The gut is home to trillions of bacteria that play many roles in human health and disease. New research described in this chapter sheds light on how certain genetic variants that increase risk of inflammatory bowel disease (IBD) may affect the way gut cells respond to those bacteria. (Left panel) The bottom half of the image shows cells that line the gut. Above the cells, indicated in a black box, is a friendly type of bacteria called *Bacteroides fragilis* (*B. fragilis*), which normally resides in the gut. (Right panel) *B. fragilis* helps keep the gut’s immune system in check by delivering certain bacterial molecules to intestinal immune cells via small spheres, called outer membrane vesicles (gold), that bud from the bacterial cells’ outer coating (green). The components of these vesicles suppress an immune reaction. However, mouse immune cells lacking functional ATG16L1 protein cannot respond to these vesicles, which could lead to an improper inflammatory reaction to *B. fragilis* and other friendly gut bacteria. In some people, the ATG16L1 gene contains variants that impair the ATG16L1 protein and are implicated in IBD. This new finding about immune cell responses to gut bacteria provides a possible link between the genetics and the biological processes underlying IBD.

*Images provided by Dr. Sarkis Mazmanian, California Institute of Technology.*

*Credited by Mark Ladinsky/Greg Donaldson/Caltech*
Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of doctor visits, hospitalizations, and disability in the United States each year. These conditions span a wide spectrum of disorders that affect the gastrointestinal (GI) tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. The latest concerted effort to address the burden of all digestive diseases combining multiple big data sources estimated that digestive disease is the primary diagnosis in a total of 72 million ambulatory care visits to physicians’ offices and hospital emergency and outpatient departments in the United States each year. In addition, an estimated 4.6 million hospitalizations with a primary diagnosis of digestive diseases and 13.5 million hospitalizations with a primary or secondary diagnosis of digestive diseases are reported. More recently, a study focusing specifically on the clinical and economic burden of emergency department visits reported 15.1 million emergency department visits with a primary diagnosis of digestive diseases and a total charge of $27.9 billion in 2007.

Some digestive diseases are common and others quite rare. Yet collectively, they strike individuals across the lifespan, exacting a significant toll on public health in terms of their effects on quality of life, years lost due to premature death, and costs associated with hospitalization and pharmaceutical and surgical interventions. NIDDK-supported scientists are vigorously pursuing research with the ultimate goal of reducing the public health burden associated with digestive diseases. Such efforts aim to determine how widespread these diseases are across the United States and in specific population groups, to identify their causes and how they progress, and to test new interventions for prevention and treatment, including drugs, surgery, and behavior modification.

Inflammatory bowel diseases (IBD), which include Crohn’s disease and ulcerative colitis, are marked by damaging inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. These diseases often strike early in life, with a peak age of onset in adolescence or young adulthood. Treatment frequently requires prolonged use of multiple drugs and may require surgery, including removal of the affected region of the intestine. Scientists are investigating the complex interactions among the genetic, environmental, immune, microbial, and other factors that contribute to, or protect against, the development of IBD. The continued discovery of predisposing genetic variations, potential autoimmune and microbial influences, and new methods to repair damaged intestinal tissue will help catalyze the design of novel therapeutic strategies. Research on controlling intestinal inflammation has potential benefits not only for patients with IBD, but also for those at risk of developing colorectal cancer.

Diseases of the stomach and intestines include some of the most common digestive diseases, such as peptic ulcer disease, which is typically caused by an infection with the bacterium Helicobacter pylori or use of non-steroidal anti-inflammatory drugs. Stomach and intestinal disorders also include functional bowel disorders, which result in symptoms of abdominal pain and altered bowel habits. For example, irritable bowel syndrome (IBS) causes pain and constipation or diarrhea. IBS more frequently affects women, who may display a different range of symptoms and respond differently from men to pharmacologic treatments for the disease. While diet and stress contribute to this disorder, its underlying causes are unknown. Gastroesophageal reflux disease, in which stomach acids rise up into the esophagus, is a common functional bowel disorder that can lead to a condition known as Barrett’s esophagus. This condition, in which cells lining the esophagus turn into an intestinal type of cell, is associated with a heightened risk of esophageal cancer—one of the cancer types still on the rise in the United States. Gastroparesis, another type of functional bowel disorder, is characterized by

delayed emptying of food from the stomach, resulting in nausea, vomiting, and abdominal discomfort. While many cases of gastroparesis are of unknown origin, a common cause is diabetes, which is thought to damage nerves leading to the stomach and controlling movement of food. Fecal incontinence, or impaired bowel control, is another bowel disorder that poses a major public health burden. Although fecal incontinence is more common in older adults, it can affect people of any age. Because it is difficult to talk about, many people suffer without seeking professional treatment for this surprisingly prevalent condition. Researchers thus aim both to examine barriers in addressing fecal incontinence and to develop improved treatment strategies.

Some digestive diseases can be triggered by the body’s reaction to certain foods. For example, in individuals with celiac disease, the immune system reacts to the protein gluten—a component of wheat, barley, and rye—and damages the small intestine. This damage interferes with the ability of the intestine to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, slower growth and short stature. The only current treatment for celiac disease is maintenance of a strictly gluten-free diet, which is difficult for many people. Diagnosis of celiac disease can be challenging, due to the non-specific and often minimal symptoms in people with the disorder. Recent and continued advances in the understanding of genes that predispose individuals to develop celiac disease may contribute to improved diagnosis in the future through genetic-based screening.

The microbes that inhabit the GI tract are important factors in maintaining or tipping the balance between digestive health and disease. These bacteria and viruses can affect long-term health and nutritional status in some surprising ways, depending on their interactions with each other, with intestinal cells, and with nutrients ingested by their human host. Scientists are gaining insights into the ways these GI microbes influence the development and function of the digestive tract and other systems throughout the body, such as those with immune and metabolic functions, as well as how the composition of the GI microbial community changes with factors such as age, geography, diet, and antibiotic usage.

The exocrine pancreas, which secretes enzymes required for digestion, is vulnerable to disorders such as acute and chronic pancreatitis and their complications. Common causes of pancreatitis include gallstones, heavy alcohol use, inherited genetic factors, and drugs. In all forms of pancreatitis, digestive enzymes attack the pancreas from within, causing inflammation, loss of function, and severe pain. Advanced pancreatitis can be debilitating and may lead to cancer or diabetes, and many cases are advanced by the time they are diagnosed because pancreatitis is difficult to detect in its early stages. Research has elucidated genetic and other factors contributing to pancreatitis that may lead to ways to treat or prevent this disorder.

The liver is an organ within the digestive system that performs many critical metabolic functions, including processing and distribution of nutrients such as fats. When the liver is functionally compromised by disease, serious adverse effects on health can occur, which sometimes leads to complete liver failure. Some liver diseases primarily affect children, such as biliary atresia (a progressive inflammatory liver disease), while others generally affect adults, such as a form of nonalcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH). In recent years, however, NAFLD has been increasingly diagnosed in children in the United States as well, concurrent with rising overweight and obesity. Some forms of liver disease are caused by viral infection, as in most cases of hepatitis, or by genetic mutations such as alpha-1-antitrypsin deficiency; others arise from diverse factors such as autoimmune reactions, drug toxicity, and other triggers, some of which are unknown. Many liver diseases, such as chronic hepatitis B and C, place individuals at elevated risk for developing liver cancer. A healthy liver is necessary for life, and the only treatment for end-stage liver disease is a liver transplant. Because the number of livers available from deceased donors is limited, research is critical to identify liver disease early, find methods to preserve liver function in people with liver disease, and develop and further study new treatment options, including experimental, cell-based approaches to liver regeneration.

The number of Americans who are overweight or obese has risen dramatically in recent decades and is now at epidemic levels. Obesity is associated with numerous diseases, including type 2 diabetes, heart disease, and cancer. Multiple factors contribute to obesity. As scientists elucidate the molecular, genetic, microbial, and environmental factors that influence appetite, metabolism, and energy storage, they are
identifying potential avenues for the development of new intervention strategies to promote safe, long-term weight loss. In addition to new pharmacologic interventions for obesity that may arise from research, existing bariatric surgical techniques are being evaluated for their long-term impacts on weight loss, obesity-associated disease, and well-being. Investigators are also continuing research to help people achieve healthy lifestyles that include physical activity and improved diet. (Additional information on NIDDK-supported research endeavors focusing on obesity is provided in the Obesity chapter.)

Other nutrition-related disorders under investigation involve specific, inherited alterations in nutrient metabolism. NIDDK-supported research has enhanced knowledge of how these nutritional disorders develop and how they can best be treated.

**GUT MICROBES IN HEALTH AND DISEASE**

**Nourishing Gut Bacteria To Help Young Children Avoid Undernutrition:** In ongoing studies of the role of gut microbes in childhood undernutrition, researchers studying children living in developing countries have shown how an “immature” community of microorganisms housed in their guts contributes to impaired growth (stunting) and how unique nutrients found in milk can interact with these microbes to foster growth. Childhood undernutrition is a leading cause of mortality in children worldwide that is attributed mainly to a lack of access to nutritious food. However, undernutrition and its consequences, such as stunted growth, impaired intellectual development, and compromised immune function, are exacerbated by other biological factors, even if nutritious food or a dietary intervention later becomes available to the children. In a previous study in Bangladesh, the same research group had observed that changes in the gut microbial community that normally occur as children mature were not taking place in undernourished children. In their latest studies on the subject, they continue their explorations of how an immature gut microbial community contributes to the growth impairments of childhood undernutrition. They also discovered that some elements of the children’s diet, namely special sugars present in milk, directly “feed” the gut’s microbes and, by extension, support healthy growth.

For the first study, the group of scientists began by analyzing fecal samples collected for a previous study of young twins and triplets, ranging from newborns to 3-year-olds, living in rural parts of another country, Malawi, to determine whether a particular set of gut bacterial species was associated with age-appropriate growth in healthy children or with reduced growth in undernourished ones. Similar to the earlier study by the group in Bangladesh, they discovered that undernourished children had a more immature community of gut microbes than their healthy counterparts. Also, the level of gut microbial maturity at 12 months of age predicted growth at 18 months.

To see whether this abnormal set of gut microbes may be contributing to growth and health problems, they looked at effects in an animal model. They transplanted gut microbes taken from 6- and 18-month-old healthy or undernourished Malawan children into young male mice raised under sterile conditions free of any microbes (“germ-free”) and fed a typical Malawan diet. They showed that the immature microbial community from undernourished children can impair growth in mice, based on measurements of lean body mass gain and bone morphology, as well as altered liver, muscle, and brain metabolism. When housed in the same cage, mice that received microbes from healthy infants were able to transmit them to other mice that had received microbes from undernourished children, thereby preventing their growth impairment. They also identified two particular strains of bacteria from the healthy infants that had helped the transplanted mice thrive. Adding these two bacterial strains to the bacteria from undernourished children before giving the mixture to mice also prevented growth impairment.

In another study, members of the same research group, together with other colleagues, analyzed breast milk samples from Malawan mothers with healthy or undernourished infants 6 months after birth to identify nutrients that might interact with the children’s gut microbes and affect their susceptibility to undernutrition. They found that sugars called sialylated oligosaccharides—which are digested not by humans, but by their gut bacteria—were less abundant in the breast milk of mothers with severely growth-stunted infants. They then tested whether sialylated oligosaccharides from cow’s milk could promote growth, using a mouse model. After transplanting fecal microbes from one of the 6-month-old stunted infants into young male germ-free mice, they fed the mice a typical Malawan diet, either with or without added sialylated oligosaccharides. They showed that mice that received...
the supplemental sialylated oligosaccharides had greater muscle mass and positive changes in bone morphology and liver, muscle, and brain metabolism. These changes indicated an improved capacity for utilizing nutrients and gaining weight, despite the context of a deficient diet and gut bacteria associated with stunting. Mice that were kept germ-free while receiving the diet with sialylated oligosaccharides did not show the same beneficial effects of these sugars. These results were also verified in another animal model, the germ-free piglet, which has a physiology closer to that of humans. Results in these animal models suggest that consumption of milk high in sialylated oligosaccharides affects microbes in the gut, which in turn may promote growth in undernourished children consuming a nutrient-deficient diet.

These studies shed light on the persistent problem of childhood undernutrition by showing that an immature gut microbial community is a direct contributor to the stunting associated with this condition affecting children worldwide. They also identify potential solutions in the form of new microbial or nutrient-based interventions—such as specific bacterial species and milk sugars—that might complement existing dietary approaches, as well as animal models in which to test them.


Early-life Exposures Affect Infant Health: Three recent studies have shown how dietary and other environmental exposures, including those that shape the internal environment created by gut microbes, are critically important during the first few years of life, with implications for a lifetime of good health. These exposures include not only the diet of the mother and child, but also other experiences that have a large impact on the bacterial populations of a child’s gut, such as antibiotic treatment and delivery by vaginal or cesarean modes. More and more, the gut microbial community is being appreciated for its effects on human health, and the first 3 years of life is an important period for maturation of this gut microbial community. For example, by training the developing immune system, gut microbes are thought to play a possible role in guarding against autoimmune diseases such as type 1 diabetes and inflammatory bowel disease, as well as other immune-related diseases, including asthma and allergies. Early disturbances in the gut microbial community from such factors as antibiotics or cesarean delivery have also been linked to an increased risk for metabolic disorders, such as obesity. Studies by three research groups have delved into how great an impact these early exposures can have on infants, potentially affecting their future health.

As part of the Healthy Start Study, researchers studied over 1,000 pairs of mothers and infants from multiple ethnic backgrounds to see how different types of foods eaten during pregnancy might affect infant body fat. The mothers were recruited during pregnancy. The researchers collected blood samples and information from the mothers on such subjects as physical activity and diet. Throughout pregnancy, participating mothers also completed several 24-hour dietary recalls online to provide a more complete picture of their diets. After delivery, information was collected in the hospital on the mothers and babies, including measurements of the infants’ length, weight, and skin-fold thickness. The researchers also estimated the infants’ body composition, including fat mass and fat-free mass. The mothers’ diet quality was measured using a scoring system based on the 2010 Dietary Guidelines for Americans. The researchers found that consuming a lower-quality diet (e.g., more fat and sodium, and fewer fruits and vegetables) during pregnancy was associated with a higher percent of fat mass in the newborns, regardless of how much the women had weighed before pregnancy. The researchers plan to continue studying these infants to figure out what effect a larger fat mass at birth has on the risk of developing obesity in childhood and later in life. This study highlights a potential way to improve the health of newborns—eating more healthfully during pregnancy.

Another research group followed the gut microbial development of 43 U.S. children during their first 2 years using genetic techniques to characterize the evolving community of bacterial species present in their stool samples during this dynamic period of development. They collected vaginal swabs, rectal swabs, and stool samples from mothers, both before and after delivery, and stool samples from the infants. Typically, infants’
gut microbes follow a developmental program of maturation with some species dominating the mix at certain stages, which continues from birth until around age 3, after which point the microbial mix resembles that of adults. The researchers identified three major phases in the development of the gut microbiome in early life, with a type of bacteria called Enterobacteriaceae dominating in the first month, a more dynamic period from 1 to 24 months of life, then a more adult-like gut bacterial community resembling their mothers’ around age 2 years. However, they observed that the predominant species in the mix were affected in early life by delivery mode (vaginal versus cesarean section), infant diet (breastfeeding versus formula feeding), and antibiotic treatment, particularly during the dynamic middle phase. After the first few months of life, infants delivered by cesarean section had less diverse and less mature gut microbial communities than those in vaginally delivered infants. With antibiotic treatment, the diversity of species in the gut also diminished, and the developmental maturation of the gut microbiotal community as a whole was delayed; however, the effect was less than that of delivery mode. Gut microbiota diversity and maturity was also reduced between ages 1 to 2 years in infants fed with formula compared to breastmilk.

A similar study focused on the gut microbial changes in 39 children living in Finland during their first 3 years of life, using more in-depth DNA sequencing of the children’s stool samples. In these children, all of whom were breastfed for some amount of time, the gut microbial community development was most rapid during the first 6 months of life. As with the study of the gut microbiota in American children, the researchers found the Finnish children who were born by cesarean section or who received antibiotic treatment had a less diverse set of bacterial species in their gut. However, unlike the American study, they found that a proportion (20 percent) of vaginally born children also showed reduced numbers of some key bacterial species, called Bacteroides, that were lacking in all of those born by cesarean. Also unique to this analysis was their ability to probe deeper into the specific strains of bacteria present within the species. Through this analysis, they could see that antibiotic treatment had an even greater impact on reducing gut microbial community diversity at the level of specific bacterial strains than it did at the species level. Antibiotic treatment was also associated with a less stable gut microbial community and an increase in antibiotic resistance genes.

More research will be needed to understand fully the long-term effects of these early exposures—from the quality of the mothers’ diet during pregnancy to disruptions within the infant gut microbiome due to delivery mode, antibiotic treatment, or feeding method—on the health and disease risk of children as they grow. For example, future studies could determine, at the level of bacterial genes and their gene products, the implications of these disruptions for gut microbial community function and, by extension, human health.


Eating Fiber for the Health of Future Generations:
Researchers have discovered that a low-fiber diet causes decreased diversity of gut bacteria in mice, as well as progressive loss of bacterial diversity in future generations. People’s gut microbiome—the collection of all microbes (e.g., bacteria, fungi, viruses) present in the gut and/or their genetic material—usually contains hundreds of different bacterial species. It is known that people who eat a Western diet (i.e., low in fiber and high in fat and simple sugars) have a less diverse gut bacterial community than people who eat a plant-based, traditional diet. In other words, some bacterial groups in the gut microbiome of people eating traditional diets are missing in those consuming a Western diet. A less diverse gut microbial community is thought to increase risk of diseases, including those of the digestive system. Researchers sought to determine what factors could be contributing to this decrease in diversity and zeroed in on dietary fiber because gut bacteria use it as a main energy source.

To study the role of dietary fiber in an animal model with a gut microbiome resembling that of humans, they first introduced human gut bacteria from a healthy

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male donor into the intestines of germ-free male and female mice—i.e., mice that do not have gut bacteria of their own. After feeding the mice a high-fiber diet for 6 weeks, the researchers switched half of the mice to low-fiber chow. After 7 weeks, they found a greater reduction in the abundance of gut bacterial groups—i.e., decreased bacterial diversity—in the low-fiber diet mice compared to mice eating high-fiber chow. When the fiber-deprived mice were switched back to high-fiber food for an additional 6 weeks, some of the bacterial groups returned while others did not. These observations suggest that consuming a low-fiber diet decreases the diversity of gut bacteria in mice, and this decrease can persist even after reintroduction of dietary fiber. Next, to examine the effect of dietary fiber on gut bacterial diversity over multiple generations, the researchers bred mice that were eating like diets to each other. They found that pups born to fiber-deprived parents had reduced bacterial diversity compared to pups born to parents eating the fiber-rich diet, even if the pups were weaned to high-fiber chow. Importantly, they also observed an increasing loss of bacterial diversity in each of four generations of the fiber-deprived mice. Feeding offspring from each generation a high-fiber diet only partially restored the lost bacteria, suggesting a progressive and permanent loss of bacterial groups over time. Only when a fecal sample from a mouse fed the high-fiber diet was transplanted into a fiber-deprived mouse were healthy levels of gut bacterial diversity completely restored.

These findings suggest that, in mice, a low-fiber diet decreases the diversity of gut bacteria that are representative of species present in humans, and this effect is compounded over multiple generations. Furthermore, reintroduction of dietary fiber can only partially restore the lost bacteria. If these findings hold true in people, the results suggest that low fiber intake may contribute to decreased bacterial diversity seen in people who eat a Western diet and that a progressive loss in diversity is possible in future generations. The researchers also propose that dietary considerations and the potential need for reintroduction of missing bacterial species should be considered in the development of strategies to modify the gut microbiome for potential therapeutic purposes.

**Understanding How Crohn’s Disease Treatments Affect Children’s Gut Microbiome:** Researchers have discovered that different treatments for Crohn’s disease have varying effects on the gut microbiomes of children and teens—a finding with implications for approaches to monitor treatment response and for potential development of future microbiome-targeted therapies. People with Crohn’s disease experience abdominal pain, diarrhea, and intestinal bleeding, and children may possibly experience stunted growth as well. Current treatments include antibiotics, immunomodulators, biologic therapies, and defined formula diets. It is known that the composition of the gut microbiome is altered in people with Crohn’s disease: there are differences in which microbes are present and at what levels. This observation suggests that the microbiome may play a role in the disease. However, it is not known how current Crohn’s disease treatments affect the composition of the gut microbiome and whether treatments restore the composition seen in healthy people. This knowledge could help scientists better understand the mechanisms by which current therapies exert their effects, thereby enabling development of more effective therapeutic strategies to improve the health and quality of life of people with Crohn’s disease.

Toward this goal, researchers analyzed fecal samples from 85 male and female children and teens with Crohn’s disease who were just starting treatment with immunosuppressive medicine or a defined formula diet, and compared them to samples from 26 healthy young people. They examined symptoms, inflammation, and changes in the gut microbiome over 8 weeks, and found that each treatment had a different effect on the composition of the gut microbiome. Treatment with the formula diet—with 90 percent of daily calories coming from the formula—substantially changed the gut microbiome within just 1 week. By 8 weeks of treatment, those who experienced benefits of the treatment—measured as reduced inflammation—had a gut microbiome that still differed from a healthy microbiome, but not by as much. In individuals who did not have therapeutic benefit, the microbiome became even more imbalanced than it was before therapy started. Additionally, after 1 week of treatment with the formula diet, the composition of the gut microbiome differed between children who ultimately responded to treatment and those who did not, suggesting that measures of the microbiome may be useful to predict who will respond.

Immunosuppressive therapy, with a compound called anti-TNFα, was found to reduce inflammation and cause the gut microbiome to become somewhat more similar to that in healthy children, although it was still altered. This finding suggests that it is possible to achieve a therapeutic effect without restoring an entirely normal microbiome. Some of the study participants were also on antibiotics, which kill bacteria. The researchers found that, apart from the effects of other treatments, antibiotic use altered bacterial species and increased levels of fungi in the gut.

Overall, the scientists found that Crohn’s disease treatments had distinct effects on the gut microbiome, and none of them fully restored the normal balance of gut microbes seen in healthy youth. These findings could open up new avenues for developing treatments for manipulating the microbiome to benefit people with Crohn’s disease. They may also potentially be used to predict who will respond to different therapies, toward a longer-term goal of personalizing treatments.

Lewis JD, Chen EZ, Baldassano RN,…Bushman FD. Inflammation, antibiotics, and diet as environmental stressors of the gut microbiome in pediatric Crohn’s disease. Cell Host Microbe 18: 489-500, 2015.

**GENETIC UNDERPINNINGS OF INFLAMMATORY BOWEL DISEASE**

**Delving into Genetics To Gain a More Personalized View of Inflammatory Bowel Disease:** Two recent studies analyzing data and records from thousands of men and women with inflammatory bowel disease (IBD) yield further insights into the multiple types of IBD and further elucidate which genetic variations make people of differing ethnicities more susceptible to the disease. These studies combined the vast data of the NIDDK’s Inflammatory Bowel Disease Genetics Consortium (IBDGC) with data from other international efforts as part of the International IBD Genetics Consortium. IBD is characterized by abdominal pain, diarrhea, fatigue, and weight loss, all caused by chronic inflammation in the gut. IBD had generally been thought to fall into two categories: Crohn’s disease, in which any part of the gastrointestinal tract may be affected by the inflammation; and ulcerative colitis, in which only the large intestine and rectum are typically affected. Because both of these diseases are influenced by genetics, there have been efforts to identify the genetic variations that are present in people with IBD. Studies over the past several years had identified 163 areas of the genome that are linked to IBD, most of which are shared between Crohn’s disease and ulcerative colitis.

One of the new studies challenged the notion that there are only two main types of IBD. The researchers examined genetic data from over 29,000 men and women of European ancestry living in Europe, North America, or Australasia who, based on previous disease classifications, had either Crohn’s disease or ulcerative colitis. However, instead of only assigning the areas of the genome to either of these diseases, they also linked the genetic regions to certain characteristics of the diseases, such as age of diagnosis, location of the inflammation, and how far the disease had progressed. The researchers found that in Crohn’s disease, three regions of the genome in particular were linked to the site of inflammation, age at diagnosis, and/or disease progression. When combining the information from all the known genetic associations, the researchers found that Crohn’s disease and ulcerative colitis have different genetic signatures; and that, in Crohn’s disease, inflammation in the ileum of the small intestine (near the junction with the colon) is genetically distinct from inflammation in the colon. These results suggest that there are actually three types of IBD: ileal Crohn’s disease, colonic Crohn’s disease, and ulcerative colitis. Knowing the type of IBD could eventually help health care providers offer targeted treatments.

Another study analyzed the genomes of about 96,000 men and women of East Asian, Indian, Iranian, European, North American, or Oceanic descent, many of whom had IBD. The large number of participants in the study enabled the researchers to identify 38 regions of the genome that are associated with IBD but had not been found in previous studies. The researchers found that variations in most of these regions were consistent across the populations from different areas of the world, which means that many treatments for IBD are likely to be effective among people with different backgrounds. However, the researchers did find variations in several genetic regions that were more common in certain populations. For example, certain variants in the NOD2 gene are strongly linked to IBD in Europeans, but these variants are not present in Asian populations. Variants in other genes are present in both populations, but appear to have different
magnitudes of effects. This information could enable care providers in the future to tailor treatments based in part on the patient’s genetic background.

These studies shed light on the genetic differences and similarities among people around the world living with IBD, pointing toward a more personalized approach to diagnosing and treating this disease.


Exploring the Genes That Keep the Gut’s Immune System in Check: Recent research into the genetics of inflammatory bowel disease (IBD) has pointed to abnormal interactions between the gut and the bacteria that inhabit it, implicating genetic defects in a process that cells use to break down microbial material. IBD is a painful and debilitating collection of diseases, including Crohn’s disease and ulcerative colitis, that are marked by inflammation and damage in the gut. The causes of IBD are unclear; however, the inflammation is believed to be caused by complicated interactions between genetic and environmental factors. In particular, research has pointed to an improper immune response to bacteria in the gut—a reaction that can be affected by human genetics. Variations in many areas of the genome have been associated with IBD, including some involved in immunity, but it has been difficult to determine how these variants might be contributing to the disease. Recently, two groups of researchers have identified how certain IBD genetic risk variants may affect the way gut cells respond to bacteria. Both groups focused on a process called autophagy, whereby damaged or unnecessary materials in cells—including bacteria and bacterial components—are packaged and broken down.

One of the research groups concentrated on the genes ATG16L1 and NOD2, both of which code for proteins that are known to play important roles in autophagy and have variants that are implicated in IBD. The scientists found that immune cells from mice lacking the ATG16L1 protein were unable to suppress inflammation when exposed to a “friendly” type of bacteria called Bacteroides fragilis (B. fragilis) that normally resides in the human gut. B. fragilis helps keep the gut’s immune system in check by delivering certain bacterial molecules to intestinal immune cells. They deliver the molecules in small spheres, called outer membrane vesicles, that bud from the bacterial cells’ outer coating. These vesicles are engulfed, packaged, and broken down by immune cells in the gut, where their components suppress an immune reaction. However, the researchers found that mouse immune cells lacking functioning ATG16L1 protein were unable to respond to these vesicles, thus potentially failing to prevent an improper inflammatory reaction to B. fragilis and other “friendly” gut bacteria. Testing this idea in a mouse model of colitis, the scientists found that mice lacking functional ATG16L1 were not protected from colitis when they were given outer membrane vesicles from B. fragilis, but mice with ATG16L1 were. Mice and cells lacking functional NOD2 also had defective responses to these B. fragilis vesicles, supporting the idea that NOD2 could cooperate with ATG16L1 in suppressing inflammation. Importantly, mice or cells from male and female IBD patients with a human genetic variant of ATG16L1 that is implicated in IBD also did not respond to these vesicles, suggesting that a failure of ATG16L1-mediated autophagy could be contributing to disease in some people with IBD.

Another team of scientists investigated the role of autophagy as a cellular defense mechanism against potentially harmful bacteria. Some types of bacteria can invade cells, causing disease, and cells typically use autophagy to package and degrade the invading microbes. Armed with this knowledge, the researchers performed genetic screening in a human cell line to identify genes implicated in IBD that are involved in both autophagy and cellular defense against bacteria. Among the genes they identified was GPR65, which has variants associated with IBD. GPR65 encodes a protein that is important for the proper function of lysosomes, which are acid-rich globules in cells that break down material packaged for autophagy. The researchers found that male and female mice without functional GPR65 protein were more prone to a disease resembling human IBD when given a type of bacteria that causes intestinal inflammation in mice. This effect was
seen when GPR65 was absent from either the cells lining the gut or the immune cells within the gut. The lysosomes of intestinal and immune cells lacking GPR65 were unable to properly degrade invading bacteria. This could be explained by the observation that the lysosomes were not positioned properly in the cell and were not as acidic as normal lysosomes. Importantly, the researchers also tested a human cell line engineered to have a genetic variant found in male and female IBD patients, and immune cells from IBD patients who have this variant, and they found that these cells were also defective in destroying invading bacteria. These results suggest that this genetic variant of GPR65 could promote IBD by crippling autophagy and cellular defense against disease-causing bacteria.

By showing that certain genetic variants identified in IBD patients can cause defects in the way cells relate to, or defend themselves from, bacteria in the gut, these results provide possible links between the genetics and the biological processes of IBD. They also open the door to future treatments that could help restore proper relationships between bacteria and the gut immune system in people with IBD.


Inflammatory bowel disease (IBD) is the collective term for a group of debilitating digestive disorders, including Crohn’s disease and ulcerative colitis, characterized by chronic inflammation in the gastrointestinal tract. IBD affects millions of people in the United States. Not only can the disease be very painful, but it is also usually accompanied by diarrhea, bleeding, and loss of appetite. Severe cases can lead to tears in the gastrointestinal tract. Despite the high burden of IBD, it has been extremely difficult to pinpoint the precise causes of the inflammation, although it appears to result from complicated interactions between genetic and environmental factors. Identifying the genetic contributions to IBD would have important consequences. Not only would it provide opportunities for genetic screening to help individuals seek treatment before symptoms become severe, but it may also shed light on future treatments by identifying possible targets for therapeutics.

The NIDDK’s IBD Genetics Consortium (IBDGC) was established in 2002 to identify genes that are involved in IBD susceptibility. In collaboration with the International IBD Genetics Consortium, of which it is a member, the IBDGC has enrolled thousands of IBD patients and identified about 200 regions of the human genome that are associated with risk of IBD. This work has yielded important new insights into the nature of the disease. For example, IBDGC researchers found variations in several genetic regions that are more common in IBD patients from certain populations across the world, which could enable tailoring of future treatments based in part on genetic background. Another study found that there are actually two genetically distinct types of Crohn’s disease, which could help guide targeted treatments in the future. Despite these advances, many of the specific genes involved in IBD, along with their respective genetic variants that contribute to IBD susceptibility, have yet to be identified. To continue investigations into the genetic underpinnings of IBD, and to build upon the successes of the initial phase of the IBDGC, support for the consortium will be renewed in 2017. A goal of the next phase is not only to continue identifying regions of the genome associated with genetic risk for IBD, but also to precisely identify specific genes and genetic variants within these regions that are involved in IBD susceptibility. The consortium will also delve into how genetic factors influence the development of IBD by investigating the functions of candidate genes.

By shedding light on the genetic foundations of IBD, the IBDGC continues to uncover a wealth of details about the potential causes of the disease. Future work by the consortium could contribute to the development of novel diagnostic and therapeutic strategies.
**PANCREATITIS RESEARCH**

**Chronic Pancreatitis: Cause, Not Gender, Determines the Disease:** A study of hundreds of people with chronic pancreatitis has found that its clinical presentation and course of treatment are determined by the underlying cause of disease, rather than by the patient’s gender, as was previously thought. The pancreas is a vital organ that secretes digestive enzymes into the small intestine where they help break down certain foods. The enzymes normally do not become active until after they leave the pancreas. However, in pancreatitis, the enzymes attack and damage the tissues inside the pancreas, leading to inflammation, severe pain, and nausea. Chronic pancreatitis, in which the inflammation is long-term and does not heal or improve, is diagnosed most commonly in alcoholic men, suggesting that gender may play a role in the susceptibility to this disease. However, recent studies have suggested that genetic variants and risk factors such as smoking, in addition to alcohol, play important roles in raising susceptibility to the disease, independent of the person’s gender. These complicating factors, along with a dearth of studies focusing on women with chronic pancreatitis, have raised the question as to how large a role gender plays in the development and treatment of the disease.

To investigate the role of gender in pancreatitis, researchers analyzed data from 521 men and women who participated in the NIDDK-sponsored North American Pancreatitis Study 2 (NAPS2). All participants had been diagnosed with chronic pancreatitis, and 45 percent of the individuals studied were women, a surprising number for what has historically been thought of as a male-dominant disease. Equal proportions of men and women reported having abdominal pain, and the use of pain medications was similar between men and women. The conditions that commonly accompany chronic pancreatitis, such as pancreatic duct stones and defects in the production of pancreatic enzymes, were also similar between men and women. However, the researchers found that alcohol was more likely to be diagnosed as the cause of pancreatitis in men than women (about 60 percent of men in the study, versus 30 percent of women). In contrast, women were more likely to have pancreatitis due to an obstruction (such as the presence of gallstones) or unknown cause. Women were also more likely than men to undergo gallbladder removal or sphincterotomy procedure, in which the circular muscles constricting the ducts draining the pancreas are severed to allow the flow of digestive juices. However, the researchers found that these procedures were more likely to be performed in women because the cause of the pancreatitis was unknown, and not because of gender differences in their diseases per se. These results suggest that it is the cause of the chronic pancreatitis—such as heavy alcohol use, gallstones, or an unknown cause—and not necessarily the patient’s gender that determines how the disease presents itself and how it is best treated. These results could help health care providers determine which patients would benefit from certain courses of treatment, including identifying any biases that may exist in the current forms of treatment chosen for patients of different genders.


**Identifying Risk Factors for Pancreatitis in Children:** In the largest study of its kind, an international group of researchers found that genetics, birth defects, and ethnicity may play important roles in the occurrence of pancreatitis in children. Pancreatitis, or inflammation of the pancreas, is accompanied by abdominal pain, nausea, vomiting, and, in severe cases, permanent tissue damage. Pancreatitis can be acute (occurring suddenly and usually self-resolving after a few days) or chronic (long-lasting). In some cases, recurring acute episodes can lead to the more debilitating chronic form of the disease. While both forms of pancreatitis are more common in adults, they can also develop in children. However, researchers have struggled to identify the factors that put young people at risk for pancreatitis, partly because the most common risk factors for adults—gallstones and heavy alcohol use—are rare in children.

The multinational INSPIRE (International Study Group of Pediatric Pancreatitis: In Search for a Cure) consortium was established to investigate the risk factors and outcomes of pediatric pancreatitis. The consortium, which has enrolled the largest cohort of pediatric pancreatitis patients to date, collected genetic, demographic, and clinical data from 301 children (girls and boys aged 19 and under) with acute recurrent or chronic forms of pancreatitis. The most common risk factor for pancreatitis in children...
was at least one mutation in any of four genes that are known to be associated with pancreatitis—*CFTR*, *PRSS1*, *SPINK1*, and *CTRC*. Mutations in *PRSS1* and *SPINK1* were more common in children with chronic pancreatitis than in children with acute recurrent pancreatitis, which means that mutations in these genes may increase the risk of transitioning from acute to chronic pancreatitis. Another risk factor found was obstruction of the pancreatic duct, most frequently by a relatively common birth defect known as pancreas divisum, in which the pancreas is drained by two smaller ducts instead of a single one. Other risk factors for pancreatitis that were identified were toxic or metabolic factors and autoimmune diseases, but they were not as common as genetic or obstructive factors. Many of the children in the study were found to have multiple risk factors for pancreatitis, suggesting that the disease may result from a complex interplay among more than one factor. The researchers also found that non-Hispanic children were more likely than Hispanic children to develop chronic pancreatitis. In addition to identifying risk factors, the INSPPIRE researchers also examined the burden of disease in children with pancreatitis. They found that children with both forms of pancreatitis endured significant abdominal pain, along with a number of emergency room visits and hospitalizations. Children with chronic pancreatitis had a higher number of emergency room visits and hospitalizations than children with recurrent acute episodes, underscoring the need to diagnose and treat pancreatitis early to avoid progression of the disease to the chronic form.

Additional research is needed to tease out how these factors drive pancreatitis development and progression in children. However, overall, the results in this study suggest that there are potential ways to screen for increased risk of pancreatitis in children, such as genetic testing, possibly providing the opportunity for early intervention before the disease develops or becomes chronic.


**IRRITABLE BOWEL SYNDROME RESEARCH**

**Pain Expectations: Altered Brain Responses in People with Irritable Bowel Syndrome:** A recent study suggests that people with irritable bowel syndrome (IBS) engage brain regions involved in threat appraisal and emotion more than healthy people do when facing an uncertain threat of pain. IBS is a functional gastrointestinal disorder that is more common in women than in men. People with IBS have chronic or recurring abdominal pain and altered bowel habits, such as constipation and/or diarrhea. The cause(s) of IBS are unknown, but it is thought that multiple signals flowing in both directions between the brain and the gut (the “brain-gut axis”) play a major role in onset and recurrence of symptoms. Studies have shown that when people with IBS are either told to expect or are actually undergoing a painful rectal stimulus, brain regions involved in pain processing and threat appraisal are much more active than they are in people without IBS. At the same time, it is suspected that brain regions involved in emotional arousal contribute to symptom hypervigilance and visceral hypersensitivity in people with IBS.

The new study investigated whether people with IBS show altered brain activity when uncertain about a future abdominal pain experience. The pain experience used in the study was an electrical stimulation delivered via patch electrodes on the abdomen. Prior to the experiments, the amount of stimulation to deliver to each individual was carefully tested to achieve a level that person considered unpleasant but tolerable. Using imaging technology, researchers then looked at the brains of men and women with or without IBS under several repetitions of three conditions: when told verbally and with a visual cue that they may expect an unpleasant external stimulus to the abdomen within a certain time frame (“cued threat”); when told verbally and with a visual cue that there would be no stimulus within a certain time frame (“cued safe”); and when told verbally that there would be no stimulus, but without a visual cue about either the stimulus or the time frame (“uncued”). The experiments were designed to increase the sense of ambiguity and the likelihood that anxiety would be heightened during the “uncued” condition, by alternating the “uncued” and “cued” conditions and leaving the electrodes attached to the abdomen at all times. The scientists found that all participants showed activation of several brain regions in response to both the “cued threat” and the “uncued” condition when compared to the “cued safe” condition. Compared to healthy people, however, people with IBS showed greater activity in brain regions involved in threat appraisal, emotional arousal, and self-consciousness during the “uncued” condition versus the “cued safe” condition. This difference was primarily seen among women with IBS compared to their
healthy counterparts. Ambiguous situations generally lead the brain to engage in developing predictive responses, especially in those with anxiety, which occurs commonly in people with IBS. These results provide clues into the role of brain response to context—i.e., uncertainty—in symptom experience, including symptom hypervigilance, in those with IBS. Future studies should help to refine these findings and flesh out the additional influence of sex and gender.


New Insights into Early-life Influences on Irritable Bowel Syndrome: New findings about stress and the brain emerging from animal studies could help advance scientists’ understanding of irritable bowel syndrome (IBS). IBS is a functional gastrointestinal disorder that is more common in women than in men. Its symptoms include chronic or recurring abdominal pain. Research has shown that people with IBS experience much greater pain or discomfort than people without IBS in response to abdominal sensations, such as the pressure of gas or stool in the gut, and that alterations to nerve pathways in the brain and gut are involved in this heightened sensitivity. Other studies suggest that stress, especially early in life, is associated with development of IBS symptoms in people, although the exact mechanisms for this risk are still incompletely understood.

Working with male and female rats, scientists have mapped brain changes brought on by stress early in life that appear to heighten responses to uncomfortable sensations in the abdomen. In these experiments, a condition of early-life stress (ELS) was created by limiting the bedding available to rat pups and their mothers for a week soon after birth. (They were then given normal bedding again.) For comparison, other rat pups were given only normal bedding, so they would not be stressed. At age 10 to 11 weeks, all of the rats were exposed to an uncomfortable sensation in the abdomen, during which both abdominal and brain responses were measured and evaluated using a number of techniques. The scientists found that, compared to the unstressed rats, ELS rats displayed heightened sensitivity in their abdominal responses to the uncomfortable stimulus. Also, while all the rats showed changes in brain activity in response to the stimulus, ELS rats displayed multiple differences from non-stressed rats in the activation of brain regions involved in pain. Furthermore, ELS rats showed an increase in functional connections between regions of the brain comprising the “pain circuit.” Finally, although male and female rats showed no differences in their abdominal responses to the uncomfortable stimulus, the researchers found sex-based differences in brain activation responses in each group. They also determined that ELS exposure affected male and female brain responses differently.

These results suggest that early-life stress does have a long-term impact on certain brain regions and pathways implicated in IBS, and that there are likely sex differences in this effect, consistent with some studies of humans. These findings in an animal model can now be used to guide further exploration of the mechanisms underlying risk for IBS in people.


Adverse Childhood Events Associated with Irritable Bowel Syndrome: A study has shown that early adverse life events are associated with irritable bowel syndrome (IBS). IBS is a functional gastrointestinal (GI) disorder that disproportionately affects women and is characterized by abdominal pain and changes in bowel habits, such as diarrhea or constipation. Patients with IBS are more likely than those without the condition to report a history of some isolated early childhood traumas, such as physical abuse, sexual abuse, or household mental illness. Previous studies have shown a relationship between childhood trauma and increased risk of chronic health conditions, including coronary heart disease, diabetes, and mental distress using a tool called the Adverse Childhood Experiences (ACE) questionnaire. However, the questionnaire had never been used to provide a more comprehensive picture of how childhood traumas relate to GI disorders, such as IBS.

To examine the link between exposure to early childhood trauma and IBS, researchers administered the ACE questionnaire to a group of 148 people with IBS as well as a similar number of healthy individuals. Study participants included both women and men, although women predominated, particularly in the IBS group. An ACE score was then generated based upon...
the participants’ responses to 18 questions within 8 separate categories of trauma, including physical, emotional, and sexual abuse as well as general trauma—the higher the score, the greater the number of traumatic childhood experiences. They found that the odds of developing IBS were twice as high in those with a history of adverse childhood experiences. Compared to healthy individuals, people with IBS had significantly higher ACE scores. When investigators examined the relationship between various types of childhood trauma and the risk of developing IBS, they found the strongest predictors to be a history of emotional abuse and a mentally ill or incarcerated family member. Furthermore, ACE scores were positively correlated with GI symptom severity—in other words, the worse the symptoms, the higher the score. In order to confirm that the ACE questionnaire is a valid tool to study IBS, the researchers compared their results to results from a different questionnaire. They found the results to be very similar, and the ACE questionnaire also provided additional information.

These findings provide evidence of a strong relationship between several types of childhood trauma and the risk of developing IBS later in life. The researchers also note that past studies have shown that psychological therapies can be helpful for people with IBS who had been abused, and thus, a better understanding of an individual’s history of adverse childhood experiences can help inform treatment strategies. While the study does have limitations, including that the study population was mainly from one geographic area, the ACE questionnaire provides a valid tool with which to measure the impact of multiple forms of early childhood trauma on IBS risk and severity.


INSIGHTS INTO ACUTE LIVER FAILURE OUTCOMES

Improved Outcomes and Survival Following Acute Liver Failure in Recent Years: Results from a national, multi-center study spanning 16 years showed that outcomes and survival have improved for people who experience acute liver failure, including those who did and did not receive a liver transplant. Acute liver failure occurs when severe liver injury takes place suddenly and without any signs of preexisting liver disease. The leading causes of acute liver failure in the United States include damage from drugs, in particular from an overdose of acetaminophen, the drug found in many commonly used non-prescription and prescription pain relievers, or from liver diseases such as viral hepatitis or autoimmune hepatitis, though the cause is unknown in about 10 percent of cases. Some people who experience acute liver failure require a liver transplant to improve their likelihood of survival. Although the demand for liver transplants far exceeds supply, those who are able to be transplanted fare well, with a better chance of survival than those who do not receive a transplant.

In the current study, researchers analyzed data collected since 1998 by the NIDDK-supported Acute Liver Failure Study Group, including more than 2,000 women and men treated at 31 liver disease and transplant centers throughout the United States. Data, including clinical features, treatments, and outcomes, from two 8-year periods—1998 to 2005 and 2006 to 2013—were collected and analyzed. They found that 3-week survival rates increased between the two 8-year periods, particularly for those who did not require or were not able to receive a liver transplant. The analysis also revealed a reduced rate of requests for liver transplants and the lessened use of interventions such as blood transfusions or ventilators, but also increased use of vasopressor drugs to restore blood pressure, between these two time periods. Therapeutic use of the drug N-acetyl-cysteine, typically used as a therapy for acetaminophen overdose, and increasingly used for other causes of acute liver failure as well, was also higher in the second period. This study documents how outcomes and survival have improved in recent years for individuals who experience the life-threatening event of acute liver failure. Further studies will be required to tease out which changes in medical practice, such as broader N-acetyl-cysteine use and improved intensive care, during these time periods may have led to these improvements.

**Understanding and Treating Liver Disease**

**Toxin Provides Clues to Disease Processes Underlying Biliary Atresia:** Two recent studies have probed the effects of a newly discovered environmental toxin to provide insights into the molecular processes that may contribute to biliary atresia, a serious liver disease of early infancy. In biliary atresia, the bile ducts that drain the liver and deliver bile acids to the intestine become inflamed and scarred, which causes a back-up of bile into the liver, resulting in jaundice and liver failure. Biliary atresia is fatal if not treated with surgery or liver transplantation. Although a rare disease, biliary atresia is still the most common form of severe liver disease in children and is the leading cause for pediatric liver transplantation. Its causes are not fully understood, but both inherited and environmental factors seem to play a role. In 2015, a breakthrough came in the form of discovery of a new plant toxin called biliatresone that caused a disease resembling biliary atresia in Australian sheep. The specific toxin was isolated using the larvae of zebrafish, which are valuable animal models due to their transulence, allowing their internal organs (including the gallbladder and bile ducts) to be readily viewed through the skin after exposure to the toxin. Once identified, researchers also confirmed the toxin’s effects using mouse bile duct cells that form spherical, duct-like structures when grown in cell culture in the laboratory. In recent experiments, these investigators have dissected the mechanisms behind biliatresone’s toxic effects on bile duct cells.

In one study, the zebrafish larvae model was used to define the biochemical pathways by which biliatresone causes bile duct toxicity. By profiling which genes were turned on in the bile ducts and livers of larvae exposed to biliatresone, they found that genes involved in protecting cells against stress, such as that caused by oxidative damage, were among those most activated as a defense mechanism against the toxin. Most striking after exposure to biliatresone were changes in genes governing metabolism of glutathione, a substance made up of three amino acids that serves as a major antioxidant in cells, specifically responsible for neutralizing toxins from outside the body. Measurements of glutathione levels in the larval cells showed that biliatresone caused a depletion in this important antioxidant, particularly in the bile ducts. Furthermore, when glutathione levels were depleted with either a chemical or genetic modification to the larvae, the bile duct cells were then even more sensitive to injury by biliatresone. Conversely, the larvae’s bile duct cells were more resistant to biliatresone injury when glutathione levels were replenished. A common strategy for boosting glutathione is the use of drug called N-acetylcysteine, which is a glutathione precursor. A second strategy is use of a chemical called sulforaphane, found in vegetables such as broccoli, that activates a master regulator of glutathione synthesis. Both of these strategies led to a decrease in biliatresone toxicity to the zebrafish bile duct cells. These findings point to glutathione depletion as playing a key role in bile duct injury from this toxin. Importantly, they also point to possible means of prevention or control of biliary atresia, such as supplementing the diets of pregnant women with glutathione precursors.

A second study focused on characterizing the toxic effects of biliatresone on functioning of bile duct cells in mice, which are closer biologically to humans than zebrafish. As in some of the pioneering research characterizing the toxin’s effects, they used spherical cultures of mouse bile duct cells, as well as intact bile ducts removed from newborn mice. Biliatresone treatment of the bile duct spheres disrupted the normal orientation of the cells and their ability to form a continuous layer, such that the spheres became leaky. Treatment of the spheres and bile ducts also resulted in scarring and blockage of the ducts. As in zebrafish, depletion of glutathione increased the toxicity and replenishing glutathione, with N-acetylcysteine or sulforaphane treatment, decreased the toxicity of biliatresone. The investigators also found that levels of the important gene regulator SOX17, which plays a role in bile duct development and maintenance, were diminished by biliatresone treatment. This work enriches understanding of some important cellular functions impaired as part of the injury to mammalian bile duct cells caused by an environmental toxin.

Although there is currently no evidence that biliatresone is the specific cause of human biliary atresia, the mechanism by which it injures bile ducts is likely similar to what occurs in humans. These studies also suggest how environmental toxins may play a role early in the disease process. Most importantly, these findings may aid in the development of new treatments or prevention strategies, either in avoiding the environmental toxins that might have similar...
Controlling Levels of Bile Acids for Liver Health:
Researchers have recently revealed an important step in the way the levels of bile acids are regulated in the liver. Bile acids, a component of bile produced in the liver, are critical for digestion and absorption of fats in the small intestine. In addition, bile acids are signaling molecules that affect metabolism. Levels of bile acids must be tightly controlled because excess bile acids can be toxic and lead to cholestasis (reduced bile flow) and liver injury. Conversely, insufficient bile acids can lead to malabsorption and malnutrition.

Previous research showed that a protein named “Small Heterodimer Partner (SHP)” plays a key role in regulating bile acid levels, but the details of this process were unknown. In a recent study, scientists discovered a clue to this process when analyzing proteins that bind to SHP when human liver cells were exposed to high levels of bile acid. The most important protein that interacted with SHP was “RanBP2.” Using biochemical and mouse liver cell experiments, the researchers found that, when exposed to high levels of bile acids, RanBP2 chemically modified SHP, facilitating SHP’s movement into the nucleus of the cell. Once in the nucleus, SHP turns off genes that are needed for synthesis and transport of bile acids. In this study, the scientists demonstrated that the chemical modification by RanBP2 was required for SHP’s activity to move into the nucleus and turn off these genes.

To study further the role of SHP, the scientists introduced a version of SHP that could not be chemically modified by RanBP2 into male mice and fed the mice bile acids. They found that bile acids levels increased in the liver, gallbladder, and small intestine of these mice, and observed pathological changes in the liver (liver cell death and increased inflammation). Thus, mice with impaired SHP proteins were unable to decrease bile acid levels to normal. In a complementary experiment, decreasing the amount of RanBP2 in mice and feeding the mice bile acids led to increased bile acid levels in the liver and gallbladder resulting in an increase in liver toxicity markers and pathological changes in the liver. These experiments indicated that the RanBP2 helps regulate bile acid levels and protects against liver damage. This study illuminated how, upon detecting elevated bile acid levels, SHP acts to reduce the levels. Developing a therapeutic that targets this pathway could be a promising direction for treatment of cholestatic liver diseases and other bile acid-related diseases.

INSIGHTS INTO THE DEVELOPMENT OF LIVER CANCER

Key Biomarker Involved in Liver Cancer Development from Multiple Causes: Scientists have discovered that activation of a protein called p62 in liver tissue already injured by diverse factors plays a central role in promoting the development of the major form of liver cancer, called hepatocellular carcinoma (HCC). HCC is known to develop in response to inflammation and damage caused by a wide range of conditions, including hepatitis B or C viral infections, alcohol abuse, and obesity—specifically the obesity-associated condition nonalcoholic steatohepatitis or “NASH,” a form of fatty liver disease. The scientific community has been searching for a common mechanism by which these diverse causes of liver injury are “precancerous” and can lead to the development of liver cancer. Recently, a group of researchers focused their attention on p62, a protein that participates in transmitting signals inside the cell and that tags damaged proteins inside cells for destruction and recycling. This protein accumulates in many forms of chronic liver disease, including those most closely linked to cancer. The investigators used several mouse models of liver disease, as well as liver samples from patients with HCC. One mouse model involved giving a carcinogenic chemical to animals genetically manipulated to lack p62. The mice that lacked p62 developed fewer liver cancers than those still producing p62. Turning to an animal model of overfeeding that mimics human NASH, the scientists fed a high-fat diet to mice with or without
Progression from fatty liver to HCC was again less in mice without p62 compared to those with normal levels of the protein. To ascertain the actions of p62 in the liver, they next engineered viruses that would infect liver cells in mice and boost their levels of p62. Infection with these viruses boosted p62 levels and caused more liver tumors in mice than did infection with control viruses that did not change the protein levels. The researchers also investigated p62’s role in liver cancer progression in humans. Using tissue samples from patients with HCC who had undergone surgery to remove the tumor, they found that patients with high levels of p62 in the remaining liver had reduced survival, likely due to HCC recurrence. This study suggests that p62 is important in the complex and varied pathways that lead from liver injury to HCC development and suggests this protein may be a promising target for future therapeutics or in providing a valuable biomarker for identifying people with HCC at increased risk of recurrence, even after surgical removal of their tumors.

Workshops: Functional Bowel Disorders and Chronic Pancreatitis in the 21st Century

The NIDDK sponsored several workshops in 2016 to identify knowledge gaps in digestive disease research that could lead to new research directions:

On June 23-24, the NIDDK convened a meeting, entitled “Functional Bowel Disorders Workshop: Future Directions in Pathophysiology, Diagnosis, and Treatment.” Functional bowel disorders (FBDs), including irritable bowel syndrome and certain types of dyspepsia (indigestion), occur when the stomach or bowels do not work properly, even though there is not an obvious physical defect diagnosed in these parts of the body. FBDs are a major health care burden in the United States. The workshop’s goal was to review recent advances in these disorders and to identify new directions for research. Among the topics discussed were new findings on the roles of gastrointestinal muscle and nerve cells in the development of FBDs. Also discussed were recent advances in the understanding of genetic and environmental factors, including the microbiome and psychosocial factors, that could contribute to FBDs. The workshop participants discussed current and emerging strategies to manage and treat FBDs, such as changes in diet and the effectiveness of current pharmaceutical therapies. New ways to diagnose FBDs were also discussed, including efforts to identify and detect physiological changes associated with FBDs.

Another workshop, entitled “Chronic Pancreatitis in the 21st Century: Research Challenges and Opportunities,” was held on July 27 to address new approaches to research on chronic pancreatitis, which is a long-lasting inflammation of the pancreas. Chronic pancreatitis is usually accompanied by abdominal pain, which can result in severe disability. The disease may also lead to other serious conditions such as pancreatic cancer or diabetes. However, pancreatitis is very difficult to detect in its early stages, so the disease is usually at an advanced stage—and difficult to treat—by the time it is diagnosed. The workshop was convened to discuss recent advances in the understanding, diagnosis, and treatment of pancreatitis, and to identify areas that should be emphasized in future research. Among the needs discussed were the identification of predisposing risk factors, such as genetic variants, and better tools to diagnose pancreatitis early and reliably. Also discussed were the development of standardized protocols to distinguish pancreatitis-induced (type 3c) diabetes mellitus from other types of diabetes, and the design of effective therapeutic strategies based on new cell culture technologies, animal models, and pain management tools. Potential future treatments for chronic pancreatitis were also discussed, such as gene therapy and new drugs that target molecules in the disease process.

Summaries of these workshops will be published in major scientific journals. The knowledge shared at these workshops will help steer research toward providing new pathways for diagnosis and treatment for these diseases.
Illuminating the Inner World of the Gut Microbiome and Its Impacts on Human Health

The human body, particularly the gastrointestinal tract, is home to a thriving community of microorganisms. Although these microbes have been the subject of scientific inquiry for many years, the last two decades have witnessed an explosion of research activity in this area, with support in part from the NIDDK. This research has illuminated the darkest corners of the human gut through breakthrough discoveries in identifying the teeming microbes it harbors and the important ways in which they influence human health and disease.

The Microbial “Organ” Within

Recent efforts to conduct a census of microbes living within humans estimate that there are as many microbial cells in the body as there are human cells, roughly 40 trillion, with the vast majority of our microbial companions residing in the colon. From the time we are colonized as infants with microbes inherited from our mothers, these fellow travelers accompany us everywhere we go and are as unique to an individual as a fingerprint. This collection of microbes is often called the “microbiome,” a term originally coined around 2001 to define the collection of microbial genetic material, but now used to refer to the entire microbial community. The microbes within and on humans include bacteria, as well as viruses, parasitic worms and protozoa, and other microorganisms called Archaea, which also inhabit more extreme environments such as hot springs and volcanoes.

Microbes found in the gut carry their own genes and perform many important functions that human cells lack, thereby augmenting the body’s genetic and biochemical repertoire. These functions include extracting energy from nutrients that human cells find indigestible, synthesizing vitamins, fine-tuning the human immune system to respond appropriately to harmful and innocuous microbes or substances, interacting with the gut lining to support its continuous cell turnover and proper barrier function to defend against pathogens, and even influencing behavior. Because the human microbial community is now thought to serve so many important purposes in the body, it has been referred to as an essential, though often underappreciated, “organ.” In recent years, new discoveries have revealed how extensively these microbial powerhouses affect human health, not only in the gastrointestinal tract where they have the greatest presence, but also in niches throughout the body such as the skin, respiratory tract, and genitourinary tract.

Through investigator-initiated efforts and participation in larger initiatives like the Human Microbiome Project, over the past few decades the NIDDK has supported a wealth of scientific advances shedding light on the gut microbiome and its functions, including its roles in nutrient metabolism and in the immune functions of the cells lining the intestine, as well as digestive diseases, obesity, and other diseases within the NIDDK mission.

Who’s Who and How Did They Come To Be There?

Scientists have made great strides in elucidating the microbial species present in the human gut and how they make this habitat their home, as a foundation for revealing their contributions to human health and...
disease. They have explored the factors affecting human gut microbial colonization, co-evolution with the microbiomes of other species and environments, and stability over time and geographical distance. This research has been facilitated by the development and use of technologies such as DNA sequencing and genomic analysis; and of pioneering work with experimental models such as germ-free animals, which are raised in a sterile environment to lack any gut microbes; and gnotobiotic mice, which have a gut microbial community that is customized, such as those transplanted with human bacteria to make their microbiomes “humanized.”

One early study used state-of-the-art DNA sequencing methods to conduct a census of bacterial communities across several body sites of health individuals, including the gut. They found that bacterial community composition varies considerably between different people, although each person’s microbiota appears to be relatively stable over time. While most of these studies have focused largely on the bacterial members of the gut microbiome during this time, scientists have also begun identifying and characterizing the viruses that live in the human intestines, including several viral types that infect bacteria, but do not harm them. Scientists also showed how time and geography affect the stability and diversity of the human gut microbiome. They found that the composition of bacterial species populating the human gut evolves with age, particularly in the first years of life, and differs among people from diverse geographic regions, potentially reflecting varying nutrition. In another study of stability of the human gut microbiome over time, researchers combined precise assessments of bacterial composition with high-throughput methods for culturing and genomic sequencing. They found that the majority of bacterial strains in an individual’s gut microbiome remains relatively stable for several years, with some fluctuations due to changes in diet and weight.

The first members of the human gut microbiome are acquired from the maternal “environment” at birth, or possibly even earlier in the womb. Exposures during this dynamic period of development early in life have been shown in the past few years to be quite influential on the establishment of the gut microbiome. Antibiotic treatment in young animals, even low-dose and short-term treatment, can dramatically alter the types of microbes present in the gut, resulting in lasting effects on metabolism, weight gain, and immune function. Recent studies of children living in the United States and Finland have expanded on this exploration to show how the gut microbiome is shaped during the first few years of life not only by antibiotic treatment, but also by delivery mode and diet, such that antibiotic treatment, formula feeding, and cesarean section delivery were associated with reduced diversity in the infants’ gut microbial communities.

Other studies have focused on the particularly powerful influence of host diet on the gut microbial community. Employing cutting-edge technology and computational methods, researchers sequenced the genomes of gut bacterial communities from humans and a wide range of other mammals to find that bacterial species differed depending on whether the animals were meat eaters, plant eaters, or omnivores. They also found that, regardless of diet, microbial communities within the guts of all animals shared a core set of bacterial genes. Scientists also looked at people’s long-term dietary patterns (such as diets high in animal protein and saturated fat, or in carbohydrates) and found that they correlate with the dominant bacterial species in their gut microbiomes. Several studies tested dietary impacts on human gut bacteria using a gnotobiotic mouse model transplanted with bacteria from human donors. Recently, researchers discovered that a low-fiber diet, in particular, reduces human gut bacterial diversity in mice and leads to progressive loss of bacterial diversity in future generations.
What Are They Doing in There?

Studies in recent years have revealed crucial insights into the myriad ways gut microbes influence host physiology and disease development. Employing cutting-edge approaches, scientists have elucidated the role of gut microbes in a spectrum of nutritional states—from the overnutrition of obesity to forms of malnutrition—and in a variety of digestive diseases.

Boosting Digestion and Immune Function

In one study, scientists characterized the functions and evolutionary adaptations of a type of Archaea called *Methanobrevibacter smithii*, which is abundant in the human intestine and known to increase the efficiency of nutrient digestion. In a germ-free mouse model, they showed that introduction of this archaeon into the gut resulted in activation of specific genes and metabolic functions. Other recent research in mice suggests that one particular type of human gut virus can confer some of the same functional benefits to its host as do gut bacteria, such as supporting normal intestinal and immune functions.

Turning to effects of the gut microbiome and host-microbe interactions on properly calibrating host immune function, researchers showed in mice that a gut cell type called a Paneth cell produces a molecule that preferentially attacks harmful, invading microbes. Another group of scientists used advanced genomic tools and data, some swimming bacteria, and see-through zebrafish to track the movements and host impacts of gut bacteria, demonstrating the bacteria’s beneficial effects on fish immunity. Scientists also found that conventionally raised mice are able to clear infection by virulent bacteria, but mice raised germ-free are not, suggesting that infection and clearance of intestinal pathogens is the result of virulence factors carried by the invaders and a competition for nutrients with other microbes. Research also showed that intestinal cells can sense potentially harmful bacteria nearby and release an antimicrobial protein to help create a protective buffer zone between the inner walls of the small intestine and the bacteria contained within. A similar study showed that another protein produced by intestinal cells contributes to creating this buffer zone by selectively puncturing the protective outer membranes of targeted bacteria. Other studies focused on one particularly “friendly” bacterial species, *Bacteroides fragilis*. Scientists found that this species actively engages with gut immune cells through molecular communication processes to maintain the “tolerance” response required for a colonization and an ongoing symbiotic relationship. Using mouse and cell models, researchers uncovered one way in which beneficial microbes in the intestine support healthy immune function in their hosts—by releasing substances called sphingolipids that keep the activity of the local immune cells in check. Recently, scientists explored how different human gut bacterial strains modulate functions such as immunity and metabolism. To do this, they transplanted gut microbes isolated from human stool samples into germ-free mice and found that several bacterial strains are associated with an expansion of immune cells in the colon, as well as an increase in fat stores.

One of the gut microbiome’s most well-recognized functions is to facilitate the digestion of nutrients that human cells are unable to metabolize. Early studies provided evidence that the polysaccharide-rich mucus gel layer of the human intestinal wall provides a matrix capable of supporting a thin layer of helpful bacteria that functions to aid in digestion of intestinal contents, as well as to augment host defenses against disease-causing organisms. Another study discovered how mouse intestinal cells detect and absorb some of the nutrients and calories produced by gut microbes’ metabolism of complex carbohydrates in the diet into short chain fatty acids. They found that the gut microbial community of obese mice is more efficient at performing this metabolic task, thereby extracting more energy from the diet.
**Obesity**

Pioneering studies in this field have been particularly prolific in investigating the role of the gut microbiome in obesity. Early studies found that conventionally raised mice have more body fat than their germ-free counterparts; when the germ-free mice were given microbes, they dramatically increased their total body fat, even while decreasing their food consumption. The scientists showed that gut microbes increase the amount of calories harvested from the diet and also boost production of liver enzymes involved in fat production. In other experiments, researchers used a “humanized” mouse model to show that two microbial species in the human gut—*Methanobrevibacter smithii* and *Bacteroides thetaiotaomicron*—have a cooperative relationship in digesting fiber that leads to more efficient nutrient absorption and energy storage as fat. Another ground-breaking study was one of the earliest to show a possible role for gut microbes in human disease; it provided evidence that the relative abundances of two types of dominant beneficial bacteria in the gut are altered in obese humans, and that their balance is restored with weight loss. Studies in lean and obese mice and humans revealed how some gut microbes not only contribute to providing extra calories by extracting more energy from food, but also modulate the biologic pathways that regulate metabolism and whether calories are burned or stored as fat. In a study of obese and lean adult twins and their mothers, researchers examined the human gut microbiota through fecal samples to determine factors associated with bacterial composition. The researchers found that obesity was associated with significantly less gut bacterial diversity than leanness. In another study, researchers showed how changes in gut bacteria play a surprising role in the progression of nonalcoholic fatty liver disease, a condition associated with obesity. Researchers revealed that gut microbes from pairs of human twins—one obese and the other lean—can transmit these body types to mice, making them gain or lose weight, in conjunction with their diets. To gain new insight into a form of gastric bypass surgery, a treatment for obesity, researchers studying a mouse model found that restructuring of the digestive tract leads to weight loss and metabolic benefits in part by altering the communities of bacteria that normally live in the intestines. Research on a large population of twins in the United Kingdom showed that genetic factors shape the composition of the gut microbial community, and that some gut microbes, such as those in the microbial family *Christensenellaceae*, may in turn affect human metabolism and propensity for weight gain. Scientists comparing different breeds of mice discovered that genetics, diet, and gut microbes acquired in different environments all interact to modify susceptibility to obesity and other metabolic conditions, such as insulin resistance.

**Malnutrition**

On the other end of the nutritional spectrum, malnutrition, particularly in children, has also been an area of intense investigation for gut microbiome researchers. A study in Malawi showed that gut microbes may play an important role in causing a severe acute form of malnutrition called “kwashiorkor” in children that persists in spite of nutritional interventions. A similar study in an impoverished urban area of Bangladesh discovered that children who are malnourished do not harbor gut bacteria typical for their age, but rather display an “immature” gut microbiome, even several months after receiving a nutritional intervention. Through further work in Malawi, researchers identified a group of bacteria in fecal samples from severely undernourished infants and children that take hold in the gut under conditions of nutrient deficiency, thwarting the body’s ability to absorb available nutrients in the diet and to fend off disease. Recently, scientists also analyzed milk samples taken from Malawian mothers with healthy or undernourished infants 6 months after birth to identify nutrients...
called sialylated oligosaccharides that specifically interact with gut microbes and affect their children’s susceptibility to malnutrition. Another recent study in Malawi analyzed the immature microbes present in undernourished children; transplanting these microbes into mice, the researchers showed that they impair growth of the mice. Giving these mice two other gut bacterial species, taken from healthy mice, improved their growth. These findings could lead to the development of interventions to modify gut bacteria in undernourished children, to improve the children’s growth and health.

**Inflammatory Bowel Disease**

The human gut microbiome has also been shown to play an important role in inflammatory bowel disease (IBD). An early study in a rat model found that the composition of gut microbes is one factor influencing IBD. Another study showed how short-chain fatty acids produced through gut bacterial fermentation of dietary fiber act on immune cells to protect against intestinal inflammation in mice. One team of scientists discovered how the gut bacterium *Bacteroides fragilis* interacts with the immune system to suppress IBD in mice by releasing a substance called polysaccharide A in small spheres, called outer membrane vesicles, which bud from the bacterial cells’ outer coating. Scientists also showed that mice with a pre-existing genetic susceptibility to intestinal inflammation fed a diet high in saturated fats from milk have altered intestinal microbial communities that occur along with changes in bile acid composition, altered immune function, and increased intestinal inflammation. These findings outline a compelling picture of how genetics, immunity, diet, and microbes interact in the development of conditions such as IBD. Other studies used mouse models to detect the relationships of microbes and immune cells to IBD and other immune-related diseases by showing that exposing pregnant mice to “friendly” bacteria shortly before delivery protected their offspring against chemically induced ulcerative colitis. Researchers also identified disease-related changes in the gut bacterial community of children with IBD, along with changes in gene activity that occurred within their gut cells, resulting in a particular microbial and genetic “signature” that could provide targets for improving diagnosis and therapy. Another group of researchers attempted to identify the bacteria associated with IBD by determining which bacteria are coated with a type of “antibody” or immune protein, called IgA, that the body produces to protect itself from foreign substances. Research on the gut “virome” points to viruses called Caudovirales inhabiting the human gut as other possible culprits in IBD. More recently, a study in children and teens showed that different treatments for Crohn’s disease, such as immunosuppressive medication or a defined formula diet, have varying effects on the gut microbiome—a finding with implications for approaches to monitoring treatment response and for potentially developing microbiome-targeted therapies. Two recent studies of the genetics of individuals with IBD have pointed to abnormal interactions between the gut and the bacteria that inhabit it in these cases, implicating genetic defects in a process called autophagy that cells use to break down microbial material.

The NIDDK continues to participate actively in the NIH’s Human Microbiome Project (HMP), which was launched in 2007 to characterize the community of microbes present in humans using DNA sequencing technology developed in large part through the Human Genome Project. Now in its second stage, referred to as the “integrative Human Microbiome Project,” the HMP is currently supporting three research projects, with the NIDDK actively co-funding and managing research to understand how the gut microbiome is altered in IBD. One project is integrating many different types of measurements of gut microbes as they change within IBD patients, including both children and adults, over time. This project is profiling the gut microbiome along with the genetics and activity of the human host to provide insights into
StoRy of DiScovery

How the microbiome interacts with the human body in patients with IBD. Also, in 2016 the NIDDK released a funding opportunity announcement to continue and expand the Inflammatory Bowel Disease Genetics Consortium (IBDGC), which will include both genetic and microbiome studies. These and other projects may help to advance understanding of how IBD develops and ultimately may be useful for informing new disease detection, prevention, and treatment strategies.

Ongoing Research Efforts

The NIDDK is continuing to support multiple avenues of research on the gut microbiome. To gain input for one recent research initiative, the NIDDK hosted a 2-day workshop in September 2014 bringing together leaders in research on the human microbiome, with the goal of identifying key research needs and opportunities for understanding how gut microbes and their interactions with the host affect human physiology and disease. Stemming in part from this workshop and its research recommendations, in 2015 the NIDDK released two funding opportunity announcements that have encouraged research the Institute is currently supporting on the human microbiome and its effects on human nutrition, obesity, and digestive and liver diseases. One project initiated in 2016 as an NIH Director’s Pioneer Award is identifying the gut bacterial species and genes behind the production of the top 100 most abundant small molecules, which may have biological activities similar to drugs. Additionally, an NIH Transformative Research Award is developing probiotics based on genetic engineering of “designer bacteria” to test as a treatment for Clostridium difficile infections, IBD, and other conditions. The NIH has also participated in broader efforts such as the White House Office of Science and Technology Policy’s National Microbiome Initiative to study the microbiomes of the human body and the environment, relating to potential applications in health care, food production, and environmental restoration. These and other future explorations of the gut microbiome have a huge potential to yield new insights about human health and promising approaches for managing disease.

Other Digestive Diseases

The gut microbiome has also been found to contribute to other forms of digestive disease. Researchers found that microbial “signatures” with certain mixes of intestinal bacteria are associated with pediatric irritable bowel syndrome, a painful condition of unknown cause. Necrotizing enterocolitis (NEC), a common and deadly form of gastrointestinal disease affecting premature infants, develops in part due to an excessive immune response to gut microbes. Studies in newborn mice showed that breast milk protects against NEC by reducing activation of a pro-inflammatory receptor on gut cells that recognizes toxic molecules on the surfaces of some intestinal bacteria. Scientists also uncovered strategies used by a particular species of food-borne bacteria to cause a form of diarrhea prevalent in infants living in developing countries, which may enable the development of new approaches to treat and prevent infant mortality caused by this intestinal infection. Additionally, researchers used genomic analysis to understand digestive disease, specifically peptic ulcer disease and gastritis, caused by particular strains of Helicobacter pylori infection, which is extremely common in the United States and other countries.
SCIENTIFIC PRESENTATION

Dr. Lee Kaplan—
Molecular Mechanisms Underlying the Beneficial Effects of Bariatric Surgery

Lee M. Kaplan, M.D., Ph.D. is director of the Obesity, Metabolism and Nutrition Institute at the Massachusetts General Hospital (MGH), and associate professor of medicine at Harvard Medical School (HMS). Dr. Kaplan graduated from Harvard University and received his M.D. and Ph.D. in molecular biology from the Albert Einstein College of Medicine. He completed an internship and residency in internal medicine and a fellowship in gastroenterology at MGH and HMS and a fellowship in genetics at the Brigham and Women’s Hospital. He is director of the fellowship program in Obesity Medicine and Nutrition at MGH, director of the Blackburn Course in Obesity Medicine at HMS, and chairman emeritus of the Campaign to End Obesity. He currently serves as Chair of the Obesity, Metabolism and Nutrition Section of the American Gastroenterological Association, Chair of the Bariatric Surgery Section, and Chair of the Clinical Committee of The Obesity Society.

Dr. Kaplan’s research is focused on the physiological and molecular mechanisms of gastrointestinal regulation of energy balance and metabolic function, and his group has pioneered the development and use of rodent models of weight loss surgery and gastrointestinal devices to explore these mechanisms. At the September 2016 meeting of the NIDDK Advisory Council, Dr. Kaplan presented findings from his laboratory’s research. The following are highlights from his presentation.

A Fresh Take on Energy Balance Regulation

Severe obesity is a chronic condition that, for many people, is difficult to treat with diet or exercise alone and increases risks for type 2 diabetes, cardiovascular disease, fatty liver disease, and many other devastating health conditions. Most current models for the regulation of energy balance—the balance between calories consumed and calories burned—are based on the idea that human behaviors drive physiological responses. That is, physical activity and the amount of food eaten drive weight loss or weight gain. However, in his presentation, Dr. Kaplan posited that an alternative, inverse model should be considered—that physiological regulation of energy balance actually drives human behaviors. In this model, the nature of the physiological inputs, such as the chemical compositions of different types of foods (nutritional intake) and the specific types of physical activity undertaken (muscle health), can affect eating and physical activity behaviors in an individual. There is growing support for this model, which predicts that the brain coordinates and integrates inputs from different systems in the body. Previous research from Dr. Kaplan’s group and others suggests that the gastrointestinal (GI) system (the gut) provides a critical function in the regulation of energy balance. Therefore, understanding the physiological characteristics of the gut could provide insights into treatments for metabolic disorders and obesity.

Bariatric Surgery as a Treatment for Severe Obesity

Bariatric surgery procedures, which alter the anatomy of the GI tract and change relationships between the gut lining and the contents of the lumen (the space inside the tubular stomach and intestines), have been performed increasingly to treat severe obesity when other interventions have not produced enough weight loss to improve health. Dr. Kaplan described different bariatric surgery procedures that have been
used in clinical practice and that induce weight loss but also have metabolic effects independent of weight loss:

- **vertical sleeve gastrectomy (VSG)**, in which a portion of the stomach is removed, leaving a sleeve or tube through which food can pass;
- **Roux-en-Y gastric bypass (RYGB)**, in which the upper stomach is connected to the middle part of the small intestine, so that food bypasses a portion of the proximal small intestine; and
- **biliopancreatic diversion/duodenal switch**, which is a more aggressive procedure that both limits nutrient-stomach interactions and includes a much longer bypass segment.

By contrast, laparoscopic adjustable gastric banding (LAGB), which reduces the opening to the stomach with an adjustable band, does not appear to have significant metabolic benefits that are independent of weight loss.

Dr. Kaplan shared long-term weight-loss and weight-regain data from different studies, to compare a lifestyle intervention with different types of surgery. Individuals who had RYGB exhibited 27 percent weight loss after 10 years, while those who had LAGB experienced about half that amount of weight loss, and those participating in a lifestyle intervention averaged only 2 percent weight loss after 10 years.

Dr. Kaplan also discussed the reduction seen in surgery-related complications. Procedural improvements and surgeons’ increased experience have led to better health outcomes for patients. He described one study by the U.S. Department of Veterans Affairs in which scientists observed a substantially lower mortality rate in bariatric surgery patients 12 years following their procedure when compared to a similar group of patients who did not undergo surgery. Other research, including the landmark Swedish Obese Subjects study, also showed reduction in mortality in people who had bariatric surgery when compared with similar people who did not.

For the past several decades, scientists and physicians viewed bariatric surgery procedures simply as physical interventions, limiting caloric intake by stomach restriction or nutrient malabsorption. Dr. Kaplan suggested a different model, in which various signals normally sent to the brain and other organs from the gut are altered as a result of GI changes from bariatric surgery. In principle, by better understanding the molecular underpinnings of bariatric surgery, it may be possible to develop therapeutics that could provide beneficial effects similar to those of bariatric surgery. Through the rest of his presentation, Dr. Kaplan discussed his research, and that of others, to determine the nature of these regulatory signals.

**Molecular Mechanisms Underlying the Effects of RYGB Surgery**

Determining the molecular and cellular changes caused by bariatric surgery requires a robust animal model in which to test hypotheses. Dr. Kaplan’s group developed a mouse model of RYGB that could be used for research, taking into consideration the minor differences between mouse and human GI systems. In a typical experiment, these mice were fed a high-fat diet to induce obesity, and divided into three groups. RYGB was performed on one group of mice, and a second group was given a sham operation as a control (referred to as “SHAM” mice). Following a recovery period, both groups were returned to a high-fat diet. SHAM mice returned to their original weights within 2-3 weeks, but RYGB mice maintained an approximately 40 percent reduction in weight for the duration of the 12-week experiment. A third group was given the sham surgery, but underfed following the recovery period (referred to as the weight-matched sham, or “WMS” group), resulting in body weights that matched those
Deeper analysis of these mice revealed that RYGB mice lost weight, in part, because of increased energy expenditure, not simply by eating fewer calories. But what is causing the increased energy expenditure that was observed? Dr. Kaplan is currently exploring some possible explanations. For example, his group has evidence suggesting that RYGB is activating brown and beige adipose tissues—two distinct types of fat that burn calories to generate heat.

These studies, taken together with many other research findings, point to an interesting new understanding of RYGB—that its mechanisms are essentially opposite to those underlying restrictive dieting. Quite differently from dieting, RYGB leads to increased energy expenditure, reduced hunger, increased satiety, and opposite responses of appetite-related hormonal signals that originate in the gut.

Weight loss through RYGB clearly involves many molecular pathways that need to be elucidated. Dr. Kaplan’s group investigated the role of melanocortin-4 receptor (MC4R), a protein found in the brain and known to help regulate energy balance and appetite. The research team compared the effects of RYGB on normal mice with the surgery’s effects on mice that lacked the MC4R gene. In both cases, SHAM mice were also used as a control. Whereas normal mice sustained a typical amount of weight loss following RYGB for this kind of experiment, mice lacking MC4R gained weight, more closely mimicking the sham surgery controls. This experiment revealed the essential role of signaling through the MC4R in mediating weight loss from RYGB, and Dr. Kaplan noted that a similar dependence of bariatric surgery on MC4R was recently observed in three patients with mutations that rendered both copies of their MC4R non-functional.

In addition to MC4R, other genes are undoubtedly playing important roles in the beneficial health effects of RYGB. Dr. Kaplan mined the sets of genes turned on or off in multiple tissues to identify other key pathways involved in the process. During their analysis, his group found RYGB altered more than one-fifth of the genes that can be turned on in the mouse. Taking a deeper look at the molecular pathways that changed after RYGB, they discovered significant changes in genes that regulate circadian rhythms—daily, rhythmic physiological changes, even at the cellular and molecular level, that adjust behaviors and bodily processes based on the day/night cycle. As Dr. Kaplan noted, several lines of research have previously linked circadian rhythms to obesity and metabolic disorders, including its effects on weight loss following bariatric surgery. For example, one study conducted several years ago examined weight loss from bariatric surgery and found that the relatively small number of patients who worked the night shift lost significantly less weight than did their counterparts who worked during the day. In addition, previous studies have shown links in mice between circadian rhythms and food intake, movement, and the gut microbiome (the community of microorganisms that reside in the GI tract) in response to a high-fat diet. Dr. Kaplan’s group is currently exploring the myriad possible mechanisms by which RYGB alters circadian rhythms, ultimately affecting weight loss and metabolism trajectories.

**Interactions Between the Gut Lumen and the Body**

Although bariatric surgery procedures differ in the way they remodel the GI tract, Dr. Kaplan pointed out that one common feature is dramatic, albeit different, changes to the composition of the contents within the lumen at every level of the gut. These contents, which include the microbiome, enzymes,
and bile acids, varied in different regions of the GI tract among mice that had different procedures. (Bile acids are chemicals released from the gallbladder into the upper portion of the small intestine, normally aiding in the digestion and absorption of nutrients; they also act as hormones, influencing metabolism and other physiological processes.) Research from Dr. Kaplan and other scientists has revealed enormous complexity in the interactions between these luminal contents and a variety of functions of the body, such as nutrient absorption, heat production, appetite, circadian rhythms, and pancreatic function. Bariatric surgery disrupts these interactions, leading to the observed physiological changes.

**Conclusions**

Hypotheses explaining the mechanisms behind bariatric surgery’s effects have evolved over the years. Initially, bariatric surgery was thought to work simply through the mechanical restriction of the GI tract to reduce the amount of food that the body could ingest or absorb. Over time, the models became considerably more complex, including physiological changes caused by bariatric surgery, and later adding the integration of specific molecular “chokepoints” (e.g., MC4R) that allow for blocking the procedures’ effects (e.g., with the MC4R mutations in mice). Dr. Kaplan suggested that global influences of bariatric surgery, as illustrated by the vast number of genes, proteins, and metabolites affected by these procedures, drive highly integrated metabolic changes throughout the body. In the early phase of this work, he anticipated that pharmacological treatment targeting a small group of pathways might be able to reproduce the effects of bariatric surgery, and his focus was on identifying those critical pathways. The sheer complexity of the physiological response to bariatric surgery has changed his perspective. Based on these more recent studies, he noted that the ideal targets of “surgicomimetic” therapy likely reside in the gut itself. He concluded that better understanding of the local GI signals that induce these complex, global physiological effects of bariatric surgery will likely provide the fastest route to new and more effective treatments for obesity, diabetes, fatty liver disease, and related metabolic disorders.
One Man’s Experience Surviving Acute Liver Failure

Monday the 29th of February, 2016, is a leap day Scott will not soon forget. It’s the day when he went from feeling he had a bad case of the flu—to the shock of learning that both his liver and kidneys were failing. In an instant, his thoughts racing as he confronted his mortality at only 34 years of age, he recalls thinking, “What if I actually do need a new liver... is this really going to happen?” Luckily for Scott, his condition had been the subject of decades of intense research efforts supported by the NIDDK, including clinical trials testing new treatments for acute liver failure.

From Aches and Pains to Organ Failure in 24 Hours

In late February of 2016, Scott was feeling ill with fever, muscle aches, and nausea. Staying home sick in Ruckersville, Virginia all week from his job as a production art manager at a T-shirt design company in nearby Charlottesville, he started to feel better, then took a turn for the worse on Sunday night. “Every single joint ached. It hurt to move,” he remembers. With a high fever that caused him to sweat through his bathrobe and sheets, and nausea limiting his water intake, he became profoundly weak. “I was walking in my kitchen, trying to make some tea, and had to put myself on the floor rather gently,” he recalls. After losing consciousness, he later woke up and remembers thinking “I’ve got to get back to bed.” When the local sheriff came by at the request of his company to check up on him, he was able to answer the door and agreed to have an ambulance called. “I just wasn’t feeling right.... I just figured I had the flu,” says Scott.

At the local outpatient emergency room on Sunday, February 28th, the staff treated his dehydration with intravenous saline. Scott called his workplace to let them know where he was and that he would likely be out for a few more days. As he understood it, the treatment plan was “…we’re going to get some fluids in you, we’re going to get you kind of stable there, and then we’re going to see if you can keep fluids down … if you can do that, we’ll send you home.” But over the next day and into early Monday, it became clear that something more serious was going on. Tests of his liver and kidney functions came back with alarming results: possible kidney and liver failure. He recalls the doctors telling him, “You look fine, but your numbers say you are not fine…. You are going to be sent to the hospital.” The staff quickly called around to area hospitals to see which intensive care units had beds available. He was ultimately transferred an hour’s drive away to Virginia Commonwealth University (VCU) in Richmond. In the early hours of Monday, February 29th, Scott was driven by ambulance to VCU, feeling awful and vomiting blood. “It was a pretty long drive,” he recalls.

The doctors were perplexed as to the cause of his sudden organ failure, particularly in someone so young without any previous history of liver or kidney problems. They questioned him repeatedly about his lifestyle and whether he had taken any medications recently, particularly the pain reliever acetaminophen. But Scott reported that he had deliberately avoided taking any acetaminophen during his illness because he was made aware of its

“Every single joint ached.... I just figured I had the flu,” says Scott. But tests of his liver and kidney functions came back with alarming results.
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potentially harmful effects on the liver by friends who were doctors. Instead, he had taken only aspirin for pain relief during the past week. They also asked him routine questions to test his mental clarity, considering that his organ failure had elevated ammonia levels in his body to a point that can cause cognitive impairment and even coma. “They kept asking, ‘What day is it? Who’s the President?’,” Scott recalls. Despite his discomfort and precarious situation, he retained his sense of humor. When asked once again by his doctors if he took drugs, he waved his hand attached to the intravenous (IV) drip, saying, “You mean this stuff?”

The medical team continued to search for clues to the cause of his organ failure. The sudden onset and marked abnormalities in his liver test results would usually point to an acetaminophen overdose, but Scott had not taken the drug. To help find out the cause, they took a liver biopsy, which showed small droplets of fat in the liver cells. Such changes are typical of a now-rare condition called “Reye syndrome,” a disease caused by a “perfect storm” of severe viral infection and aspirin. Aspirin in the recommended dosage is usually harmless, but can cause problems if taken during infections with chicken pox or influenza, particularly at higher doses. Though the doctors were not entirely sure that aspirin was the cause, they proceeded with treating his organ failure. Scott was put on dialysis and a special diet for his kidney failure. For his liver, he was treated with a drug called N-acetylcysteine, which is a safe and effective treatment when given early for acute liver failure. Also, on the afternoon of Tuesday, March 1st, they offered him the opportunity to participate in a clinical trial testing another, new treatment for acute liver failure. Though he was reluctant at first to take an experimental drug, after thoroughly reviewing the paperwork and discussing it with the study doctors, he agreed to participate.

As a backup, his doctors also set up meetings the following day, a Wednesday, with the liver transplant committee, to prepare for the worst-case scenario: the need for a life-saving organ transplant, assuming a donor liver would be available. That Wednesday night, with the situation clearly dire, he finally called his family, whom he had been reluctant to contact for fear of upsetting them unnecessarily.

Acute Liver Failure and Reye Syndrome

Acute liver failure or “ALF” is relatively rare in the United States, but can be caused by over-the-counter and prescription drugs, dietary supplements, and herbal remedies. Its most common cause in this country is the over-the-counter pain reliever acetaminophen. However, in Scott’s case, he had avoided taking acetaminophen during his illness, taking aspirin instead. Aspirin, the most commonly used pain-reliever or fever-reducer in the world, is a rare, but sometimes life-threatening, contributor to ALF. ALF from aspirin is called “Reye syndrome;” it was first reported by Dr. Douglas Reye in Australia in 1963 in children who had recently had a severe viral infection such as influenza B or chicken pox. Subsequently, more and more reports of Reye syndrome in children came in from around the world, peaking in the 1970s and 1980s. In the United States, staff of state health departments, the Centers for Disease Control and Prevention, and others, including NIH staff, reviewed case reports and conducted careful epidemiological surveys that linked Reye syndrome to the use of aspirin during the early phase of viral illness, mainly in children. This led to wide-scale public warnings in the 1980s advising that aspirin not be used in children, after which reported cases in the United States fell precipitously, from more than 500 per year before 1986 to less than 2 cases per year since then. Although rare, the syndrome is still seen from time to time, almost always in children, but sometimes in young adults.

“You look fine, but your numbers say you are not fine…. You are going to be sent to the hospital,” Scott recalls his doctors saying.
Reye syndrome is marked by dysfunction in the mitochondria—structures within cells that generate their energy—causing a build-up of fat in the liver and lactic acid in the blood. This likely occurred in Scott when he took aspirin during his infection with a flu-like virus. Along with the acute liver failure of Reye syndrome, ammonia levels rise in the blood and enter the brain, where they can cause swelling, as well as confusion, altered consciousness, and even coma. The syndrome also causes depletion of another energy source within the liver: glycogen, a stored form of glucose, as the body tries to compensate for the failing mitochondria. If appropriate medical care is not received, the syndrome can swiftly turn fatal. Fortunately, the effects of Reye syndrome can reverse spontaneously once aspirin is stopped. Its negative consequences can be managed by supporting the patient during the dangerous period of severe liver and kidney failure, so that the injury is not permanent and the organs recover with time.

STOPping Acute Liver Failure in Its Tracks

In Scott’s case, his doctors attributed his acute liver failure to an adult form of Reye syndrome, caused by his use of aspirin in combination with his flu-like viral infection. After consenting to participate in the clinical trial, called “STOP-ALF,” he was treated over the next few days with an experimental drug called ornithine phenylacetate, delivered intravenously, in addition to the other standard medical treatments he received. Based on promising results from prior research, the doctors hoped that the drug would detoxify the ammonia buildup caused by his failing liver and thereby protect his brain while the liver and kidneys slowly recovered. The doctors continued to monitor his liver and kidney functions, which soon started to improve, obviating any further discussion of a transplant. His mother stayed nearby and acted as an advocate for Scott, taking notes during visits from the doctors and handling calls to his health insurance company. During his second week in the hospital, his liver function numbers were back to normal, while the kidneys took some additional time to recover. By the time he left the hospital on March 15th, his liver had fully recovered, but he was scheduled to come back for more dialysis. Fortunately, at that later appointment, he was informed that his kidneys had improved to the point where dialysis was no longer required. He has continued to return to VCU to check in with his doctors there.

The STOP-ALF trial is part of a larger research effort supported by the NIDDK called the Acute Liver Failure Study Group, a group of clinical centers throughout the country committed to advancing understanding of acute liver failure and improving its care. The Study Group has documented the increasing frequency of acute liver failure due to drugs in the United States. In 2009, the Study Group published results of a large clinical trial showing that N-acetylcysteine was successful as an early treatment for non-acetaminophen-related acute liver failure, a finding that led to the main course of treatment chosen for Scott. More recent work by the Study Group highlights the steady improvement over the past several years in outcomes and survival for people experiencing acute liver failure, particularly in those who do not receive a liver transplant, which may be due in part to wider use of the N-acetylcysteine treatment.

Another NIDDK-led research effort, the Drug-Induced Liver Injury Network, collects and analyzes data from people with severe liver injury caused not only by over-the-counter and prescription drugs, but also by alternative medicines, such as herbal products and dietary supplements. This research has helped doctors to better understand and diagnose liver

“If it helps other people,” Scott says of his participation in the STOP-ALF clinical trial, “I’m more than happy to participate.”
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injury caused by drugs and other agents. The NIDDK also partners with the NIH’s Library of Medicine on the “LiverTox” website (http://livertox.nih.gov/), which features sample cases of people with drug-induced liver injury based on the Network’s data, as well as a database summarizing liver injuries caused by drugs, including aspirin, acetaminophen, and various herbal and dietary supplements.

Life After Surviving Acute Liver Failure

All signs indicate that Scott has made a full recovery from his liver and kidney injury. In mid-April 2016, he was able to return to work and conquer the “tsunami” of email received during his absence. He has also been able to get back to enjoying his hobbies, including building model cars and seeking real project cars to work on. However, his stamina remains limited, as he notices in his regular activities, such as when he uses his push lawnmower on his half-acre property. Although he is cleared to take a reduced dose of acetaminophen as needed for everyday pain, he avoids all over-the-counter pain relievers—especially aspirin—as well as alcohol, to protect his recovering liver. “If I get a headache, I will grin and bear it,” he states resolutely.

A silver lining of Scott’s experience with acute liver failure and the extended hospital stay is his renewed commitment to better health. “I was a smoker, and so I said … maybe I should use this as an opportunity to quit,” he says. Now, in the time he previously used for smoke breaks, he instead takes a walk around his office building. He also eats more healthfully after his experience on the special renal diet. He continues to have his health monitored by his primary care physician and the doctors at VCU.

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Overall, his experience with participating in the STOP-ALF clinical trial was a positive one that Scott would recommend to others who might find themselves in similar circumstances. “If it helps other people, I’m more than happy to participate and do follow-up,” he says, though he urges anyone considering enrolling in a clinical trial to be fully informed, including asking the medical staff any questions they might have. “It was a scary experience,” he says, summing up his ordeal with acute liver failure. “But I definitely had faith in the doctors,” he says appreciatively. And, he adds, “every time I’ve gone back to do the follow-up, I’ll check in on the ward and see some of the nurses there just to thank them.”