EDITORIAL

Diversity: From Diet to Flora to Life

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Discussions of diversity bring about a wide variety of social, political, emotional, and intellectual responses. Biodiversity, or biological diversity, describes the variety and population of all life forms in a given place—plants, animals, bacteria, viruses, fungi, etc. Biodiversity also describes the structures and functions that sustain this variety and allow it to adapt to changing environmental circumstances. The human gastrointestinal microbiota is one of the most complex and diverse ecosystems on the planet, with each of us offering a home to more than 100 trillion organisms, 150 to 300 species, 100 times more DNA than each human cell, and an array of essential functions without which we could not live.

I live in the Blue Ridge Mountains of western North Carolina, one of the richest and most diverse broadleaf temperate forests in the world. With more than 158 species of trees (more than there are in all of Europe), more than 4000 species of plants and 2000 species of fungi, my home ranks highest amongst the ecosystems for total floral diversity as well as the highest total of flora and fauna species in North America. The unique microhabitats and geologic stability that fostered this biodiversity have given way to modernity, and we have seen more that 80% of the habitat in this ecosystem altered over the past 100 years. So too we have seen alterations in the diversity of the gut microflora within the human inhabitants over the same period of time.

Like the Blue Ridge, the (micro)organisms within human gastrointestinal tract create a diverse ecosystem. Interacting with the human body, the "gut microbiome" provides for a variety of functions, including: providing energy for gastrointestinal cells, supporting digestion, synthesizing vitamins, educating the adaptive immune system, acting as modulators of inflammation, inhibiting pathogenic bacteria via biofilms, antimicrobial peptides and competition for nutrients, and stimulating immune response. In communicating with my patients, I use the example of when we go for "a walk in the woods" we observe a loss of diversity within the flora of these hills and mountains. We see imbalance within the ecosystem as manifested in changes, such as the American Chestnut blight of the last century (a fungus killed more than 4 billion trees) and the parasitic Hemlock Woolly Adelgid, which has killed more than half of the hemlock trees in the past 30 years. When there is loss of diversity within the gut microflora, we find this imbalance leads to myriad diseases within our patients.

Only 10 years ago it was believed that, once established in infancy and early childhood, the gut microflora was stable within an individual throughout his or her life. It has been noted that a variety of factors affect the early development, distribution, and diversity of microflora, including: family history, gestational age, mode of delivery, place of delivery, type of infant feeding, age of solid food intake, exposure to antibiotics, and early dietary exposure.[1] Early colonizers are often aerobes such as *Staphylococcus*, *Streptococcus*, and *Enterobacteria*, followed later by anaerobic colonizers such as *Clostridia*. After these early stages, the microbiota of breast-fed infants is dominated by *Bifidobacteria*. With weaning, the introduction of solid food promotes a stable adult-like microbiota, characterized by a remarkable biodiversity. Patients followed longitudinally are noted to have increasing diversity and stability across the principal phyla: Major = Bacteroidetes (including *Prevotella*) and *Firmicutes* (including *Clostridia*, *Lactobacilli*, and *Streptococci*); Minor = Actinobacteria (including *Bifidobacteria*), Proteobacteria (including *Enterobacteria*), and Verucomicrobia.[2] Methanogenic archaea, eucaryotes (mainly yeast), and viruses are also present.

Culture-based studies have led us to assume that humans share most of the same species of gut microbiota. However, the Human Microbiome Project has demonstrated that there is *not* a uniform "core microbiota" with theme and variation. To illustrate this point, while each person carries at least 160 of the nearly 1500 species identified, there was not a single species of bacteria that was present in every one of the 242 individuals studied *and* most healthy people carried some quantity of pathogenic bacteria.[3] In contrast, metabolic pathways are stable and there is a consistency across the functional components of the microbiome (metabonomics) even though diversity and abundance vary widely across the taxonomic distribution (metagenomics) of microbiota. In healthy people, the microbiome supports functions of digestion, motility, carbohydrate degradation, vitamin and amino acid synthesis, short chain fatty acid (SCFA) production, immune modulation, detoxification, bile acid metabolism, etc—all of which occur despite the fact that there are vast differences in microbiota distribution across individuals.[3] When comparing with environmental ecosystems, we can see other temperate broadleaf forests in eastern China that have many similar functions but are comprised of different species that have evolved independently. There is more than one way to maintain health and balance.

DeFillippo et al followed up on the early work of Gordon and Ley[4] to evaluate the co-evolution of microbiota across different human diets. The gut microbiome of children in Burkina Faso (whose diet was low in fat and sugar, and high in plant starch, fiber, and polysaccharides) were compared with those of children in Italy

(whose diet was high in animal protein, fat, sugar, and refined starch and low in fiber). The African fecal microbiome contained genes that could break down cellulose and plant fiber and consisted of higher amounts of Bacteroidetes phyla, compared with higher quantities of the Firmicutes phyla within the Western European children.[5]

The overall composition of the gut depends on the age, health, diet and even geographical location of the individual.[6] We see that the distribution and diversity of the gut microbiome is principally mediated by our diet and nutritional status. Claesson and the Cork ELDERMET Project performed a unique analysis of elderly patients in different settings, from community to hospital to short-term rehabilitation to long-term care facilities. They were able to observe that as microbiota diversity declined, so did health status. Further analysis showed that these microbiome changes across institutions correlated with decreasing dietary diversity, as defined by the Healthy Food Diversity index. Diet-driven microbiome change correlates with health declines (ie, frailty) in aging.[7]

As seen in the aforementioned elderly and cross-cultural evaluations, Wu also found that the microbiota composition in Americans was associated with diet—ie, *Bacteroides* was more prevalent in animal-based diets and increased *Prevotella* was associated with plant-based diets.[8] The European METAHIT Consortium (similar to the US Human Microbiome Project) postulated in 2011 the idea of three distinct "enterotypes,"[9] a gastrointestinal analogue to common blood types, but further analysis and debate have blurred this distinction.[10] As seen in the above studies, the animal-based diet correlates with the *Bacteroides* enterotype and the plant-based diet is associated with the *Prevotella* enterotype. The proposed *Ruminococcus* enterotype now seems less likely to be a separate entity and has been bundled by some researchers as a variant in the animal-based diet with *Prevotella* predominance.[11]

Long-term dietary patterns clearly influence the taxonomy and the functional capacity of the microbiome. It is now clear that a short-term macronutrient change (two diets, 5 days each + washout period[12] was able to rapidly change the gut microbiome; with reductions in *Prevotella* on the animal-based diet as well as increases in the secondary bile acid, deoxycholic acid (DCA), which is derived from microbial metabolism. (See the related case report in this issue.)

With the growing awareness of gut microbiome composition and function, experiments have begun to emerge that evaluate microbiome diversity (also referred to as "richness") to stratify metabolic risk profiles. Low richness/diversity of gut microbiota has been reported in patients with inflammatory bowel disorder (IBD), elderly patients with inflammation and in obese individuals, but the differences of richness within these groups or among non-obese individuals were not previously detected. As the composition of the gut microbiota seems to be rather stable over long periods of adulthood, its richness may well be a characteristic feature of an individual.[13] Further studies have extended these assessments of diversity (ie, richness) to demonstrate that dietary interventions improve genomic richness (high gene count vs low gene count) and alleviate phenotypic indicators of inflammation.[14]

These studies have established that there are multiple mechanisms to evaluate diversity. Research measures of alpha diversity (mean species diversity within a habitat) and beta diversity (the diversity between habitats) are useful when measuring 100s of species and 100s of metabolites. To date, these research measures do not provide the essential clinical tools to evaluate gut microbiome diversity amongst and within patients. This is a curious consideration, for we do recognize that macroscopic balance will recapitulate the microscopic ecosystem holographically.

To wit, current attempts to measure aerobic and facultative anaerobic bacteria from stool culture give us only a small window into the overall richness and abundance of the gastrointestinal microbiota. The gut microbiota is a robust system that interconnects and is controlled by multiple factors and at multiple levels. When the entire system is out of balance (ie, decreased alpha diversity), we find that measures such as stool culture, DNA probes, distribution of short chain fatty acids,[15] and β-glucuronidase activity[16] can give us a window into the richness/diversity and abundance of the entire gut ecosystem. The clinical implications of this are vast, as we can gain insight into human-microbe homeostasis via measures of bacterial function (SCFA,[17] β-glucuronidase); microbiologic identification of aerobes/anaerobes (culture, MALDI-TOF); and 16S-RNA–based measures (FISH, tRFLP, PCR, MicroArray, PyroSequencing)[18] of bacterial organizational taxonomic units (OTUs), phyla, genera, species, and strains.

So too, when we take our walk in the woods, we can feel the difference between an old growth forest and a second (or third) growth forest even if we are unable to identify a single species. This has implications for recognizing imbalance of the gut microbiota through clinical symptoms, such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), atopic illnesses like eczema and asthma, and numerous autoimmune diseases. These clinical syndromes and diseases have imbalanced gut microflora (often termed *dysbiosis*) as an essential, though not necessarily causative, factor.

As we consider the entire system of the gut microbiome, we recognize that point-source interventions, such as a singular probiotic, are not likely to provide a sufficient leverage point to shift the entire system. Likewise, antibiotics can cause widespread changes within the gastrointestinal tract and microbiome but they do not tend to alter the underlying conditions (diet, environment, stress, genes, etc) that provided the conditions for imbalanced bacterial growth in the first place. While antibiotics and probiotics may alter the

distribution of gut microbiota in the short term, only few studies have demonstrated long-term clinical effects of these agents; and when they have, such as the impressive anti-inflammatory effects of VSL-3 in ulcerative colitis,[19] it is with massive doses (3.6 trillion colony forming units) and multiple (8) pleomorphic species/ strains.[20] As we unravel the molecular basis of human-microbe interactions, we are beginning to understand how to select probiotics for different indications based upon their metabolic, antiinflammatory, immune-modulatory, and antimicrobial effects.[21]

Diversity promotes self-organization and healing within an ecosystem; it is a source of evolutionary potential, innovation, resilience, opportunity, and change. Promoting diversity requires us to look at the components that provide the context for diversity to arise initially. When we evaluate the development of the gut microbiome, we see that diversity and stability arise between the ages of 2 to 4 years—at the time when children begin to eat solid food! Diet is the ultimate driver of diversity of the gut microbiota, as noted by phenotypic (ie, metabolomics) and structural/ population (ie, metagenomics) assessments. One review notes that dietary changes explain 57% of the total structural variation in gut microbiota, whereas changes in genetics accounted for no more than 12%.[22] Western high-sugar diets alter populations and transform healthy gut microbiota toward disease-inducing configurations,[23] while the fermentation of prebiotic foods can produce a significant shift in the gut microbiota toward balance.[24]

It should come as no surprise to any of us that "We are what we eat." The myriad gastronomic delights and availability of foods from any ecosystem across the world at any moment has moved the human body and its microbiome away from a direct relationship to "eating right with the seasons."[25] The diversity of phytonutrients across a spectrum of type, color, source, and preparation is necessary to promote the diversity of microbiota, which is necessary for the redundancy and resiliency necessary to optimize gut function. In this manner, we move upstream—from the gut to the mouth to the diverse and beautiful bounty of nature—to provide the FOOD necessary for health and well-being. Enjoy!

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