Supplement Article

The impact of nutrition on the human microbiome

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Diet-derived carbohydrates that are not fully digested in the upper gut, known as nondigestible carbohydrates, provide a major source of energy for bacteria that colonize the human large intestine. It is well established that dietary intake of nondigestible carbohydrates influences microbial fermentation and total bacterial numbers in the colon. Recent evidence from molecular ecology has also shown that the amount and type of nondigestible carbohydrates (e.g., resistant starch, non-starch polysaccharides, and prebiotics) influences the species composition of the intestinal microbiota both in short-term dietary interventions and in response to habitual long-term dietary intake. Interindividual variation in gut microbiota may, in part, reflect differences in dietary intake, but the response of the gut microbiota to dietary change can also differ among individuals. As a better understanding is gained of the impact of different groups of bacteria on host metabolism, the ability to manipulate the microbiota through diet should provide a route for delivering health benefits.

INTRODUCTION

It is now recognized that the human body has 100 trillion microbes in the gut – tenfold greater than the number of cells in the human body – and they amount to perhaps 2 kg in mass. The microbiome is an interface between food, the different fuels absorbed, and the human body. This microbiome is also in contact with the dominant pool of the body's immune cells and with the second largest pool of neural cells in the body, the largest being located in the brain. The microbiome appears to play a major role in health and disease and has been cited as being involved in a number of clinical problems, including the frailty of the elderly, inflammatory bowel disease, irritable bowel syndrome, colorectal cancer, and gut-derived infections. More generally, there are proposed links between the gut microbiota and appetite control, energy balance, obesity, diabetes, immune function, allergies, behavioral perturbations, cardiovascular disease, and cancers such as stomach cancer. Most of these diseases are escalating in prevalence as the role of infectious diseases declines, so it is now important to address the array of potential contributions of the microbiota to these medical problems, particularly since some of them advance to a stage at which they become irreversible. The diet might be expected to have a strong influence on the gut microbiota and to be able to modify the impact of the microbiota upon health, with either beneficial or deleterious consequences. It is important, therefore, to establish whether the intestinal microbiota can be thought of as essentially static within an adult individual, or whether it is subject to dietary control.

Most molecular approaches estimate the proportions of different bacterial groups rather than absolute population sizes, but ideally, changes in both community composition and population sizes should be considered. The composition of the community is assumed to be relevant to health because it determines the ratio of different microbial metabolites, the ratio of harmless commensal organisms to potential pathogens, and the relative production of proinflammatory versus anti-inflammatory signals received by the immune system. Arguably, however, absolute bacterial population densities are more important, since it is these that determine the absolute production rates and concentrations of metabolites and signals of microbial origin. It should also be noted,

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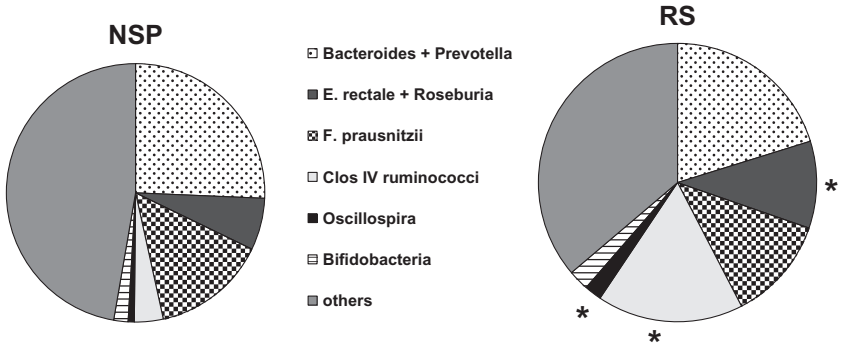

Figure 1 **Impact of diets enriched in non-starch polysaccharides (NSP; wheat bran) or resistant starch (RS; type 3) upon the human fecal microbiota.** Mean values for percent total bacterial 16S rRNA genes (estimated by quantitative PCR) are shown for 14 obese male volunteers for samples taken between 2 and 4 weeks after a switch to each diet (in a crossover design). Groups that showed a significant increase ($p < 0.001$) on the RS diet are indicated by asterisks. Full details are given in Walker et al.[7] Abbreviations: *E. rectale, Eubacterium rectale; F. prausnitzii, Faecalibacterium prausnitzii*; Clos IV ruminococci, ruminococci belonging to clostridium cluster IV (Ruminococcaceae).

therefore, that dietary intake can potentially alter overall microbial population densities – with consequences for the host – without necessarily altering the species composition of the fecal microbiota.

RESPONSE OF THE MICROBIOTA TO CHANGES IN NONDIGESTIBLE CARBOHYDRATE INTAKE

A fraction of normal human dietary intake remains undigested in the small intestine and passes through to the large intestine. This fraction may be increased by rapid gut transit[1] but is mainly determined by the chemical and physical nature of dietary components. "Nondigestible" components include plant cell wall polysaccharides (including cellulose, xylan, and pectin) and certain storage polysaccharides such as inulin and oligosaccharides that contain bonds that are resistant to mammalian hydrolytic enzymes. Even dietary starch includes an important component ("resistant starch") that is not fully digested in the small intestine, for example, because of retrogradation or starch granule structure.[2]

This nondigested residue provides the major source of diet-derived energy for the growth of microorganisms in the large intestine[3] and therefore has the potential to profoundly influence microbial ecology and competition between species within the colonic microbial community. Such changes are difficult to document in humans who follow normal, uncontrolled diets, but they can be observed in carefully controlled dietary studies. Decreased total carbohydrate intake in weight-loss diets is necessarily accompanied by some reduction in dietary fiber and resistant starch. The provision of such diets to obese volunteers has been shown to result in decreased concentrations of microbially produced short-chain fatty acids in fecal samples, together with a significant decrease in the proportion and total numbers of bifidobacteria and butyrate-producing *Lachnospiraceae* related to *Roseburia*.[4] Significant impacts of low-carbohydrate weight-loss diets upon colonic metabolism and fecal microbiota composition have also been reported in other studies by Brinkworth et al.[5] and Ley et al.[6] The effect of varying the major type of nondigestible carbohydrate has also been investigated recently in 14 obese male volunteers on weight maintenance diets[7] (Fig. 1). In most individuals, an increase in certain bacterial groups was detected in fecal samples by quantitative PCR within 3–4 days of changing to a diet high in resistant starch, with ruminococci related to *Ruminococcus bromii* increasing, on average, by tenfold to reach populations as high as 25% of total bacterial 16S rRNA. Increases were reversed by a subsequent diet low in resistant starch. Responses were also individual specific, however, with some individuals who apparently lacked *R. bromii*-related bacteria showing no response for this group. Intriguingly, the two individuals with the smallest ruminococcal populations also failed to fully ferment dietary resistant starch, suggesting that ruminococci might play a key role in the fermentation of this substrate. A significant impact of dietary resistant starch upon *R. bromii*-related bacteria has also been reported in other recent human studies.[8,9] Interestingly, there are also indications that the type of resistant starch may influence the groups of bacteria that show a response,[9] indicating that the effects of diet on the colonic microbiota are likely to be subtle and highly intricate.

Prebiotics are selected nondigestible carbohydrates that are chosen for their intended benefits to health through modulation of particular microbial groups in the gut.[10] Inulin-derived prebiotics, for example, have been shown to result in significant increases in the representation of bifidobacteria[11] and *Faecalibacterium prausnitzii*[12]

among fecal bacteria in humans; once again, however, responses were often individual specific, depending on the initial composition of the microbiota before the intervention.

While these and other studies show that deliberate modulation of the diet by inclusion of nondigestible carbohydrates can modify the gut microbiota composition, by no means are all groups of bacteria responsive. Changes are likely to apply mainly to groups that have specialist nutritional niches, whereas bacteria that are more nutritionally versatile (Sonnenburg et al.[13]) or that depend largely on host-derived substrates may be less affected. This means that diet-driven changes in specific groups may not always be immediately apparent from analysis of whole datasets derived by sequencing. For example, Tap et al.[14] concluded that habitual diet had a minor influence on the overall microbiota profiles in 17 subjects, although a more recent study has reported an association between intake of fat, protein, and carbohydrate and the frequency of *Bacteroides*- and *Prevotella*-dominated microbiotas (enterotypes) in 98 volunteers.[15] Gnotobiotic animal models have also been used to demonstrate the responses of individual species to dietary components in a simplified community.[16]

IMPACT OF DIET ON THE GUT ENVIRONMENT

In the cases discussed above, a particular nondigestible carbohydrate is assumed to stimulate specialist groups of microorganisms that possess particularly high affinity and degradative activity for that substrate. In addition, however, there is considerable potential for secondary stimulatory effects. These will arise, for example, from cross-feeding of partial breakdown products and fermentation products formed by the primary degrading organisms.[17–19] This is a possible explanation for some of the positive associations between species that have been revealed by metagenomics.[20] A third, more general mechanism, however, arises from the undoubted influence that diet composition has upon the gut environment. An increase in dietary fiber intake increases gut transit, total bacterial numbers, and concentrations of fermentation products.[1] At the same time, increased colonic fermentation results in a decrease in the pH in the proximal colon resulting from high concentrations of short-chain fatty acids.[21,22] A one-unit decrease in pH (from 6.5 to 5.5) has been shown to have a profound selective effect upon the colonic microbial community in fermentor simulations supplied with soluble polysaccharides, with a tendency to suppress *Bacteroides* spp. and to promote butyrate-producing gram-positive bacteria.[23] Fecal samples are assumed to reflect the consequences primarily of residence in the higher pH environment of the distal colon, but diet-driven changes in pH and gut transit can nevertheless be expected to influence the composition of the fecal microbiota.

INTERPLAY BETWEEN GEOGRAPHIC AND NUTRITIONAL FACTORS

Major differences in the fecal microbiota between children in Africa and children in Italy were reported recently, based on analysis of amplified 16S rRNA gene sequences.[24] Notably, the *Bacteroidetes* phylum was more abundant in the African children, while *Firmicutes* were relatively higher in the Italian group. Furthermore, the dominant *Bacteroidetes* differed between the two groups, with relatives of *Prevotella* spp. predominant in the African children and *Bacteroides* spp. in the Italian children. Major differences in staple dietary intake, notably a total higher fiber intake in the African children, were also reported and proposed to explain these differences in the dominant colonic microbiota, although direct supporting evidence was not presented. Interestingly, however, a recent investigation involving 98 volunteers concluded that fecal enterotypes rich in *Bacteroides* (first proposed by Arumugam et al.[20]) were associated with habitually high intakes of protein and animal fat, whereas those rich in *Prevotella* were associated with higher carbohydrate intake.[15] It has been suggested recently that the culturability of dominant gut bacteria may be higher than was previously thought.[7] Nevertheless, the cultural representation of the dominant fecal microbiota in human populations outside the developed world may still be very low.

CONCLUSION

The application of molecular tools that allow rapid analysis of microbial communities is starting to yield new insights into the impact of diet on the microbiota of the human colon. These range from targeted approaches such as quantitative PCR to community-wide metagenomic analyses. Human studies that involve careful dietary control have revealed that the populations of certain groups of colonic bacteria can be reversibly enhanced by specific nondigestible carbohydrates, including resistant starches and prebiotics. This suggests that normal day-to-day variation in dietary intake must cause continual shifts in microbial community composition, although these will be more difficult to document for a number of reasons. It also seems reasonable to expect that differences in staple diet composition and intake between different communities and geographic regions will contribute to differences in microbiota composition. It must be emphasized, however, that the relative contributions of diet, host genetics, environmental

exposure, and early microbial inoculum to such variation have yet to be fully established.

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