Diet, Gut Enterotypes and Health: Is There a Link?

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Abstract
The human gut contains a vast number of microorganisms known collectively as the ‘gut microbiota’. Despite its importance in maintaining the health of the host, growing evidence suggests the gut microbiota may also be an important factor in the pathogenesis of various diseases, a number of which have shown a rapid increase in incidence over the past few decades. Factors including age, genetics, and diet may influence microbiota composition. We used diet inventories and 16S rDNA sequencing to characterize fecal samples from 98 individuals. Fecal communities clustered into previously described enterotypes were distinguished primarily by levels of Bacteroides and Prevotella. Enterotypes were associated with long-term diets, particularly protein and animal fat (Bacteroides) versus simple carbohydrates (Prevotella). Although the distinction of enterotypes as either discrete clusters or a continuum will require additional investigation, numerous studies have demonstrated the coexclusion of the closely related Prevotella and Bacteroides genera in the gut microbiota in healthy human subjects, where Prevotella appears to be a discriminatory taxon for residence in more agrarian societies. Ultimately, the impact of diet on the human gut microbiota may be an important environmental factor involved in the pathogenesis of disease states that show a rapidly increasing incidence in industrialized nations.

Introduction

Human microbiomes are very distinctive amongst various body sites and are composed of not only bacteria but also other microorganisms including prokaryotic organisms such as Archaea, microeukaryotes such as fungi, and viruses,
principally bacteriophage – the latter being amongst the most abundant biologic entities in the biosphere. In this review, the term ‘microbiota’ will be used to denote the compilation of bacterial microorganisms within a specific environment, whereas the ‘microbiome’ refers to not only the bacterial taxa but also their collective genomes. The human gut microbiota is a densely populated bacterial community with approximately $10^{11}$ organisms per gram of fecal weight composed of over 1,000 species, most of which are obligate anaerobes [1, 2], with a collective genome size 150-fold greater than that of its human host [1]. Although there are over 50 bacterial phyla on Earth, human-associated bacteria largely belong to one of four phyla, Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes. Mammalian hosts have coevolved to exist with our gut microbiota in a mutualistic relationship where we provide a uniquely suited environment in return for physiological benefits provided to us by our gut microbiota [3]. Examples of the latter include fermentation of indigestible carbohydrates to produce short-chain fatty acids that are utilized by the host, biotransformation of conjugated bile acids, synthesis of certain vitamins, degradation of dietary oxalates, hydrolysis of urea by urease activity, participation in host nitrogen balance and education of the mucosal immune system [3].

**Association between Gut Microbiota and Human Disease**

Despite the importance of the gut microbiota in maintaining the health of the host, growing evidence suggests that it may also be an important factor in the pathogenesis of a variety of diseases, particularly those that have shown a rapid increase in incidence over the past few decades. These include both type 1 and type 2 diabetes mellitus, atherosclerosis, asthma, colon cancer, and inflammatory bowel disease (IBD), to name a few [4]. Advances in genomic technology, principally DNA sequencing and SNP mapping used for genome-wide association studies combined with biocomputational algorithms, have revealed the genetic underpinnings of these complex disease processes. In most circumstances, the contribution of host genetics to the risk of disease development is significantly less that 50%, implicating the importance of environmental influences [5]. The observation that these diseases have shown a steadily increasing incidence over the past several decades, the geographic distribution of disease clustering in industrialized nations, and immigration studies revealing the adoption of disease risk of the host country within 1 or 2 generations, all emphasize further the importance of environment in the pathogenesis of these diseases.

Interestingly, inflammation has been strongly associated with many of these ‘westernized’ disease processes. In addition to IBD and asthma, which are prin-
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Diseases due to unrestrained immune processes, T1DM has been associated with type 1 interferon production and altered T cell signaling suggesting an autoimmune response, and insulin resistance, the hallmark of T2DM, is associated with an inflammatory response in adipose tissue [6]. Even obesity and atherosclerosis have been associated with chronic inflammation with elevations of serologic markers such as C-reactive protein.

Although a causal relationship has been demonstrated primarily in animal models, and a functional effect in human disease is currently lacking, the role that the gut microbiota plays in the establishment of host immunity together with its effects on the inflammatory response suggest that continued investigation may lead to direct evidence for the role of the microbiota in at least some of these disease processes. Indeed, early life exposure to the gut microbiota and its effects on the development of immunologically based disease has recently been demonstrated in an animal model [7].

Determinants of Gut Microbiota Composition, Dysbiosis and Inflammatory Bowel Disease

Using our current understanding of disease pathogenesis in IBD as a paradigm, functional genomics has revealed a complex interaction between host innate and adaptive immunity that provide protection against microbial invasion yet demonstrates tolerance to colonization with the microbiota at mucosal surfaces (recently reviewed in Khor et al. [8]). In the case of IBD, the loss of mucosal tolerance, together with a defect in protective host innate immunity to a dysbiotic microbiota, leads to an unrestrained mucosal immune response, the hallmark of this disease process. Indeed, of all the chronic disease states currently associated with the gut microbiota, the evidence for a causative role in the pathogenesis of IBD is the strongest.

Dysbiosis of the gut microbiota, an alteration of the microbial community structure associated with disease, has been consistently observed in patients with IBD. Although the dysbiosis may simply be a result of the inflammatory process [9], it may play a role in the pathogenesis of disease where there is an increase in potentially harmful bacteria and a reduction in more protective bacterial species [10]. Functional evidence for a role of the dysbiotic microbiota in the pathogenesis of disease is supported by animal models where colitis, or predisposition to disease, can be transferred to wild-type mice using genetically defined disease mice as gut microbiota donors [11, 12].

The notion that an alteration in the composition of the gut microbiota is a possible etiologic factor in the predisposition to immunologically mediated dis-
ease has been proposed as one of the environmental factors that may play a role in the increasing incidence of the diseases associated with the gut microbiota mentioned previously [13]. Indeed, many aspects of our environment have been changed dramatically over the past few decades concurrent with the increasing incidence of these disease processes. Elements of the modern lifestyle that have been postulated to result in changes in the gut microbiota include improved sanitation, vaccinations, increased antibiotic use, decline in parasite infections, caesarean section, decline in *Helicobacter pylori*, smaller family size, refrigeration, less crowded living conditions, sedentary life styles, food processing, and dietary changes. The impact of host genetics on the gut microbiota may also play a role, but evidence is largely based on studies in model organisms such as rodents and has been reviewed recently [14]. The impact of host genetics on the gut microbiota in healthy human populations, based on current evidence [15], may be relatively modest, but further studies are needed.

**Short- versus Long-Term Diet, Enterotypes and Gut Microbiota**

The impact of diet on the composition of the gut microbiota begins early in life. Colonization of the gut begins at birth and, following an initial chaotic community structure during the first year of life, the human gut microbiota becomes more stable and adult-like [16] concurrent with the introduction of solid foods into the diet [17]. Several studies have explored the impact of diet on the newborn gut microbiota and have compared breastfeeding with formula feeding. A consistent finding has been the higher proportion of *Bifidobacteria* in breastfed infants as compared to formula-fed infants [18–21].

Although studies have examined the impact of diet on the gut microbiome of various mammalian species in a cross-sectional fashion [22] as well as in a mouse intervention study [23], until very recently, there have not been any studies broadly examining the association between diet and the composition of the adult human gut microbiota. We recently reported two separate experiments, whereby the association between diet and the human gut microbiota was evaluated in a cross-sectional and short-term interventional study [24]. Dietary questionnaires used to assess dietary consumption and 16S rRNA gene sequencing to determine the composition of the gut microbiota in 98 healthy human subjects, revealed a statistically significant association between overall diet and the composition of the gut microbiota. Spearman correlation coefficient associations between bacterial taxa and micronutrients revealed that major nutrient categories clustered independently with relative proportions of bacterial taxa where ‘fat’ and ‘fiber’ were inversely correlated as were ‘amino acids’ and ‘carbohydrates’.
An analysis was also performed to determine whether previously described clustering of human subjects into three gut microbiota groups, termed ‘enterotypes’, was dominated by *Bacteroides*, *Prevotella*, and *Ruminococcus* genera [25]. The analysis revealed the presence of two ‘enterotypes’ dominated by *Bacteroides* and *Prevotella*. Long-term diets high in animal protein and fats and low in carbohydrates, similar to a westernized diet, were associated with high levels of *Bacteroides* and low levels of *Prevotella* genera. By contrast, diets high in carbohydrates but low in animal protein and fat were associated with the inverse pattern, higher levels of the *Prevotella* and lower levels of *Bacteroides*. This relationship was observed with a dietary questionnaire that probed long-term diet but not with a 24-hour dietary recall instrument measuring short-term diet.

These observations are also consistent with a study comparing the gut microbiota of children from a village in the West African country of Burkina Faso with those in Europe [26], as well as a more recent study comparing the gut microbiome of residents in the agrarian Malawi and Amerindian societies with residents in the US [27], where the inverse relationship between *Bacteroides* and *Prevotella* genera were also noted. Although the abundance of *Prevotella* could be considered a discriminative taxon associated with the residence within an agrarian society, the fact that these associations were also observed in residents of the US [24] supports the notion that the observed inversely related proportions of *Prevotella* and *Bacteroides* may be, in part, due to diet. In total, these four studies suggest that long-term diet helps to distinguish a gut microbiota community, or enterotype that is associated with a westernized diet rich in *Bacteroides*, from an enterotype associated with an agrarian diet where the bacteria of the *Prevotella* genus predominates.

A second study to evaluate the impact of a short-term dietary intervention with either a low fat/high fiber or high fat/low fiber on the human gut microbiota revealed that the gut microbiota responds to a dietary change within 24 h but does not lead to a switch in enterotype clustering [24]. Although the short-term diet-induced alteration in the gut microbiota was highly statistically significant, the effect was modest relative to inherent intersubject variability in gut microbiota composition. Furthermore, we did not find any evidence that short-term diet reduced this intersubject variability.

**Enterotypes, Enterogradients and Disease**

Clearly, it is important that additional studies be performed to address numerous critical issues. These include studies to determine whether or not long-term diet can lead to enterotype switching and studies to determine whether or not
the *Bacteroides* versus *Prevotella* enterotypes exist as distinct entities or rather represent a continuum (or ‘enterogradient’) where the observed dietary associations occur at the extremes. There has been considerable discussion in the literature about the discreteness of enterotype clustering, where some datasets support this notion while others do not [28]. Perhaps better described as an enterogradient or a ‘trade off’ between abundance of *Bacteroides*- and *Prevotella*-dominant gut microbial communities, it currently appears that these two genera do not coexist well within the gut environment [29]. An analysis of the recently published NIH-sponsored Human Microbiome Project dataset revealed that organisms that are phylogenetically related and functionally similar tend to coexist within the same environment consistent with niche-driven community structures. Coexclusion of *Bacteroides* and *Prevotella*, taxonomically and functionally similar genera, within the gut is an exception perhaps suggesting competition within the same niche [29].

The ability to distinguish a gut microbiota based upon a westernized versus a more agrarian diet may be of importance in the interpretation of studies associating various disease states that are more prevalent in industrialized nations with the gut microbiota. For example, the gut microbiota may augment the development of atherosclerosis through the production of certain metabolites of dietary lipid phosphatidylcholine that are associated with the risk for the development of CVD. Using a targeted approach to identify plasma metabolites which predict CVD in patients, Wang et al. [30] identified a novel pathway linking dietary lipid intake, intestinal microbiota and atherosclerosis. Foods rich in phosphatidylcholine are a major source of choline. Catabolism of choline by the intestinal microbiota results in the formation of the gas TMA (trimethylamine) that is metabolized by the liver to form trimethylamine oxide and augments the development of atherosclerosis in animal models, thus providing the first link between dietary lipid intake, the intestinal microbiota, and the risk for the development of atherosclerosis [30]. Consistent with this notion, the consumption of choline is positively correlated with the human gut microbial enterotype rich in *Bacteroides* that is associated with a westernized diet [24].

Ultimately, it will be important to determine whether individuals with a *Bacteroides*-predominant gut microbiota have a higher incidence of diseases associated with a western diet, and whether long-term dietary interventions can stably switch individuals to a gut microbiota that is *Prevotella* predominant. If the abundance of *Bacteroides* is ultimately shown to be causally related to disease, then long-term dietary interventions may allow modulation of an individual’s enterotype/enterogradient to improve health. Alternatively, if causal associations are not established, the abundance of *Bacteroides* versus *Prevotella* may still have utility as a prognostic biomarker of disease.
Diet, Gut Microbiota and Inflammatory Bowel Disease

Certain nongenetic factors associated with the development of IBD may be due, in part, to their effects on the gut microbiota. Environmental factors that may alter the composition of the gut microbiota include diet, the use of antibiotics, and geographic location. Population-based studies suggest that IBD is unevenly distributed throughout the world with the highest disease rates occurring in industrialized nations [31, 32]. One theory, the hygiene hypothesis, suggests that humans living in more industrialized countries are exposed to fewer microbes or less complex microbial communities at an early age leading to the development of an immune system less able to ‘tolerate’ exposure to the microbial-laden environment in later life resulting in inappropriate immune activation. Consistent with this notion is the possible role of diet in light of the differences in access to clean water and availability of food refrigeration in underdeveloped parts of the world. Alternatively, a westernized diet rich in animal fat and protein while low in fiber, may alter the gut microbiome in a way that increases the risk for the development of IBD.

Regardless of the mechanism, there are reasonable data to support a role for diet in IBD pathogenesis. Several investigators have examined the association of dietary patterns and the incidence of IBD [33, 34]. For example, the authors of a systematic review concluded that high dietary intake of total fats, polyunsaturated fatty acids, omega 6 fatty acids, and meat were associated with an increased risk of Crohn’s disease and ulcerative colitis; high fiber and fruit intakes were associated with a decreased Crohn’s disease risk, and high vegetable intake was associated with a decreased ulcerative colitis risk [34]. These studies support a potential role for dietary patterns in the pathogenesis of IBD. As proof of principle, the consumption of milk fat has been shown to alter host bile acid composition thereby promoting the expansion of the sulphite-reducing pathobiont *Bilophila wadsworthia*, resulting in an exacerbation of colitis in IL-10 knockout mice [35]. Together with the recent data characterizing the impact of diet on the gut microbiome and its association with enterotypes [24], it is tempting to speculate that the alteration of gut microbiota community structure through the consumption of an agrarian versus westernized diet may play a role in either reducing or increasing, respectively, the risk for the development of IBD.

Conclusions

In this review, we highlighted that the gut microbiota is an important factor in the pathogenesis of certain diseases focusing on those associated with the consumption of a westernized diet. Although studies now suggest that diet has an
impact on the human gut microbiota, there is clearly much to be learned. The abundance of *Bacteroides* versus *Prevotella* may be an oversimplification of alternative states of the gut microbiota in response to diet. Indeed, a recent study of environment and dietary effects on the composition of the elderly gut microbiota revealed six ‘coabundance groups’, two of which included *Bacteroides* - and *Prevotella*-predominant communities [36]. Additionally, associations between the gut microbiota and human disease, including the impact of diet, do not prove cause-and-effect relationships. Indeed, most data supporting a functional effect of an altered microbiota on host physiology are based primarily on murine models. Although such studies provide fundamentally important information about disease mechanisms demonstrating ‘proof of principle’, the degree to which they reflect human pathophysiology awaits further investigation.

**Disclosure Statement**

F.D.B, J.D.L. and G.D.W. declare no conflicts of interest.

**References**
