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Report

The Microbiome Of Aging And Age-Related Disease Conference

By Ben Best

The **microbiome** refers to the full complement of microbial organisms inhabiting the human body, including bacteria, protozoa, fungi, and viruses.[1] There are about 10 times as many microbial cells as there are human cells in the body.[2] The great majority of these organisms reside in the gut, particularly the colon. The human colon typically contains almost half a pound of bacteria,[3] which function as if they were another human organ. There are many thousands of times more bacteria in the colon than there are in the small intestine, just as there are many thousands of times more bacteria in the small intestine than there are in the stomach.[4,5] Beneficial effects of bacteria in the gut include displacement of harmful bacteria, synthesis of vitamins, degradation of fibrous foods, maintenance of intestinal wall integrity, and stimulation of the immune system.[6-10]

Ingested food typically spends less than an hour in the stomach, where acidic conditions are very unfavorable to most microorganisms. Food being digested typically spends two to six hours in the small intestine where most absorption occurs.[11] Thereafter, food remnants (mostly fiber) reach the large intestine (colon, large bowel), where they remain an average of about 40 hours for the many bacteria to ferment the fiber that cannot be digested in the small intestine.[12] The sulfur component of gas resulting from fermentation gives an unpleasant odor to flatus and feces.[13] Human feces composition is about three-quarters bacterial.[14]

On October 16 to 19, 2014, what may have been the world's first conference on the impact of the microbiome on aging was held near San Antonio, Texas. The conference was organized by the Barshop Institute, an organization dedicated to curing the diseases of aging. The Director of the Barshop Institute, Dr. Nicolas Musi, attended the sessions and gave a presentation.

The Human Microbiome Project


Nierman

William Nierman, PhD, (Director, Infectious Disease Program, J. Craig Venter Institute, California) reported on some of the results of the Human Microbiome Project (HMP). The HMP was initiated in 2007 by the National Institutes of Health as a follow-up of the Human Genome Project. The mandate of the HMP was to sequence the genomes of microorganisms inhabiting five major body areas: gastrointestinal tract, oral cavity, skin, urogenital/vaginal, and respiratory tract.[15] The J. Craig Venter Institute, where Dr. Nierman works, was one of the four research centers designated to do HMP genome sequencing.[16]

More than **99%** of the microorganisms identified in the colon were bacteria from the ***Bacteroidetes*** and ***Firmicutes*** phyla of bacteria—out of the dozens of possible bacterial phyla that exist.[17] Aside from bacteria producing short-chained fatty acids from fiber, there were organisms converting the hydrogen gas produced by fermentation into methane.[17]

The skin offers a more harsh habitat for organisms than other areas of the body, which means fewer species of bacteria reside there.[18] As in other areas of the body, some species of bacteria on the skin are protective by preventing overgrowth of

harmful species.[18] Oily areas of the skin can attract acne-causing bacterial strains,[18,19] which can be treated with topical ginseng, pine, or black currant.[18]

Staphylococcus overgrowth on the skin is associated with **atopic dermatitis** (also called atopic eczema, "atopic" meaning hypersensitive), a condition that affects about **15%** of American children.[20] Atopic dermatitis is the most common inflammatory skin disease.[21] Atopic dermatitis is associated with growing up in an excessively sanitary environment that is believed to counteract the development of a healthy immune system (ie, the hygiene hypothesis).[22] One study found that children who received antibiotics early in life have a **40%** increased risk of developing atopic dermatitis.[23] Conversely, childhood exposure to furry pets or farm animals was associated with reduced risk (although this is partly because allergy-sensitive families will avoid having furry pets).[24] Vitamin D supplementation has been shown to reduce atopic dermatitis.[25] Paradoxically, mineral baths can be beneficial, whereas hard water may be harmful.[25]


Weinstock

George Weinstock, PhD, (Professor, Jackson Laboratory for Genomic Medicine, Maine) was a Principal Investigator of the Human Microbiome Project.[26] In particular, Dr. Weinstock was Principal Investigator for studying the "human virome" (the cumulative genetic makeup of viruses inhabiting the human body).[27] Although viruses can cause disease, the typical healthy human carries many persistent viruses that cause no harm, and may even be protective.[27] Dr. Weinstock reported on the microbiome of the mouth and the vagina.

As with other areas of the body, studying the organisms that inhabit the oral cavity is difficult because the majority of them cannot be cultured in a laboratory.[28] Oral bacteria are linked to a variety of diseases, including diabetes, stroke, pneumonia, and cardiovascular disease.[28] A correlation has been found between bacteria in dental plaque and bacteria in atherosclerotic plaque.[29] Dietary sugar increases acidity in the mouth, which encourages the growth of acid-producing (cavity-producing) bacteria.[30]

A healthy vagina is dominated by ***Lactobacillus*** bacteria that turn lactose and other sugars into lactic acid.[31,32] In contrast to the mouth, a slightly acid environment is protective in the vagina because it suppresses the growth of harmful bacteria.[33] Displacement of *Lactobacilli* by other classes of bacteria results in **bacterial vaginosis**, a condition that affects **8** to **23%** of women of reproductive age. Symptoms include a vaginal discharge having a fishy odor, although about **40%** of women do not have these symptoms.[34] The condition is present in over **70%** of sex workers, and is associated with sexually transmitted disease.[35] Although irrigating the vagina (douching) is imagined to be hygienic, the practice increases the risk of bacterial vaginosis, so douching should be avoided.[36]

Chronic Inflammation And The Microbiome


Curiel

Tyler Curiel, MD, (Professor of Medicine, University of Texas Health Sciences Center) is concerned with the effects of inflammation in the gut, which can lead to inflammatory bowel disease[37] as well as cancer of the colon and rectum.[38,39]

Bacteria are generally classified as either gram-negative or gram-positive on the basis of whether they take a Gram stain. **Gram-positive** bacteria have a thick layer of **peptidoglycan** protecting the cell membrane, whereas **gram-negative** bacteria have an exposed cell membrane displaying **lipopolysaccaride** (**LPS**), a carbohydrate-fat complex.[40,41] LPS is a potent inducer of inflammation,[42] which is why it is called **endotoxin**. LPS causes intestinal inflammation.[43] Olive oil has been shown to protect mice against septic shock induced by LPS.[44]

Dietary fiber in the colon is fermented to produce the short-chain fatty acids **acetate**, **propionate**, and **butryate**. Acetate

absorbed into the bloodstream from the colon is an energy source for muscle, heart, kidney, and brain. Butyrate mainly remains in the colon, where it is the major energy source for colon cells.[45] In mice fed a high-fat diet, butyrate supplementation prevented obesity and insulin resistance.[46] Human patients with inflammatory bowel disease have been effectively treated with butyrate enemas.[47,48]

Dr. Curiel described a study showing that the short-chain fatty acid butyrate boosts anti-inflammatory action of the immune system.[49] Butyrate leads to epigenetic changes in immune system cells (macrophages) that reduce the secretion of inflammatory factors (like IL-6).[50] Butyrate reduces intestinal permeability and the infiltration of harmful molecules, like LPS, into the bloodstream from the colon.[51] Butyrate given orally as a doubly enteric-coated tablet was an effective treatment for irritable bowel syndrome.[52] Propionate and acetate also have anti-inflammatory effects.[53,54]


Musi

Nicolas Musi, MD, (Director of the Barshop Institute, and Professor of Medicine at the University of Texas Health Center) discussed the role of dietary fat in inflammation, obesity, and aging. Both aging and obesity are characterized by chronic inflammation.[55] The innate immune system reacts to lipopolysaccharide (LPS) in the cell membranes of gram-negative bacteria by an inflammatory response.[56,57] As a defense against bacteria, this form of inflammation is protective—in contrast to the chronic inflammation associated with obesity and aging. Chronic inflammation causes the muscle wasting (sarcopenia) so often seen with aging.[58]

Toll-like receptors on immune system cells (macrophages) and fat cells are detectors that trigger inflammation in response to the fat (in LPS) in bacterial cell membranes.[59] Unfortunately, these receptors also produce an inflammatory response to forms of fat other than what is found in bacterial cell membranes.[60] Infusion of free fatty acids or LPS into mice has been shown to increase inflammation and, as a consequence, increases insulin resistance and obesity.[60,61] Healthy men given a high-calorie diet showed increased plasma LPS.[62] LPS in the bloodstream of type II diabetics was found to be about **75%** higher than in healthy subjects.[63]

An inflammatory effect is seen in healthy humans after eating a high-fat, high-carbohydrate meal—but not after eating a meal rich in fruit and fiber.[64] Not all fat has this effect, however. In fact, supplementation with the omega-3 fatty acid eicosapentaenoic acid (EPA) has been shown to reduce inflammation and muscle wasting.[65]


Franceschi

Claudio Franceschi, MD, (Professor of Immunology, University of Bologna, Italy) is best known for his concept of chronic inflammation as the cause of aging and aging-related disease (ie, inflammaging).[66,67] The innate immune system recognizes peptidoglycan in the cell wall of gram-positive bacteria and lipopolysaccharide (LPS) in the cell membrane of gram-negative bacteria to produce an inflammatory response that fights the bacteria.[68] LPS is more inflammatory than peptidoglycan.[69] In a healthy immune system, the inflammation ceases after the bacteria have been eliminated, but aging results in an increasingly unhealthy immune system characterized by chronic inflammation.[70,71] Mice fed a high-fat diet showed a higher proportion of gram-negative (LPS-containing) bacteria in their gut, greater intestinal wall permeability, and a higher concentration of LPS in their bloodstream.[72,73] Fat may assist in transporting LPS from the gut into the bloodstream.[62] Excess body fat also causes chronic inflammation, leading to atherosclerosis, insulin resistance, high blood pressure, and other aging-related diseases.[74]

Dr. Franceschi believes that an aging-related decline in short-chain fatty acids, especially butyrate, plays an important role in age-related chronic inflammation.[75,76] Butyrate is the preferred energy source for cells lining the colon, and those cells produce mucin that protects the walls of the colon. When the colon cells do not get enough butyrate, the inflammatory bacterial components peptidoglycan and LPS continuously leak out of the colon into the blood stream, resulting in chronic

inflammation.

The Microbiome In Model Organisms


Cabreiro

Filipe Cabreiro, PhD, (Lecturer, University College, London, UK), like many researchers studying the microbiome, uses nematode worms and fruit flies as model organisms. Model organisms are used because their biology is known in great detail and can be easily manipulated, and because they have such short life spans. Nematodes rely upon their gut bacteria for nitric oxide, which enhances stress resistance and longevity.[77]

Dr. Cabreiro has found that the antidiabetic drug **metformin** has effects apart from lowering blood sugar. Metformin activates the energy-sensing enzyme AMPK in both nematode worms and fruit flies, but extends the life span of nematodes, not fruit flies.[78,79] Metformin alters bacterial metabolism in such a way as to restrict the amino acid methionine.[80] Methionine restriction has also been shown to extend life span in rats.[81] Dr. Cabriera established that the effect of metformin on nematode life span is dependent upon the strain of bacteria in the worms. He suggested that the lack of a life span extension effect in fruit flies is due to different bacteria in the fruit flies.[80]

Dr. Cabreiro also cited literature emphasizing the intimate relationship between humans and their microbiota. Germ-free mice exhibit numerous defects in the development of their immune systems.[82] As the human immune system develops it learns to distinguish between self and non-self. At the same time, however, the human immune system learns that the microbiome is part of the self, which protects the microbiome from attack by the immune system.[83] Specific bacteria have been shown to be important for the development of the human immune system.[84,85]

H. pylori is one of the few bacteria that can tolerate the acidic conditions of the stomach.[86] Although *H. pylori* can cause stomach ulcers, the bacterium has many beneficial effects, including regulation of stomach acid and stomach hormones controlling appetite.[87,88] Although the great majority of Americans once had *H. pylori* in their stomach, now only a few percent do, as a result of overuse of antibiotics in childhood. The result has been an increase in obesity and acid reflux disease.[87]

Studying The Colon Microbiome


O'Toole

Paul O'Toole, PhD, (Professor, University College Cork, Ireland) has studied the difference between colon microbiota in the elderly compared to the microbiota in the colon of healthy adults living in Ireland. By phyla (major divisions of bacterial type), he found healthy adults to have **51%** *Firmicutes* and **41%** *Bacteroidetes*, whereas he found **40%** *Firmicutes* and **57%** *Bateroidetes* in the elderly.[89] Studies in different European countries show very different changes in bacteria phyla populations with aging.[75] Differences in species below the phylum level may account for the seemingly contradictory results. Obese mice and humans show fewer *Bacteroidetes* than those who are lean.[90] A mouse study showing greater weight gain with a high-saturated-fat diet than with a high-unsaturated- fat diet found that the saturated-fat-diet elevated the *Firmicutes*-to-*Bacteroidetes* ratio.[91]

Dr. O'Toole found that institutionalized elderly had less bacterial diversity in their colon than non-institutionalized elderly, and this lack of microbial diversity correlated with frailty.[92] Dr. O'Toole addressed the effect of high antibiotic use among the elderly and others. Antibiotics can be life saving, but there can be negative side effects. A major function of some bacteria is to prevent overgrowth of harmful bacteria. Diarrhea resulting from antibiotic treatment is sometimes due to the bacterium

Clostridium difficile. Further antibiotic treatment is often futile.[93] For decades, stools from healthy patients have been inserted into the rectum of patients suffering from *C. difficile* overgrowth after antibiotic treatment.[94] Recently, stools from lean donors have been shown to increase insulin sensitivity when transplanted into the colon of metabolic syndrome patients.[95] Also recently, stool substitutes consisting of collections of known bacterial species ensured to be free of pathogenic microbes have been used with success.[96]


O'Keefe

Stephen O'Keefe, MD, (Professor, University of Pittsburg, Pennsylvania) is a specialist in inflammatory bowel diseases (ulcerative colitis and Crohn's disease) and colorectal cancer. Crohn's disease is not an autoimmune disease, but instead is a combined result of both genetics and intestinal bacteria.[97,98] Inflammatory bowel disease leads to colorectal cancer in the range of about **8** to **18%** of cases.[99]

According to the American Cancer Society, colorectal cancer is the third leading cause of cancer death in males and females in the United States: breast (female) and prostate (male) are first, lung/bronchial is second, and colon/rectum is third. Although colon cancer is up to **25%** more common among African Americans than Caucasian Americans, native Africans rarely get colon cancer.[100] Bile acids, which are normally synthesized in the liver from cholesterol, function to emulsify fats in the intestine. But with a high-fat, low-fiber diet, certain colonic bacteria can lead to an increase in toxic secondary bile acids **lithocholic acid** and **deoxycholic acid**, which can increase colon cancer risk.[101] Comparing colon fluid samples, Dr. O'Keefe's team found that lithocholic acid was **3.3 times** higher in African Americans than in Africans, and deoxycholic acid was **5.1 times** higher.[100]

Butyrate is chemopreventive and inhibits the survival of colon cancer cells.[102] Calcium in milk or supplements has been shown to precipitate toxic bile acids, and reduce the risk of colon cancer.[103,104] Chlorogenic acid in coffee and blueberries can protect the colon from toxic bile acids.[105] Conversely, dietary pro-inflammatory omega-6 fatty acids (found in many vegetable oils and prevalent in the Western diet) and vitamin D deficiency increase inflammation and risk of colon cancer.[106]

In comparing the gut bacteria from rural African children with European children, Dr. O'Keefe's team found much greater diversity in the bacteria of the Africans, and a relative absence of inflammatory bacteria.[107] Much of the African bacteria were from the genus *Prevotella*, whereas this genus was virtually absent in the Europeans. Aside from diet, Dr. O'Keefe attributed these differences to greater use of antibiotics and Caesarean section birth in Europe, as well as reduced breast feeding by Europeans.[107]Short-term antibiotic treatment can alter microbiota for years.[108]

When babies are born naturally, they acquire their first microbiota from their mother's birth canal. [109,110] When born by Caesarean section, their first bacterial exposure is generally from the mother's skin. [110] Caesarian birth has been associated with greater vulnerability to childhood asthma.[111,112] Birth by Caesarian section has greatly increased in recent decades, currently accounting for nearly a third of births in the United States, **40%** of births in China, and nearly half of births in Brazil.[113]Although Caesarean section can be life-saving in specific situations, the American College of Obstetricians and Gynecologists recently expressed concern that Cesarean delivery is overused.[114]

Breastfeeding provides an infant not only with microorganisms from the mother, but with growth factors, oligosaccharides, and more whey protein than is found in cow's milk. [115] The proportion of children who were ever breastfed is lower in the US than in many other countries.[116] Compared to formula feeding, breastfed infants have less diarrhea, less inflammatory bowel disease, and possibly fewer food allergies.[115,117] Oligosaccharides in breast milk reportedly contribute to brain development.[118]

Good Diet For The Microbiome


Marco

Maria Marco, PhD, (Assistant Professor, Food Science and Technology, University of California) described the benefits of a diet high in fiber and digestion-resistant starch. Foods such as potatoes, rice, pasta, bread, noodles, sugars, and breakfast cereals are rapidly digested, which rapidly increases blood sugar that stimulates insulin release. Blood sugar then quickly drops resulting in a repeating cycle of hunger and eating—leading to obesity, insulin resistance, and type II diabetes. By contrast, split peas, lentils, black beans, artichokes, raspberries, rolled oats, and other foods high in fiber or resistant starch are very slowly digested and have important health benefits.[119] Short-chain fatty acids (especially butyrate) produced in the colon by bacteria fermenting dietary fiber prevent inflammation and help prevent colon cancer.[119]
Mice fed digestion-resistant starch show better glucose tolerance and increased levels of hunger-reducing peptides.[120,121] A human study showed similar benefits.[122] Weight cycling (repeated loss and regaining of body weight) is associated with bone loss, an effect that can be minimized by a diet high in digestion-resistant starch.[123]

Probiotics And Prebiotics

Fiber and digestion-resistant starch are called **prebiotics**. Prebiotics are components of food that cannot be digested by the stomach or small intestine, but are fermented in the colon to provide additional nutrients and health benefits.[124,125]

By contrast, **probiotics** are live bacteria that provide health benefits when taken orally.[126] Probiotics able to survive the acidic environment of the stomach include acid-producing bacteria from the genus ***Lactobacillus*** and the genus ***Bifidobacterium***.[127-129] *Escherichia coli* and *Streptococcus* from the birth canal are predominantly the first bacteria in a newborn, but upon breastfeeding, these bacteria are mostly displaced by *Bifidobacteria*.[5] *Bifidobacteria* can account for **95%** of gut bacteria in healthy, breast-fed babies.[130] The decline of *Bifidobacteria* with age may contribute to aging-associated disease.[131]

Probiotics have been used in medicine to prevent or treat antibiotic-associated diarrhea.[132] Probiotics given to mothers prenatally were shown to reduce the incidence of atopic eczema in their children by about half.[133] Probiotics given to the elderly were shown to enhance immune system function.[134-137] Probiotics fed to rats and mice were shown to prevent diet-induced obesity and prevent insulin resistance.[138-140] Probiotics fed to mice increased longevity by suppressing chronic inflammation.[141]

Prebiotics are digestion-resistant chains (polymers, oligosaccharides) of sugar molecules. Such oligosaccharides occur naturally in chicory, asparagus, onions, soybeans, and milk.[142-144] Prebiotics promote the growth of *Bifidobacteria* in the colon.[145] Prebiotics have been shown to protect rats from colon cancer.[146,147] In humans, prebiotics have been shown to reduce appetite,[148] improve insulin sensitivity, and promote weight loss.[149]

Fermentation of fiber produces gas, but this effect was reduced for longer polymers (chains) of sugar molecules.[150] Alpha-galactosidase (an enzyme that may be taken orally) has been shown to reduce the volume of gas resulting from fiber fermentation in the colon.[151,152] Concern about flatulence from eating beans is reportedly based on an exaggerated perception of the effect,[153] and ignores the health benefits. Mixing prebiotics can reduce gas.[154] Dietary meat increases the unpleasant odor in gas resulting from sulfur compounds,[155] which can cause DNA damage (increasing cancer risk).[156]

Conclusion

Existing and new information about the importance of our microbiota suggests everyone should supplement with a high-quality probiotic on a daily basis. Considering the harm that can be caused to the microbiota by the use of antibiotics, it

seems prudent to avoid antibiotics when possible and to supplement with probiotics following an antibiotic treatment cycle.

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