Diet Can Act through Gut Microbiota To Affect the Host Susceptibility to Disease

*Diet is a powerful but noninvasive tool for manipulating gut microbiota and thus preventing and treating both acute and chronic diseases*

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Summary

- Microorganisms of the gastrointestinal tract are increasingly recognized as critical players in host health and disease.
- Diet can profoundly affect how opportunistic pathogens respond to changes in the gastrointestinal tract of the host, influencing their ability to cause disease.
- Commensal gut microorganisms are particularly critical in defending against opportunistic infections in immune-naïve newborns.
- Researchers are unraveling how interactions between diet and gut microbiota drive obesity and metabolic diseases.
- Diet can alter the gastrointestinal microbiota, sometimes driving the development of cancer, and other times reducing the risk of cancer within the host.

Gut microbes are increasingly recognized as critical players in host health and disease. These microbes respond to their surrounding environment, which is heavily influenced by host diet. In some cases dietary compounds or minerals may directly thwart or induce virulence in opportunistic pathogens. In others, sugars, fats and fibers serve as food sources that alter the make-up of microbial populations. These diet-dependent impacts on gut microbes have profound influences on host biological processes and susceptibilities to diverse infectious and non-infectious diseases, from metabolic diseases to cancer. During the 2016 ASM Microbe conference, held in Boston last June, researchers discussed various dietary influences on host microbiota and disease, pinpointing specific bacterial species and mechanisms of pathology. These reports highlight diet as a powerful and non-invasive tool to manipulate gut microbiota for preventative and therapeutic treatment of both acute and chronic diseases.

Dietary Compounds Protect Against Opportunistic Infections

The gut hosts numerous microbial species that typically reside commensally, but under certain conditions, can become virulent, proliferate and cause invasive infections. Researchers report that diet can profoundly impact how such opportunistic pathogens respond to their environment within the host, influencing their ability to cause disease.

Compounds found in cranberries thwart key virulence mechanisms used by uropathogenic *Escherichia coli* (UPECs) to cause recurring urinary tract infections. Specifically, flavinoids called proanthocyanidins, found in a variety of fruits including cranberries, black currants, grapes, acai, apples and cocoa beans, act as natural defenses against plant pathogens, but retain activity against human pathogens as well. “Cranberry proanthocyanidins are natural compounds that can prevent key virulence mechanisms used by UPECs, which cause the majority of UTIs,” says Wen-Chi Chou of the Broad Institute, Boston, Massachusetts.
These natural products interfere with bacterial fimbriae, also known as “attachment pili,” which mediate pathogen adhesion to host cells and surfaces, the first step to invasive infection. *E. coli* use fimbriae to adhere to host epithelial cells in the gut, which acts as a reservoir from which they can repeatedly invade the urinary tract. Proanthocyanidins block UPEC adhesion to various epithelial cell, including colonic, bladder and uro-epithelial cells, thwarting both colonization and invasion by the pathogen in laboratory studies. These natural compounds are even effective against multi-drug resistant strains, blocking the adhesion of clinical isolates to uroepithelial cells by 70%, suggesting a potential alternative treatment in the face of multi-drug resistant infections.

Proanthocyanidins may also protect against other opportunistic infections by interfering with common attachment and motility mechanisms of potential pathogens. Fimbriae are critical to Staphylococcal and Streptococcal virulence. In addition, these compounds impair bacterial motility by downregulating flagellin genes, impairing colonization by *E. coli, Psuedomonas aeruginosa* and the uropathogen *Proteus mirabilis*.

In a collaboration between the Broad Institute of MIT and Harvard (Boston, MA) Washington University of St. Louis (St. Louis Missouri), The Fred Hutchinson Institute (Seattle, Washington), and Ocean Spray, Chou and his colleagues are investigating whether proanthocyanidins may have multifaceted effects by also reducing the prevalence of commensal gut species that can break down these protective compounds. “Cranberry juice may help the gut microbiome prevent recurrent UTIs by removing (species) which shield pathogenic *E. coli* from the host-healthy effects of proanthcyanidins,” says Chou.

Commensal gut microbiota are particularly critical in defending against opportunistic infections in immune-naïve newborns, says David Mills of the University of California, Davis. Primarily dominated by Bifidobacteria that digest human milk oligosaccharides (HMOs), this community employs a hording strategy to dissuade gut invaders. Species such as *B. longum subsp. longum, B. breve, B. pseudocatenulatum, and B. longum subsp. infantis* internalize milk sugars and digest them inside the cell, preventing neighboring bacteria from scavenging on breakdown products. This strategy sequesters essential nutrients away from potential pathogens, essentially starving them out.

“The ‘internalize, then degrade’ approach for HMO consumption adopted by the majority of infant-borne Bifidobacteria can be viewed as an ingenious strategy for protecting the neonate,” says Mills. “These bacteria prevent growth of competitor strains by simple sequestration of available sugars in the colon.” Indeed, infants with higher proportions of these Bifidobacterial species have lower levels of unutilized sugars in their feces, as well as fewer dysbiotic scavengers such as Enterobacteriaceae.

However, premature births disrupt the establishment of this beneficial microbiota, causing a dysbiosis dominated instead by Bacteroides species. These species are “external eaters” that instead secrete digestive enzymes into the gut lumen, and then internalize the smaller pieces. This strategy provides other species with a free meal, crossfeeding pathogens that otherwise lack the enzymes necessary to digest the larger sugars. Mouse, piglet and human studies suggest that Bacteroides digestion of oligosaccharides releases fucose sugar monomers critical to overgrowth of opportunistic pathogens such as Enterobacteriaceae, *E. coli*, and Klebsiella. In human infants, these pathogens may lead to necrotizing enterocolitis, a life-threatening condition that afflicts 10% of premies, with 20-30% mortality rates, says Mills.
Infant diet may play a critical role in preventing or correcting such dysbiosis. Mills notes that while breast milk promotes microbiota dominated by “internal eaters,” disturbances like antibiotic treatment and formula feeding could disrupt this community to favor “external eaters” and pathogen overgrowth. “Microbiota liberated host sugars facilitate post antibiotic expansion of enteric pathogens,” he says. In these circumstances, formula feeding could unintentionally endanger the infant by providing nutrients to neighboring pathogens. “This problem might be alleviated by the use of synbiotics (a combination of pre and probiotics) in which the probiotic component is known to readily consume the oligosaccharides provided,” he suggests. Thus, pairing of specific dietary sugars with “internal eaters” could protect at-risk infants from enteric pathogens.

**Dietary Fats and Fibers can Shape Gut Microbial Populations to Influence Host Metabolic Disease**

Gut bacteria have been implicated in obesity and metabolic disease in numerous studies. While these metabolic conditions were originally blamed on poor dietary choices, influences of Western diets, and even host genetics, researchers are beginning to unravel the interplay between diet and gut microbiota that drives these diseases.

Human patients and animal models show a dysbiosis of gut bacteria dominated by Firmucutes, particularly of the class Erysipelotrichi. One species specifically, *Clostridium ramosum*, may be to blame for promoting metabolic disease, says Michael Blaut of the German Institute of Human Nutrition, Potsdam, Germany.

This species blooms in response to high fat and western diets in mice and rats, and is over-represented in the gut of humans with obesity and type II diabetes. Blaut finds that mice with human microbiomes containing *C. ramosum* gain more weight and have higher body fat than animals lacking *C. ramosum*.

“*C. ramosum* promoted diet-induced obesity, probably by enhancing nutrient absorption,” says Blaut. Indeed, animals with *C. ramosum* had upregulated glucose transporter 2 (*glut2*) and fatty acid translocase (*cd36*) in the intestine, enhancing the uptake of obesogenic sugars and lipids, thereby affording the host greater food efficiency. “Upregulation of *glut2* and *cd36* transcription in the small intestinal mucosa in gnotobiotic mice harboring intestinal *C. ramosum* indicates that this organism promotes body fat accumulation through enhanced intestinal glucose and lipid absorption,” says Blaut.

Dietary changes may help to correct this microbial dysbiosis and associated metabolic diseases by starving out *C. ramosum* in favor of health promoting gut microbiota. “Diet as the main energy source for intestinal bacteria, influences microbiota composition, (which in turn) promotes the development of metabolic disease in a diet-dependent manner,” says Blaut. Reducing fat intake, or alternatively, supplementing high fat diets with fiber sources can favor the growth of beneficial Bifidobacterial species at the cost of *Firmicutes*. In mice, supplementation with the dietary fibers oligofructose or inulin restores metabolic function, improving symptoms like glucose tolerance, insulin sensitivity and body fat mass, finds Blaut.

Dietary fibers like oligofructose can also improve other aspects of metabolic disease via effects on gut microbiota. Non-alcoholic fatty liver disease (NAFLD) is a hallmark of metabolic disease in obese,
type II diabetic patients, characterized by high cholesterol and fatty acid accumulation in the liver that can lead to serious liver complications like hepatitis, fibrosis and cirrhosis. Nathalie Delzenne of the Universite Catholique de Louvain, Belgium, finds that many of these symptoms can be corrected in mouse models simply by feeding animals fructo-oligosaccharides (FOS), a non-digestible, fermentable fiber source. “FOS supplementation leads to several beneficial health effects, including decreased triglyceridemia in humans and a reduction in hepatic steatosis (NAFLD) in numerous rodent models of obesity,” she says.

Fructo-oligosaccharides are known to modulate gut microbiota by favoring the growth of health promoting Bifidobacterium species, which Delzenne finds has profound effects on host gene expression and fat metabolism. “The gut microbiota is able to regulate fat storage and host metabolism, via the induction of expression of key enzymes and factors controlling lipogenesis in liver tissue,” she explains.

Specifically, Delzenne finds reduced expression of enzymes involved in cholesterol biosynthesis, reflected in lower cholesterol levels in both the blood and liver of FOS-fed mice. In addition, triglyceride levels were much improved, although triglyceride synthesis pathways were unaffected. “Lower accumulation of triglycerides in the liver… resulted from increased fatty acid oxidation. Several enzymes involved in fatty acid mitochondrial, microsomal and peroxisomal oxidations were upregulated in the liver,” says Delzenne.

These extensive changes in lipid metabolism can be traced back to transcription factors and micro-RNA that control gene expression. “The gut microbiota modulates gene expression in the liver tissue by influencing key metabolic regulators that are interrelated to each other, namely SREBP-2, miR-33 and PPAR-a,” says Delzenne. She finds that peroxisome proliferator activated receptor-a (PPAR-a), a transcription factor that controls fatty acid oxidation genes, is activated in the livers of FOS-fed mice. Meanwhile, the expression of the micro-RNA miR-33, which inhibits fatty acid oxidation, and the transcription factor sterol regulatory element binding protein 2 (SREBP-2), involved in cholesterol homeostasis, are both reduced. Altogether, these changes improve lipid profiles.

Gut microbiota derived metabolites and influences on gut hormones are likely the communications conduit from the gut to the liver. Delzenne notes that Bifidobacterium produce conjugated linoleic acids, which have been shown to reduce NAFLD symptoms in several rodent models by activating PPAR-a. In addition, specific bacterial metabolites, such as bioactive lipids, trigger the secretion of gut hormones that influence host glucose, lipid and energy metabolism. Indeed, Delzenne finds enhanced production of the gut derived hormone glucagon-like peptide 1 (GLP-1), which stimulates fatty acid oxidation pathways and reduces lipid accumulation in the liver. “We propose that those modifications in (metabolic) gene expression involved miR33, SREBP-2 and PPAR-a in the liver tissue itself and gut derived hormones such as GLP-1 as a metabolic relay between the gut and liver,” says Delzenne.

Blaut and Delzenne suggest that dietary fibers offer a non-invasive strategy to correct diet-induced microbial dysbiosis and the resulting metabolic diseases that currently plague Western society. “Our data underline the advantage of targeting the gut microbiota by colonic nutrients in the management of liver disease,” says Delzenne.
Dietary conditions can also alter gastrointestinal microbiota with carcinogenic consequences. In some cases, dietary factors stimulate certain species to secrete carcinogenic toxins. In others, dietary influences alter microbial populations to favor inflammatory species which promote tumorigenic conditions within the host.

Salt is an essential mineral, but at high concentration it can cause a Mr. Hyde to Dr. Jekyll transformation in *Heliobacter pylori*. “*H. pylori* adapts to particular environmental conditions associated with change of diet,” says Timothy Cover of Vanderbilt University. *H. pylori* is primarily asymptomatic, residing in the stomachs of approximately 50% of the population worldwide. However, a high salt diet induces toxin secretion that contributes to gastritis, peptic ulcers and even stomach cancer, according to Cover and his graduate student Rhonda Caston.

At high salt concentrations *H. pylori* mounts a generalized stress response and upregulates various virulence factors, including the toxin CagA(cytotoxin-associated gene A product). CagA is carcinogenic to epithelial cells lining the stomach, altering various aspects of cellular physiology. “These include changes in epithelial cell differentiation resulting in an invasive phenotype, enhanced proliferation and inhibited apoptosis, activation of gastric stem cells and degradation of the p53 tumor suppressor,” says Cover. “CagA functions as a bacterial oncoprotein.”

In addition, CagA is highly antigenic, triggering mucosal inflammatory responses and generating chronic inflammation known as gastritis. Over prolonged periods, gastritis promotes additional cell damage and pathogenesis that leads to peptic ulcers and gastric cancer.

*H. pylori* also upregulates the virulence factor VacA(vacuolating cytotoxin A), another carcinogenic toxin. “Increased risk of gastric cancer associated with strains producing more active forms of VacA may be a consequence of several actions, including the capacity to stimulate gastric epithelial cell injury, alter parietal cell function and gastric acidification, and interfere with immune cells function,” says Cover. VacA targets a variety of immune cells, including T cells, B cells, eosinophils, mast cells and dendritic cells, contributing to inflammation in the stomach and intestinal mutations that precedes cancer.

These discoveries provide a molecular explanation for epidemiological studies which link high salt diets to increased risk of gastric cancer in humans. In fact, in studies of humans and rodents examining both factors, *H. pylori* infection and high salt diets synergize to exponentially increase cancer risk.

“Several dietary risk factors for gastric cancer directly impact *H. pylori* virulence,” says Cover. In addition to high salt diets, iron deficiency can cause similar changes in *H. pylori* pathogenicity. “In response to iron starvation conditions, several virulence genes were found to be differentially expressed, including cagA and vacA,” says Cover. Iron-starved *H. pylori* also upregulate type 4 secretion system pili responsible for injecting the CagA toxin into gastric epithelial cells, thereby causing greater carcinogenic damage. In addition, these iron-starved bacteria are more antigenic, inducing higher levels of mucosal inflammation that promotes tumorigenesis.

These studies reflect observation in patients. “Strains from patients with low serum ferritin levels induced more robust inflammatory responses,” says Cover. “Collectively these results suggest that...
prolonged exposure of *H. pylori* to low iron conditions *in vivo* leads to stable changes in bacteria, including increased activity of CagA mediated (pathogenic) phenotypes.” Indeed, iron-deficiency anemia is a well-known risk factor for gastric cancer in humans.

Gut microbiota can also influence the development of cancers outside of the gastrointestinal tract, including skin, liver, breast and lung cancers. “Recently it has been demonstrated that the microbiome could also impact the occurrence and progression of cancer at extra-intestinal sites,” says Laure Bindels of the Universite Catholique de Louvain, Belgium.

Dysbiosis in gut microflora promotes gut permeability and produces systemic inflammation that fuels tumor growth at distant sites. Bindels examines mouse models of leukemia and liver cancer, and finds signature microbial changes with decreased Lactobacillus species and increased Enterobacteraceae and *Parabacteroides goldsteinii*. Bindels corrects this dysbiosis through diet, by administering the probiotic *L. reuteri* 100-23, known for its anti-inflammatory properties, and pre-biotic nutritional fibers that support its growth. This synbiotic approach restores gut barrier function, and decreases inflammatory cytokine production. The impact on tumorigenesis is remarkable, reducing hepatic cancer cell proliferation and improving animal morbidity and mortality. “The synbiotic approach prolonged survival, a relevant primary outcome for nutrition therapy in cancer,” says Bindels.

These gut microbial alterations also influence cancer-associated muscle wasting, fat loss and anorexia known as cachexia, which results when the body shuts down in the face of various terminal illnesses. “Cachexia is a serious but often neglected consequence of many chronic diseases, (and afflicts) up to 50-80% of patience with advance cancer,” says Bindels. Cachexia undermines the patient’s ability to fight the disease, exacerbating morbidity and accelerating their decline. Bindels’ synbiotic treatments reduce muscle wasting and molecular markers thereof in cachexic animals, contributing to their improved outcomes. “Nutritional modulation of the microbiome by combined pre and probiotic approaches resulted in the reduction of cancer cell proliferation and an improvement of cachectic features, thus leading to prolonged survival,” says Bindels. Currently there are few therapeutic options for patients experiencing cachexia, and Bindels is optimistic that this non-invasive, dietary approach to modulate gut microbiota could help to alleviate their symptoms and cancer progression.