered via cesarean section.¹⁵

Despite exposure in utero, the majority of microbes that will colonize the infant gut are acquired post partum. The initial colonization pattern is thought to be chaotic, and increasing evidence suggests that environmental exposures early in life, including diet, are responsible for these variations.¹⁶ Gut colonization in an infant occurs in a succession of stages. Early on, the gut becomes colonized by primarily aerobic organisms, such as enterobacteria, staphylococci, and streptococci, many

of which have the potential to be pathogenic. These early colonizers begin to change the gut environment, paving the way for colonization by an increasingly anaerobic community of microbes.¹⁷ The structure of the gut community continues to change over the first year of life and thereafter in response to external factors such as diet and antibiotic use.^{18,19} Weaning, breastfeeding status, and the successive introduction of different types of food all correspondingly affect the infant gut microbiome and immune system.²⁰

Infant Feeding Method

Breast milk is the optimal food for infants, as it meets all their nutrition and physiologic requirements. Human milk contains protein, fat, and carbohydrate as well as immunoglobulins and endocannabinoids.²¹ Breast milk is not sterile, as it contains as many as 600 different species of bacteria including beneficial *Bifidobacterium breve*, *Bifidobacterium adolescentis*, *Bifidobacterium longum*, *Bifidobacterium bifidum*, and *Bifidobacterium dentium*.²² In addition to containing lactose, the carbohydrate component of human milk also contains oligosaccharides, which comprise the third largest solid component.²³ Human milk oligosaccharides are indigestible polymers formed by a small number of different monosaccharides that serve as prebiotics by selectively stimulating growth of members of the genus *Bifidobacterium*.²³ Some of the beneficial effects of breast feeding over formula feeding are attributed to the effects of oligosaccharides on the beneficial bacteria, as an increased proportion of Bifidobacteria is noted in breast-fed infants compared with those fed formula.²⁴ Bifidobacteria have been linked with strengthening gut mucosal protection through activities against pathogens.²⁵ Bifidobacteria have been shown to increase the production of immunoglobulin A, which is correlated with modulation of the intestinal immune system.²⁶

Schwartz et al²⁰ studied breast-fed and formula-fed infants and their mothers. Stool samples from each infant were collected; microbial DNA was extracted and sequenced; and messenger RNA (mRNA) was isolated from stool containing host gut exfoliated epithelial cells and processed for microarray analysis. The investigators found the microbiome of breast-fed infants to be significantly enriched in genes associated with virulence functionality and demonstrated a multivariate correlation between the gut flora genes associated with bacterial pathogenicity and the expression of host genes associated with immune and defense mechanisms. Interestingly, the operational taxonomic unit (OTU) composition and genetic potential of the microbiota differed between breast-fed and formula-fed infants. The researchers suggested their findings indicate that human milk promotes the mutualistic crosstalk between the mucosal immune system and the microbiome in the maintenance of intestinal homeostasis. More research is needed to better determine the mechanisms by which Bifidobacteria produce these effects.

Aerotolerance of the intestinal microbiota also seems to differ between breast-fed and formula-fed infants. Aerobic organisms are more common in the feces of breast-fed infants, whereas anaerobic and facultatively anaerobic organisms, which preferentially use anaerobic glycolysis, are more frequently identified in feces of formula-fed infants.²⁷ Bacteroides and Clostridia colonization differs between the 2 types of feeding, with breast-fed infants characterized with lower concentrations of both.²⁸⁻³⁰

With a better understanding of the composition of human breast milk, developments of complex infant formulas have attempted to mimic its nutrition value, making them an acceptable alternative for mothers unable to breast-feed. Infant formulas are not a perfect substitute for human milk, since they lack bioactive compounds contained in breast milk known to affect nutrient absorption and digestion, immune protection, and defense against potentially pathogenic microbes.²¹ Unfortunately, it is difficult to mimic the actions of these bioactive compounds. While infants fed a formula enriched with oligosaccharides have been shown to harbor more Bifidobacteria in the feces, more evidence is needed for affirmation that infant formulas designed to mimic breast milk are beneficial.³¹

Changes in the Gut Microbiome With Age

Revealed by metagenomic analysis, gut microbiota composition transforms throughout early stages of human development and is influenced by the diet.³² When an infant's diet comprises breast milk and formula, this is reflective in that the microbiome has minimal diversity and is enriched in genes to facilitate lactate utilization.³³ A shift in the functional capacity to preferentially use plant-derived glycans occurs prior to the introduction of solid foods. When a child is around 3 years of age, the bacterial composition resembles that of an adult and remains stable until old age, when variability in community composition increases.³⁴ In terms of ecological succession, the *Bifidobacterium*-dominated microbiota of the infant changes over time into the Bacteroidetes- and Firmicutes-dominated microbiota of the adult.³⁵ This distribution remains fairly stable throughout adulthood in the absence of perturbations, such as long-term dietary changes or repeated antibiotic usage.

Declines in dentition, salivary function, digestion, and intestinal transit time may affect the gut microbiota upon aging.³⁶ There are notable differences in the microbiota in elderly people compared with young adults, with relative proportions of Bacteroidetes predominating in elderly people compared with higher proportions of Firmicutes in young adults.³⁷ Elderly people are also noted to have significant decreases in Bifidobacteria, Bacteroides, and *Clostridium* cluster IV.³⁸ Variability varies greatly among individuals, ranging from 3% to 92% for Bacteroidetes and 7% to 94% for Firmicutes.³⁴ However, the microbiota is less variable within individual subjects.³⁵

Alterations in gut microbiome are associated with health concerns pertaining to elderly people, such as frailty. A significant reduction in microbial diversity, with reduced composition of Lactobacilli, Bacteroides/Prevotella, and *Faecalibacterium prausnitzii* and increased proportions of *Ruminococcus*, *Atopobium*, and Enterobacteriaceae, was observed in people with high frailty scores.³⁹ Claesson et al³⁹ investigated the links between diet, environment, health, and microbiota in 178 older people (≥ 65 years) and found an association between gut microbiome diversity and functional independence. Decreased microbial diversity was noted in individuals living in short- or long-term residential care compared with those living in the community, and this difference was associated with increased frailty, decreased diet diversity, and health parameters as well as increased inflammatory markers (serum tumor necrosis factor- α , interleukin [IL]-6, IL-8, and C-reactive protein).³⁸ Dietary patterns in residential location correlated with separations based on microbiota composition, with the most discriminating food types being vegetables, fruit, and meats. Complete linkage clusterings revealed 4 dietary groupings: low fat/high fiber and moderate fat/high fiber included 98% of the community and day hospital subjects; and moderate fat/low fiber and high fat/low fiber included 83% of long-stay subjects.³⁹ Significant associations between several health and frailty measurements were found, with minimum variability among community dwellers, but within the long-stay subjects the most significant associations were related to functional independence, Barthel index (functional assessment), and nutrition, followed by blood pressure and calf circumference. The authors speculated that the latter may be attributable to the influence of diet and/or the microbiota on muscle mass and sarcopenia and hence frailty.

Influences on the Gut Microbiome

Geography

Geographical location and ethnicity have been shown to be determinants of the diversity and overall composition of microbiota. A 2013 study performed by Prideaux et al⁴⁰ looked at Caucasian and Chinese subjects in the United States and Hong Kong and found that microbial composition differed between countries and between ethnicities within the same country. In an elegant study, Yatsunenکو et al⁴¹ characterized bacterial species in fecal samples from 531 healthy children and adults from the Amazonas of Venezuela, rural Malawi, and U.S. metropolitan areas, including monozygotic and dizygotic twins.⁴² All populations studied showed shared features of gut microbiome development during the first 3 years of life, including age-associated changes in the genes involved in vitamin biosynthesis and metabolism. Phylogenetic composition of fecal microbiota was significantly altered between individuals living in the different countries, with pronounced separation occurring between the U.S. and the Malawian and Amerindian

gut communities; this was true for individuals aged 0–3 years, those aged 3–17 years, and adults. However, there was also separation between the non-U.S. populations. Bacterial diversity increased with age in all 3 populations, with the microbiome of the U.S. population having the least diversity. Microbiome datasets from breastfed children ($n = 110$: 24 babies [0.6–5 months old], 60 children and adolescents [6 months to 17 years old], and 26 adults) were analyzed to determine which bacterial taxa changed monotonically with increasing age within and between the 3 sampled populations. In all babies, 16S rRNA sequences mapped to members of the *Bifidobacterium* genus, and while Bifidobacteria dominated fecal communities during the first year of life, they proportionally declined during this period. Also noted were age and population-related changes in metabolism. A total of 476 enzymes were identified as being significantly different in U.S. versus Malawian and Amerindian breastfed babies ($P < .001$). The most prominent differences involved pathways related to vitamin biosynthesis and carbohydrate metabolism. Malawian and Amerindian babies, but not adults, had higher representation of enzymes that were components of the vitamin B₂ (riboflavin) biosynthetic pathway. Compared with adult microbiomes, baby microbiomes were enriched in enzymes involved in the scavenging of glycans represented in breast milk and the intestinal mucosa (mannans, sialylated glycans, galactose, and fucosyl oligosaccharides), with several of these genes significantly overrepresented in Amerindian and Malawian baby microbiomes compared with U.S. subjects. Interestingly, urease gene representation was significantly higher but decreased with age in Malawian and Amerindian baby microbiomes, as opposed to subjects from the United States, where it was low from infancy to adulthood. Urea, which comprises up to 15% of the nitrogen present in human breast milk, is broken down by urease to ammonia, which can then be used for microbial biosynthesis of essential and nonessential amino acids. Urease also has a crucial role in nitrogen recycling, particularly when diets are deficient in protein-making urease, which is potentially advantageous to both the microbiome and host when dietary nitrogen supply is suboptimal.⁴² A typical U.S. diet is rich in protein, whereas diets in Malawian and Amerindian populations are dominated by corn and cassava. The differences between U.S. and Malawian/Amerindian microbiomes can be related to these differences in diet. The enzymes that were the most significantly enriched in U.S. fecal microbiomes parallel differences observed in carnivorous versus herbivorous mammals.⁴³ Interestingly, several enzymes involved in the degradation of amino acids were overrepresented in adult U.S. fecal microbiomes, including aspartate, proline, ornithine, and lysine, as were enzymes involved in the catabolism of simple sugars (glucose-6-phosphate dehydrogenase and 6-phosphofructokinase), sugar substitutes (L-iditol 2-dehydrogenase, which degrades sorbitol), and host glycans (α -mannosidase, β -mannosidase, and α -fucosidase). By contrast, Malawian and Amerindian microbiomes had an overrepresentation of α -amylase, which

participates in the degradation of starch, reflecting their corn-rich diet. Perhaps reflecting a fat-rich diet, the U.S. microbiomes had an overrepresentation of enzymes involved with vitamin biosynthesis (cobalamin, biotin, and lipoic acid), as well as in the metabolism of xenobiotics (phenylacetate CoA ligase), which participates in the metabolism of aromatic compounds, and mercury reductase, and in bile salt metabolism (choloylglycine hydrolase).

To obtain insights into commonalities and differences between gut microbiomes across different populations, Arumugam et al⁴⁴ sequenced 22 European metagenomes from Danish, French, Italian, and Spanish individuals who were selected for diversity and combined them with previous sequencing results from Japanese and Americans to total 39 individuals. The investigators found that despite the vast number of species residing in the gut and their interindividual variability, the microbiota composition can be classified into at least 3 distinct groups, or enterotypes. The enterotypes contain functional markers that correlate with individual features such as age and body mass index (BMI). For example, starch degradation enzymes such as glycosidases and glucan phosphorylases increase with age, which could be a reaction to decreased efficiency of host breakdown of dietary carbohydrates with age.⁴⁵ Three marker modules, 2 of which were adenosine triphosphatase (ATPase) complexes, correlated strongly with the hosts' BMI, supporting the link found between the gut microbiota's capacity for energy harvest and obesity in the host.⁴⁶ The authors concluded that these functional markers might be used for diagnostic and perhaps even prognostic tools for numerous human disorders: for instance, colorectal cancer and obesity-linked comorbidities such as metabolic syndrome, diabetes, and cardiovascular diseases.

Food Supply

Diet has emerged as one of the most relevant factors in influencing the gut microbiome. Significant and meaningful changes in the gut microbiota have been associated with dietary alterations, primarily consumption of dietary fiber from fruits, vegetables, and other plants. A diet that is varied and complex is associated with a more diversified microbiome. Globally, the microbiome composition is noted to be different among different populations and cultures. De Filippo et al⁴⁷ compared the gut microbiota of children aged 1–6 years living in a village of rural Africa, in an environment that resembles that of Neolithic subsistence farmers, with the gut microbiota of western European children (Florence, Italy) of the same age, with dietary habits and living conditions typical of the developed world. Traditionally, the African children are breast-fed until the age of 2, after which their diet is predominantly vegetarian: low in fat and animal protein and rich in starch, plant polysaccharides, and fiber. All food resources are completely produced locally, cultivated and harvested nearby the village. The dietary content of carbohydrate, fiber, and nonanimal protein is very

high. Animal protein intake is low and consists of mainly chicken and termites during the rainy season. The European children were breast-fed for up to 1 year of age, after which they ate a typical Western diet high in animal protein, sugar, starch, and fat and low in fiber. Average caloric intake differed between the 2 populations (African children: 1–2 years old, 672 kcal/d; 2–6 years old, 996 kcal/d; European children: 1–2 years old, 1068 kcal/d; 2–6 years old, 1512 kcal/d). Multiplex pyrosequencing of the 16S rRNA gene revealed that 94.2% of the sequences in all of the African and European samples belonged to the 4 most populated bacterial phyla, namely Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria, in agreement with previous studies describing such phyla as those contributing to the majority of human gut microbiota.⁴⁸ Relevant differences were found in the proportions of 4 phyla: Actinobacteria and Bacteroidetes were more represented in African than in European children's microbiota (10.1% vs 6.7% and 57.7% vs 22.4%, respectively), whereas Firmicutes and Proteobacteria were more abundant in European than in African children (63.7% vs 27.3% and 6.7% vs 0.8%, respectively). Firmicutes were twice as abundant in the European children as evidenced by the different ratio between Firmicutes and Bacteroidetes (Firmicutes/Bacteroidetes ratio \pm SD, 2.8 ± 0.06 in European and 0.47 ± 0.05 in African). While short-chain fatty acid (SCFA)-producing bacteria were found in both populations, bacteria (*Xylanibacter*, *Prevotella*, *Butyrivibrio*, and *Treponema*) that use xylane, xylose, and carboxymethylcellulose to produce high levels of SCFAs and have a protective role against gut inflammation⁴⁹ were found exclusively in the African children. Notably, the African children had higher levels of total SCFA than the European children, with 4 times higher levels of butyrate and propionate. The authors concluded that a correlation exists between polysaccharide-degrading microbiota and the calories that the host can extract from his or her diet, potentially influencing the survival and fitness of the host, suggesting that the microbiome of the African children co-evolved with their diet to assist with energy harvest by producing high levels of SCFA.

Microbial enrichment has been associated with diets high in fruits, vegetables, and fiber compared with a Western diet rich in fat, sugars, and animal protein and depleted of fiber. Zimmer et al,⁵⁰ using classic microbiological culture, analyzed the fecal flora of a large group of healthy volunteers on a strict vegetarian or vegan diet and compared them with age- and gender-matched subjects consuming an omnivorous diet. The fecal microbiota of vegetarian and vegan subjects showed significantly lower microbial counts of Bacteroides, *Bifidobacterium*, *Escherichia coli*, and Enterobacteriaceae species and lower stool pH compared with omnivores. Compared with an omnivore diet, a vegetarian/vegan diet is associated with a higher carbohydrate and fiber content in which the undigestible polysaccharides can be fermented into SCFA by the gut microbiota. Production of SCFA is associated with decreasing luminal pH. The fact that *E coli* and Enterobacteriaceae do not thrive in

lower pH ranges (5.5–6.5) and that they prefer proteins as their energy source may explain the lower counts in the subjects consuming a vegan/vegetarian diet. Depleted microbial biodiversity of the gut microbiota in people consuming a Western diet is associated with increasing incidence of obesity, coronary vascular disease, metabolic syndrome, and certain malignancies. Detailed discussion of each of these associations is beyond the scope of this paper. At a minimum, the diversity of the gut microbiome may be a future biomarker of long-term consumption of a “healthy” versus “unhealthy” diet, which may be linked to potential for disease development.

Stress

Stress is defined as an organism's total response to environmental demands or pressures. There are several different types of stressors, such as acute or chronic, acute on chronic, or repetitive acute. Stress can be predictable and controllable as well as unpredictable and uncontrollable, mild or severe, and can occur in or out of context.⁵¹ Both the perception of stress and the persistence of its consequences vary between people. Stress contributes to susceptibility to disease and disabilities, therefore representing a severe economic burden.

Psychological and physical stressors activate the hypothalamic-pituitary-adrenal (HPA) axis. This results in a series of hormonal responses including release of corticotropin-releasing hormone, which induces the release of corticotropin systemically, which then stimulates glucocorticoid synthesis (cortisol) in the adrenal cortex.⁵² Additionally, catecholamines (noradrenaline and adrenaline) are released following psychological and physical stressors. The GI tract and, more recently known, the gut microbiota are sensitive to stress and stress mediators. Enteric bacteria respond to the release of stress-related neurochemical mediators by the host, which can influence the response to a bacterial infection.⁵³ Recent theory suggests that bacteria act as delivery vehicles for neuroactive compounds and therefore can affect the physiologic characteristics of the host by providing neurochemicals.⁵⁴

Physiological Stress—Exercise

High-intensity exercise is a physiological stressor that can lead to GI distress. Some reports indicate that 30%–90% of distance runners have experienced intestinal problems related to exercise.⁵⁵ The degree of intestinal distress can range from mild to severe, and symptoms include nausea, vomiting, abdominal angina, and bloody diarrhea. High-intensity training has been associated with reduced GI blood flow, tissue hyperthermia, and hypoxia, leading to possible alterations of microbiota and gut barrier. Athletes often have different dietary patterns from nonathletes depending on the type of activity in which they participate. In a study of professional rugby players during a regulated environment of preseason training, Clarke et al⁵⁶ demonstrated the impact of exercise and associated dietary

changes on the gut microbiota. Enhanced gut microbial diversity was significantly higher and was positively correlated with increased exercise and dietary protein intake in athletes compared with size-matched (high BMI, $\sim 30 \text{ kg/m}^2$) and age- and gender-matched (lower BMI, $<25 \text{ kg/m}^2$) nonathletic control groups, with fewer differences seen between the 2 control groups. Interestingly, when compared with controls, the athletes also exhibited lower inflammatory markers and improved metabolic markers. Athletes consumed more calories, with a higher percentage of protein, and “grazed” when eating throughout the day compared with nonathletes. Microbiota diversity measures were positively correlated with protein intake and plasma creatine kinase levels, a marker of extreme exercise, suggesting that both diet and exercise influenced changes in microbial diversity. The athletes’ increased microbial diversity was reflected by the presence of representatives of 22 phyla of bacteria in contrast to 11 and 9 phyla in the low- and high-BMI controls, respectively.

Several potential mechanisms may explain how physical activity and fitness might modify the microbiota. Abrupt exercise produces multiple metabolites and inflammatory mediators, whereas habitual exercise and fitness lead to suppression of basal inflammatory cytokines, suggesting a regulatory loop between exercise biology and host immunity.⁵⁷ Regular exercise has an anti-inflammatory effect, which improves the immunological profile in type 2 diabetes mellitus, coronary artery disease, peripheral arterial disease, and obesity.⁵⁸⁻⁶¹ In animal models, repeated exercise results in reduced proinflammatory cytokine expression and increased anti-inflammatory IL-10 expression.^{62,63} Regular exercise also decreases colonic oxidative insult in a rat model of colitis.⁶⁴

Contrary to the benefits of regular exercise, prolonged excessive exercise can negatively affect intestinal function. As high-intensity exercise can lead to prolonged intestinal hypoperfusion, intestinal ischemia may ensue. Increased intestinal permeability can result, making the gut susceptible to endotoxin translocation.⁶⁵⁻⁶⁷ Probiotic supplements, in conjunction with other dietary strategies, have been studied in athletes as a means to improve gut health, as characterized by decreased symptoms of nausea, cramping, bloating, and diarrhea as well as improved immunity. Primarily supplements containing *Lactobacillus* and/or *Bifidobacterium* species have been used. Products have been provided from 1 to 6 months, before and/or after exercise or a competition, and at varying doses (10^9 – 10^{12} colony-forming units per day). Some studies report clinical results of improved outcomes of upper respiratory tract illness⁶⁴⁻⁶⁹ and GI illness⁷⁰⁻⁷² as well as immunological measures and outcomes.^{67,68,73-76}

Physiological Stress—Critical Illness

As seen in high-intensity exercise, intestinal hypoperfusion resulting from redistribution of splanchnic circulation severe enough to cause gut ischemia and mucosal injury often occurs

in the critically ill. In the critically ill, the gut has an important role in promoting infectious complications and multiple organ dysfunction syndrome. This is due to deteriorated intestinal epithelium, altered gut immune system, and dysfunctional metabolic activities of commensal bacteria.⁷⁷ Shimizu et al⁷⁸ evaluated the gut microbiota and gut environment (fecal pH and presence of organic acids) in patients with systemic inflammatory response syndrome (SIRS). In comparison to healthy controls, patients with severe SIRS had significantly lower total anaerobic bacterial counts (especially 2- to 4-log fewer commensal *Bifidobacterium* and *Lactobacillus*) and 2-log higher potentially pathogenic *Staphylococcus* and *Pseudomonas* group counts. Concentrations of total organic acids, in particular the SCFAs acetate, propionate, and butyrate, were significantly decreased in the patients, whereas pH was markedly increased. This group of researchers further investigated the impact of fecal pH in critical illness in 138 trauma patients.⁷⁹ Patients with acidic or alkaline feces were noted to have decreased *Bacteroides* and *Bifidobacterium* species. The incidence of bacteremia in patients with an acidic or alkaline fecal pH was significantly higher than those with a fecal pH in the normal range ($P < .05$ vs normal range). The incidences of both bacteremia and mortality were associated with an increased pH of 6.6. When the pH level was increased or decreased by 1, the incidence of bacteremia more than tripled and mortality more than doubled. Total SCFA (propionate, butyrate) concentrations decreased with pH >6.6 ; lactic, succinic, and formic acids were increased in acidic feces, which is notable as these are produced by Enterobacteriaceae.⁸⁰ Whether these changes are a cause or consequence of SIRS is yet to be determined. While these findings suggest that fecal pH could be a risk factor marker, the study has limitations because the investigators used culture-based interrogation of the microbiota and because the pH was not specified by GI region. Alterations in the gut microbiome have been shown to occur within 6 hours of a metabolic insult, and the microbiome fails to return to microbial patterns seen in healthy controls.⁸¹

Numerous investigators have attempted to modify the gut microbiome and improve clinical outcomes in the critically ill by providing probiotics, prebiotics, or synbiotics.⁸²⁻⁸⁹ Details of these studies are beyond the scope of this review. Methods of dosing, supplement duration, and supplements used vary among studies, making general recommendations difficult. Several meta-analyses and systematic reviews have been performed evaluating probiotics in the critically ill.⁸⁹⁻⁹¹ The choice of the studies included in the analysis will determine the overall outcome of the study. Generally, it seems that probiotic supplementation in the critically ill favorably improves outcomes. However, the issues of heterogeneity and lack of good-quality studies are highlights of systematic reviews of probiotic use in critically ill patients. Given that unrecoverable gut dysbiosis occurs early after metabolic insult and considering the potential mechanisms of action of certain probiotic strains, further rigorous study on probiotic provision in the critically ill deserves attention. Future

studies should provide (1) clarification of viable probiotic strain(s) provided and their dosage, route, and timing of delivery; (2) clear definitions for the outcomes that investigators are attempting to modify (ventilator-associated pneumonia, diarrhea); and (3) data on the effect of probiotic supplementation on meaningful clinical endpoints (mortality, duration of mechanical ventilation, other infections, length of stay).

Psychological Stress

The discovery of hormonal regulation of digestion initiated the concept of the gut-brain axis.⁹² The interaction of psychological factors and altered gut function via the gut-brain axis, where brain and gut symptoms reciprocally influence each other's expression, is a conceptual framework of functional GI disorders (irritable bowel syndrome [IBS], functional dyspepsia). Early life stressors (eg, psychological, sexual, and/or physical abuse) have been suggested as important contributors to the pathogenesis of functional GI disorders.⁹³⁻⁹⁶ This is a crucial developmental period when the gut microbiota is diversifying, thus making the gut particularly vulnerable to these stressors.

Animal models of maternal separation, a model commonly used for early life stress, induce prolonged HPA axis hyperactivity,⁹⁷⁻¹⁰³ anxiety-like behavior,^{101,104-106} visceral hypersensitivity,¹⁰⁷⁻¹¹⁰ and altered cholinergic activity in the gut,^{111,112} along with increased intestinal permeability.^{107,113-116} This model results in a dysfunctional gut-brain axis and produces phenotypes found in patients with IBS.^{106,116-118} This animal model also yields gut dysbiosis in addition to other physiological and behavioral features of IBS.¹¹⁸⁻¹²⁰ Various means to restore gut dysbiosis with probiotics have been investigated and appear to improve the negative stress-induced effects.^{107,111,120-122} Morphologic changes also result from maternal separation, with increased goblet cells in the crypts of proximal colon and a subsequent increase in mucus secretion and a thinner mucosal layer.¹¹² Thus, changes in the gut microbiota in maternally separated animals result from changes to physiological¹¹²⁻¹¹⁴ and morphological¹¹² characteristics of the gut.

Although the exact mechanisms are unknown, data suggest that stress, whether acute or chronic, creates a dysbiotic gut microbiome which then may induce anxiety and depression.⁵² It seems that metabolites produced by the gut microbiota might modulate brain biochemistry and behavior.^{54,123-125} Through regulation of the vagus nerve, behavior can be altered by the gut microbiota, which then affects neurotransmitter metabolism^{126,127} or other undefined pathways.¹²⁸ Thus, future therapy for patients with IBS with comorbid depression or anxiety may include targeted probiotics and/or synbiotics, either alone or as an adjuvant to traditional therapy.

Pharmaceuticals

The pharmacokinetics of most commonly used drugs vary substantially, as do the responses of ill persons to therapeutics.

The gut microbiota assists in the conversion of inactive therapeutics (eg, prodrugs) and dietary bioactives into their active forms.¹²⁹⁻¹³¹ For example, a medication prescribed for ulcerative colitis, sulfasalazine, remains inactive until it reaches the distal gut. This prodrug consists of an anti-inflammatory 5-aminosalicylic acid (5-ASA) molecule connected to a sulfapyridine molecule through an N-N double bond. Gut microbiota encoded azoreductases cleave the N-N double bond to release active 5-ASA.¹³¹ Likewise, foods such as fruits, vegetables, cereals, and coffee contain conjugated hydroxycinnamates, antioxidant and anti-inflammatory compounds that are activated following microbial biotransformation.¹³² Therefore, the question arises whether variation in medication response among people is due to alterations in their gut microbiome.

The host possesses many mechanisms to protect against ingested pathogens, including an acidic gastric environment, optimal bile flow, peristalsis, and the gut microbiota. Current data suggest that the gut microbiota protects the host from pathogens by competing for binding sites and nutrients and by direct inhibition through release of inhibitory molecules.¹³³ When these protective mechanisms are disrupted, an imbalance in the gut microbiota can occur.

Gastric Acid Suppression

Several conditions such as intestinal dysmotility, altered GI anatomic features, immune deficiencies, and hypochlorhydria¹³⁴ are predisposing factors for the development of small intestinal bacterial overgrowth (SIBO).

While hypochlorhydria can be a result of *Helicobacter pylori* colonization and aging, many people also take medications to reduce their gastric acidity for stress ulcer prophylaxis or gastric esophageal reflux disease. The pH of gastric acid in the stomach lumen is normally 1.5–3.5; maintaining intragastric pH above 3.5–5.0 prevents gastric mucosal injury and may be facilitated with histamine 2 receptor antagonists, sucralfate, and proton pump inhibitors (PPIs).¹³⁵ PPIs are known to alter the GI bacterial population in 50% of patients on long-term treatment with any type of effective antisecretory drug.^{135,136} Lombardo et al¹³⁷ conducted a study in 200 patients with gastroesophageal reflux disease receiving PPI treatment, 200 patients with IBS not receiving PPI treatment, and 50 healthy control subjects not receiving PPI treatment >10 years and showed that SIBO occurs more frequently in long-term PPI users than patients with IBS or control subjects. The investigators found that the incidence of SIBO was significantly different ($P < .001$) in patients using PPIs (50%) compared with patients with IBS not using PPIs (24.5%) and healthy control subjects (6%). According to a multivariate analysis including 5387 elderly subjects, the presence of diarrhea was significantly associated with use of PPIs (odds ratio [OR] 2.97; 95% confidence interval [CI], 2.03–4.35) and antibiotics (OR 4.58; 95% CI, 1.95–10.73).¹³⁸ Theisen et al¹³⁹ found that the suppression of gastric acid with omeprazole led to a high prevalence of

SIBO, leading to a markedly increased concentration of unconjugated bile acids. Conversely, a retrospective chart review completed in 1191 patients with glucose hydrogen breath testing (GHBT) evaluated whether this value positively correlated with patients receiving PPI therapy.¹³⁴ Interestingly, GHBT was positively associated with older age and antidiarrheal use but not with PPI use. Therefore, the authors concluded that PPI use was not associated with the presence of SIBO as measured by GHBT. Lombardo¹⁴⁰ challenged these results, making a point that PPI use has become very common, both prescription and over-the-counter forms, and many patients may not consider nonprescription use as a medication and not disclose it to their physician. The authors were unable to confirm actual use of PPIs due to the nature of a retrospective chart review. Additionally, because PPI duration is related to the incidence of SIBO, the lack of knowledge regarding how long the patients had taken a PPI leads to a speculation: If they only took the PPI for a short time, would it have an influence on GHBT results? Given the protective role that gastric acidity has in regard to protecting the host against ingested pathogens, it is plausible that prolonged use of gastric acid suppressants contributes to the incidence of SIBO and that clinicians should be judicious in recommending their use.

Antibiotics

Antibiotic therapies target not only pathogenic microorganisms but also the host-associated microbial communities in the gut. Most antibiotics have broad-spectrum activity, so they can be used to treat many diseases. Thus, although antibiotics are designed to target pathogenic organisms, related members of the microbiota are also affected, leaving a lasting negative effect on the gut microbial community long after use of the antibiotics has ceased.¹⁴¹ Antibiotics can also promote the expansion of antibiotic-resistant strains, which can act as a reservoir for resistance genes in the gut microenvironment.¹⁴² Decreased diversity in the microbiome typically follows antibiotic treatment, and even though most of the microbiota returns to pretreatment levels, some members are lost from the community indefinitely.¹⁴² As microorganisms can be dependent upon other colonizers for the provision of nutrition, secondary metabolites, or removal of toxic waste products, alterations in microbial codependence can leave detrimental effects.¹⁴³ The antibiotic spectrum of activity will influence the shift in gut microbiota composition. Additionally, the administration dose of the antibiotic is important in determining the ecological impact it will have on the microbiota. While the subtherapeutic antibiotic dosing often used by the agriculture industry prophylactically and to promote animal growth does not reduce the total bacterial mass, this use is criticized as it shifts the composition of the microbiota and promotes dangerous levels of antibiotic resistance.¹⁴⁴ Similarly in humans, antibiotic therapeutic doses are designed to minimize these effects, but despite these efforts, subsets of the microbiota can shift to

that of increased colonization by opportunistic pathogens such as *Clostridium difficile* and *Candida albicans*. Noteworthy is that these effects of antibiotic delivery are not limited to oral delivery. Intravenously delivered antibiotics can affect the gut microbiota as they become incorporated into bile and secreted into the intestine via the biliary system.¹⁴⁴

Gut microbial community alterations can result in dysregulation of host immune homeostasis and an increased susceptibility to disease. Host-microbial interactions are very specific, and antibiotic therapy causes alterations or loss of highly coevolved processes.^{145,146} Antibiotic-induced changes that are important to microbial regulation of host immunity include loss of bacterial ligands that are recognized by the host, loss of specific bacterial signals, and alterations in the metabolites produced by the microbiota such as SCFAs.¹⁴⁷ SCFAs are beneficial for gut health because they serve as a primary fuel source for the colonocyte, are involved with water and electrolyte absorption, help to maintain the intestinal barrier, and modulate cell proliferation, differentiation, growth, and apoptosis.¹⁴⁷ Provision of butyrate during broad-spectrum antibiotic therapy mitigated the negative effects of antibiotics on gut health, notably the mRNA and protein expression for several anion exchangers, butyrate receptor, and transports as well as protein expression of intestinal tight junction proteins.¹⁴⁸

Changes in gut immunity increase host susceptibility to infection by pathogens. For instance, treatment with metronidazole, an antibiotic that targets anaerobic bacteria, reduces the integrity of the mucus layer and accelerates mucosal attachment of *Citrobacter rodentium*.¹⁴⁹ Changes in gut microbiota structure and function after antibiotic treatment create a metabolic environment that favors *C difficile* germination and colonization and associated infectious diarrhea.¹⁵⁰ While antibiotic therapy is the major risk factor for *C difficile* colonization, the accepted treatment for *C difficile* infection is further antibiotic therapy, initially with metronidazole and then vancomycin, if *C difficile* persists.¹⁵¹ Repeated antibiotic use is often necessary in the treatment of recurrent *C difficile* infections, which unfortunately causes further disruption of an imbalanced microbiota.¹⁵² Chang et al¹⁵² identified that patients with recurrent *C difficile* infection possessed a microbiota characterized by markedly reduced overall diversity compared with controls and with patients who had an initial episode of the infection. *C difficile* infection is debilitating to patients and extremely costly, with symptoms ranging from diarrhea to fulminant colitis, toxic megacolon, and death.¹⁵³ Fecal microbiota transplantation (FMT) is the process by which a homogenized stool sample from a healthy donor is administered into the GI tract of an individual. This therapy is now becoming a sought-out treatment to eradicate *C difficile* colonization and correct gut dysbiosis. FMT has been shown to be a successful treatment option to eliminate recurrent and refractory *C difficile* infection and to reestablish a healthy colony in certain patients.¹⁵⁴ With its 91% primary and 98% secondary cure rates, FMT is gaining

increased interest among researchers as a treatment option for other GI disorders.¹⁵⁴

Perspective and Future Directions

With technological advances, knowledge regarding the gut microbiome has expanded from earlier beliefs.^{155,156} The topics covered in this review are examples of what is currently understood regarding how the human gut microbiome is established and how certain life encounters alter the composition, but they are by no means inclusive. The influence of the gut microbiome on health and disease is one of the most exciting areas of science. Learning more about how breaches of the gut microbiome early in life, coupled with further insults throughout life, can influence health may provide new insights for treatments of many challenging syndromes and diseases (eg, fibromyalgia, irritable bowel disease, and autism). We are just beginning to uncover how things we ingest can influence pathological processes. Future research should be directed to the gut microbiome as a biomarker for dietary intake and disease development, the effect of probiotic supplementation on clinical endpoints, and bacterial therapy for gut health, immunity, and therapeutic treatments. Newly available and developing technologies to uncover the metabolon provide the path of exploration to this exciting frontier. The horizons are broad, with exciting developments sure to come.

Statement of Authorship

G. A. Cresci and E. Bawden contributed to conception/design of the work; contributed to acquisition, analysis, and interpretation of the data; drafted the manuscript; critically revised the manuscript; gave final approval; and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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