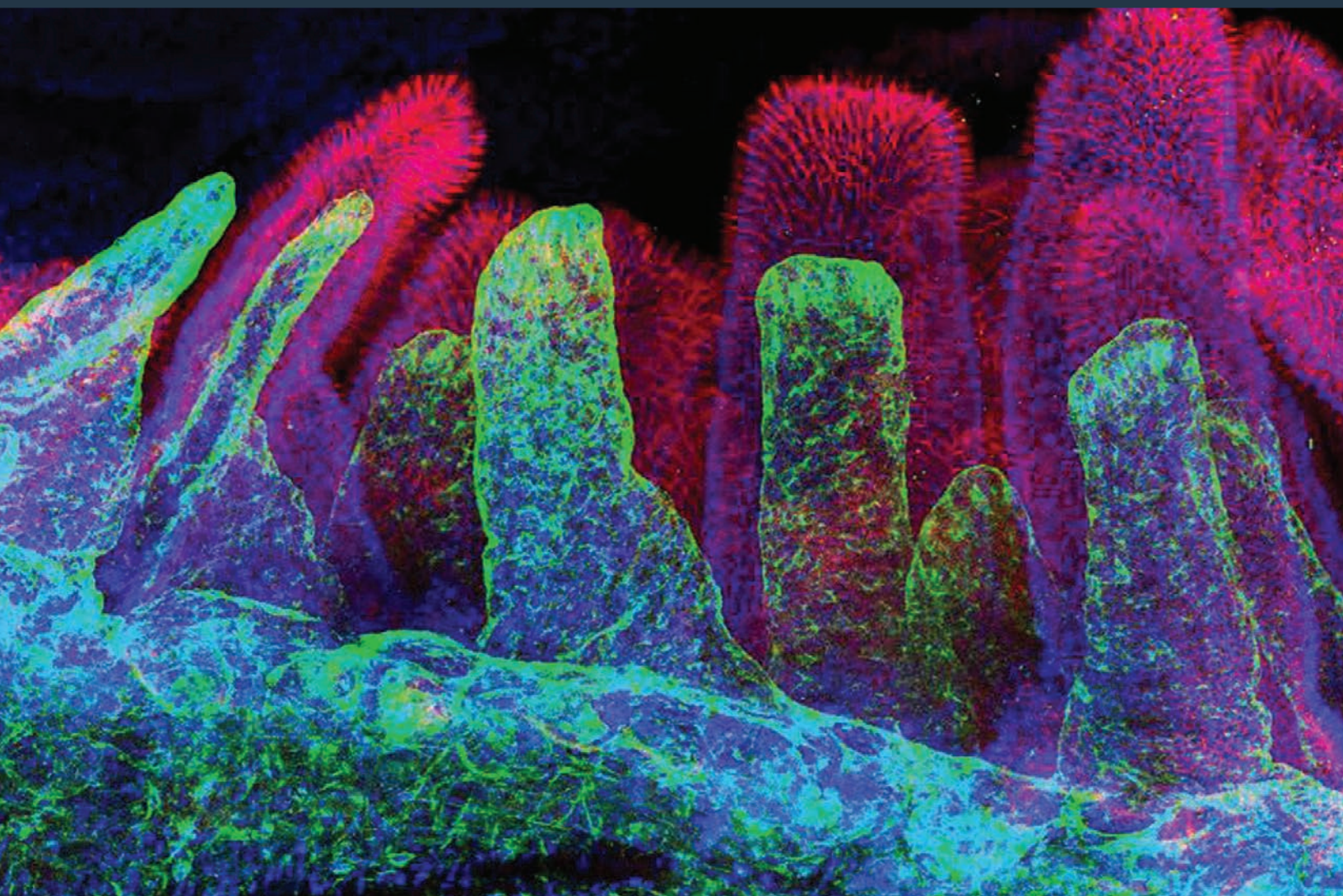


NIDDK

Recent Advances & Emerging Opportunities

January 2019



U.S. Department of Health and Human Services
National Institutes of Health
National Institute of Diabetes & Digestive & Kidney Diseases

Cover caption:

The intestinal lining is constantly renewing itself, on average every week in humans, requiring a constant supply of new cells formed from resident stem cells. NIDDK-supported researchers are steadily uncovering the complex network of cells and signals that support the intestinal stem cells and thus enable the process of intestinal renewal. For example, the cover image shows the convoluted inner surface of the small intestine, here in mice, with microscopic projections called villi surrounded by deep pits called crypts, at the bottom of which reside the intestinal stem cells. As described in this publication, the research associated with this image has shown that relatively rare intestinal cells called telocytes, labeled green, regulate growth signals sent to nearby stem cells in the crypts found in the intestinal lining, labeled red. This finding shows how essential these rare cells are for supporting the intestinal stem cells in their important work of continually regenerating the intestine.

In the past year, the NIDDK has engaged in a series of discussions to identify how best to advance research on regenerative medicine, in the intestine and other organs that relate to the Institute's mission, through a forum as part of its National Diabetes and Digestive and Kidney Diseases Advisory Council meetings. The NIDDK's Council meets three times a year to advise the Institute about its research portfolio, which includes the NIH's second largest portfolio of grants in regenerative medicine. As detailed in the "Scientific Presentations" included throughout this publication, the year-long Council forum on regenerative medicine featured talks by leading scientists in the field of regenerative medicine that touched on all three of the Institute's major programmatic Divisions, focusing on pancreatic β cells, the intestine, and the kidney. These discussions were designed to spark innovative approaches by the Institute to advance future discoveries in regenerative medicine.

Image courtesy of Professor Klaus Kaestner, Ph.D., M.S., Thomas and Evelyn Suor Butterworth Professor in Genetics, Associate Director of the Penn Diabetes Research Center, Associate Director of the Penn Center for Molecular Studies in Digestive and Liver Diseases, Director of the Next Generation Sequencing Center, University of Pennsylvania Perelman School of Medicine. Shoshkes-Carmel M, Wang YJ, Wangenstein KJ,... Kaestner KH. Subepithelial telocytes are an important source of Wnts that supports intestinal crypts. Nature 557: 242-246, doi: 10.1038/s41586-018-0084-4, 2018. Reprinted with permission from Springer Nature, Copyright 2018.

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Message from the Director



Image courtesy of GW School of Medicine and Health Sciences

As the Director of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), I am pleased to present this annual report highlighting the research efforts and programs supported by the Institute. The NIDDK has a broad research responsibility that includes some of the most common, debilitating, and costly conditions affecting Americans. These conditions include diabetes and other endocrine and metabolic diseases; liver disease and other digestive diseases and conditions, such as inflammatory bowel disease and irritable bowel syndrome; nutritional disorders; obesity; kidney diseases, such as polycystic kidney disease and glomerular disease; urologic diseases and conditions, such as interstitial cystitis/bladder pain syndrome, prostatitis, and urinary tract infection; and blood diseases.

The 19th edition of this report highlights recent NIDDK-supported scientific advances, such as:

- Discovery of a new biomarker to help diagnose biliary atresia, a potentially life-threatening liver disease in children

- Assembly of a dynamic picture of the human body's molecular response to fluctuations in body weight, including how weight gain can affect health
- The finding that existing medications approved for treating pediatric type 2 diabetes do not prevent rapid progression of prediabetes or recent onset diabetes in young people
- Research demonstrating that a primarily home-based therapy for irritable bowel syndrome can be as effective as clinic-based therapy, while also being less expensive and more accessible
- New insight into chronic kidney disease that may help identify people with the disease who are at increased risk of death
- New research on calorie consumption and calorie burning in early pregnancy in women with obesity, which could help reduce racial disparities in pregnancy outcomes
- Multiple insights from studies on the community of bacteria inhabiting the gut, which could inform development of new therapies for gastrointestinal infections and other digestive diseases
- Identification of two different molecules that can limit the growth of microbes that cause urinary tract infections
- New insights into red blood cell development that have allowed an in-depth examination of how blood cells mature
- Development of an innovative way to study a crucial family of proteins in a living animal in real time
- The finding that treatment with an immune system-suppressing medicine preserved insulin production for at least a year in people with newly diagnosed type 1 diabetes

In addition to reporting on recent advances, this publication traces the multi-step path to research

achievements through several “Stories of Discovery” and “Scientific Presentations.” These essays illustrate how major new discoveries that have greatly advanced biomedical science and are benefitting human health often emerge from incremental insights gained from research investments spanning many years and even multiple research disciplines.

This report also includes personal stories of those who have given time and effort to participate in NIDDK-sponsored clinical research or whose lives have been transformed by biomedical research. A mother and daughter talk about how the latter’s diagnosis with celiac disease led them to find ways to thrive with the disease and share that knowledge with others. A woman speaks of participating—for herself, her children, and future generations—in a study testing whether medications can stop or reverse progression of prediabetes or recent-onset diabetes. A man tells of his decades-long involvement with a landmark study investigating the benefits of early, intensive blood glucose control on development of complications in people with type 1 diabetes. A man describes taking part in a study seeking to use a new, collaborative model of health care, enhanced by implementing a novel technology platform, to improve outcomes in people with multiple chronic diseases.

The NIDDK is continuing its efforts to ensure that knowledge gained from its research is disseminated to health care providers, patients, and the public. We develop science-based information on diseases and disorders within the NIDDK mission and distribute it through our information and education

The efforts featured in this publication reflect the core mission of the NIDDK, including the Director’s guiding principles:

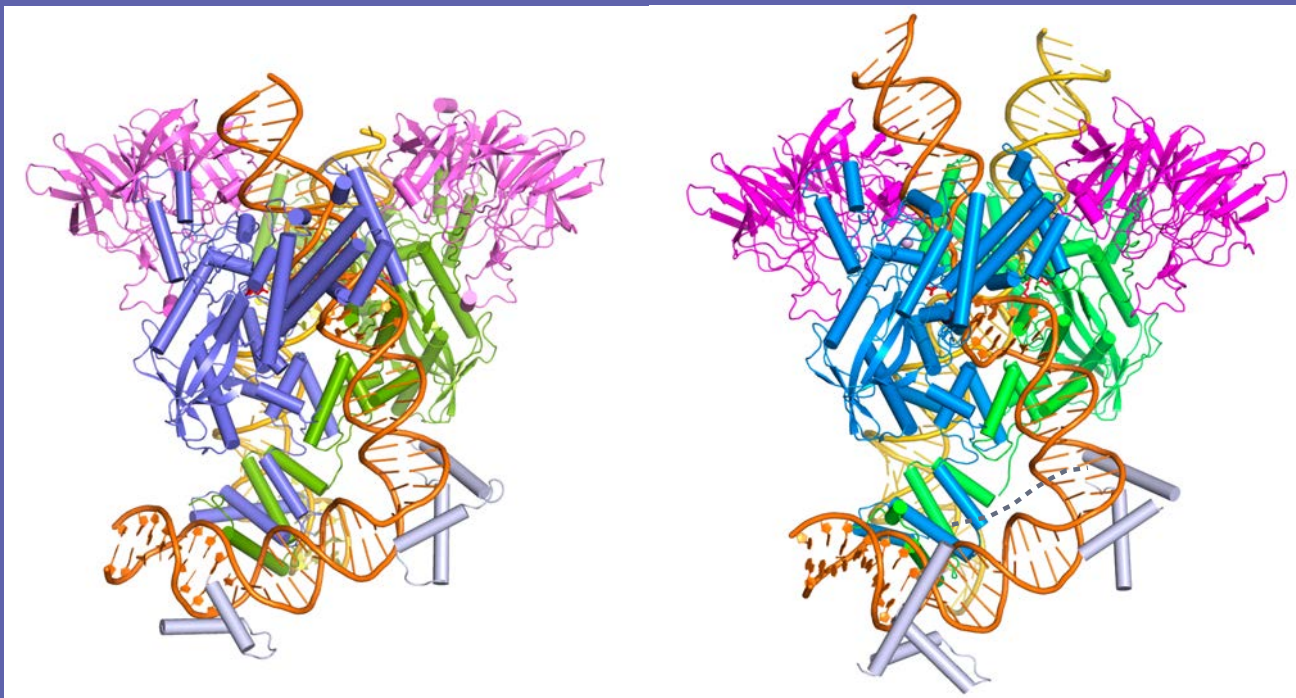
- Maintain a vigorous investigator-initiated research portfolio
- Support pivotal clinical studies and trials
- Preserve a stable pool of talented new investigators
- Foster exceptional research training and mentoring opportunities
- Ensure knowledge dissemination through outreach and communications

More information on how the NIDDK’s activities support these core values can be found in the “NIDDK Funding Trends and Support of Core Values” section at the end of this report and on our website at www.niddk.nih.gov

programs and our website. I invite you to visit us at www.niddk.nih.gov. Health information, news, and scientific advances related to NIDDK research are also available on our Twitter feed: [@NIDDKgov](https://twitter.com/NIDDKgov)

This report reflects only a fraction of the immense body of NIDDK-funded research across the country, performed by basic scientists, clinical investigators, and patient volunteers. Moving forward, we remain committed to supporting these important areas of research and translating scientific discoveries into improvements in the health and quality of life of all people.

Griffin P. Rodgers, M.D., M.A.C.P.
Director
National Institute of Diabetes and Digestive
and Kidney Diseases
National Institutes of Health
U.S. Department of Health and Human Services



Determining the three-dimensional structure of a protein allows researchers to better understand its role in health and disease and identify the regions that are important for function. The proteins RAG1 and RAG2 are critical for generating a diverse population of antibodies that defend us against pathogens but could also be culprits in autoimmune diseases. This antibody diversity is generated by a process called “V(D)J recombination,” in which segments of DNA are cut and rejoined by the RAG1/RAG2 protein complex to produce a variety of new combinations, a process that is essential for maturation of the immune system. Scientists in the NIDDK’s Intramural Research Program have determined the three-dimensional structures of the RAG1/RAG2 protein complex before (left panel) and during (right panel) the process of cleaving its target DNA. Computer modeling revealed that the RAG1 (shown in blue and green) and RAG2 (shown in pink) proteins undergo significant rearrangements in three-dimensional space, cleaving the DNA in a nutcracker-like motion. The DNA molecule itself (shown in orange and yellow) also contorts significantly during the process. A better understanding of the dynamics of the RAG1/RAG2 complex during V(D)J recombination could yield important molecular insights into antibody production, as well as the diseases caused by immune system dysfunction.

Image courtesy of Drs. Wei Yang and Martin Gellert, NIDDK. Kim M-S, Chuenchor W, Chen X,... Yang W. Cracking the DNA code for V(D)J recombination. Mol Cell 70: 358-370, doi: 10.1016/j.molcel.2018.03.008, 2018. Reprinted by permission from Elsevier, copyright 2018.

Cross-Cutting Science

Medical advances are not usually achieved in great, intuitive leaps. More often, new prevention strategies, treatments, and cures result from a long, gradual accumulation of knowledge from years of scientific research. Insights into the fundamental biologic building blocks and processes of an organism—its genes, the proteins they encode, the inner workings of cells, and the ways cells communicate with each other—can have broad and far-reaching implications. Indeed, many significant advances in our knowledge of disease and disease treatment can be traced to laboratory studies whose relevance to health could not have been fully known or appreciated at the time they were conducted.

With the development of innovative scientific technologies and the emergence of new scientific disciplines as talented and creative research teams join together to tackle ever more complex challenges, new opportunities to make exciting discoveries arise each day. Described in this chapter are several recent studies, each of which spans multiple areas within the NIDDK research mission, and a workshop on understanding how the nervous system regulates metabolism. The insights gained through this research can be expected to further scientific progress in many research areas, for today's discoveries may hold the seeds of tomorrow's cures.

IMAGING AN IMPORTANT CLASS OF MEMBRANE PROTEINS

New Mouse Model Illuminates Timing and Location of Activation of a “G protein-coupled Receptor” Important in Inflammation: Scientists have developed a novel mouse model that enables visualization of G protein-coupled receptor (GPCR) activation in a living animal in real time. GPCRs are one of the largest and most diverse families of proteins. They are involved in most physiological functions, detecting a variety of signals outside the cell that activate GPCRs, leading to cellular responses. They are implicated in many diseases, and it is estimated that nearly a third of approved pharmaceutical drugs target GPCRs. Therefore, characterizing the dynamics of their activation is critical to understanding the

important role of this protein family in health and disease. In this study, scientists in the NIDDK's Intramural Research Program created a genetic mouse model that enables imaging of a member of the GPCR family that is involved in inflammation and other responses, called S1P₁, in real time in a living animal.

To create this model, the researchers took advantage of the ability to split a protein, called luciferase, that is derived from fireflies and can produce fluorescent light. They tethered one part of luciferase to S1P₁ and linked its complement to a protein that binds activated S1P₁ (β -arrestin2). Thus, when S1P₁ was activated, β -arrestin2 was recruited to and bound the activated receptor, bringing the two luciferase pieces together and producing a faint glowing signal that could be detected with a special microscope. To test the model, the researchers sought to establish the timing and anatomical location of S1P₁'s activation during inflammation triggered by the bacterial toxin lipopolysaccharide (LPS). They found that injection with LPS activated S1P₁ systemically and with distinctive timing for different parts of the mouse's body. For example, after 24 hours, the signal was strongest in the head and chest of the mouse, including in the brain. At 72 hours, it was strongest in the abdomen. This example demonstrated that this model can reveal the locations and dynamics of S1P₁ activation. This strategy could be used to create a library of other GPCR models to gain new knowledge of the biological role of GPCRs in normal and

disease contexts toward new drug development for these important targets.

Kono M, Conlon EG, Lux SY, Yanagida K, Hla T, and Proia RL. Bioluminescence imaging of G protein-coupled receptor activation in living mice. *Nat Commun* 8: 1163, doi: 10.1038/s41467-017-01340-7, 2017.

IMMUNE SYSTEM REGULATION

Clues to the Maturation of the Immune System from Three-dimensional Structures of Proteins RAG1 and RAG2: Scientists in the NIDDK's Intramural Research Program have determined the three-dimensional structures of proteins, known as RAG1 and RAG2, in complex with the DNA they target and cut in a process critical to immune system function. Because animals encounter a wide variety of potential infectious agents, the immune system must be able to generate a large and diverse population of antibodies to recognize these various invaders. This diversity is generated by a process called "V(D)J recombination," in which segments of DNA are cut and rejoined to produce a variety of new combinations, a process that is essential for maturation of the immune system. The proteins RAG1 and RAG2 function as the "cleavers" of DNA during V(D)J recombination and have been shown to be critical to the function of the immune system, as demonstrated by the over 60 mutations in the genes encoding RAG1 and RAG2 that lead to severe combined immunodeficiency (SCID) in humans or a milder type of immunodeficiency called Omenn syndrome. Understanding their three-dimensional structures, therefore, could provide insight not only into the critical process of V(D)J recombination, but into human disease as well.

The NIDDK scientists used two different methods to "visualize" at high resolution the RAG1 and RAG2 proteins in complex with DNA: cryo-electron microscopy and X-ray crystallography. By modifying DNA pieces that bound to the protein complexes, the scientists were able to "freeze" the complex just before the process begins and at a critical point in time during the process. They found that the RAG1 and RAG2 proteins undergo significant movements in three-dimensional space during the process, cleaving the DNA in a nutcracker-like motion. In addition, the recombination signal sequences (RSS) in the DNA molecule itself also contort dramatically, which

could help explain why the RAG1/RAG2-targeted RSS tend to be rich in sequences that are physically able to bend and deform. The findings from this study provide important insights into how RAG1 and RAG2 participate in the maturation of the immune system and may lead to a better understanding of the molecular basis of SCID and Omenn syndrome.

Kim M-S, Chuenchor W, Chen X,... Yang W. Cracking the DNA code for V(D)J recombination. *Mol Cell* 70: 358-370, doi: 10.1016/j.molcel.2018.03.008, 2018.

Friend, Not Foe: Good Bacteria That Promote Skin Immunity and Tissue Repair: Researchers have discovered that non-disease-causing bacteria that live on mammalian skin are sensed by the immune system to promote protection from environmental pathogens and induce tissue repair. As the body's most exposed surface, the skin is in constant communication with a multitude of microbes, including bacteria, viruses, and fungi, and it is the first line of immunological defense. The researchers previously showed in mice that skin association with certain microbes leads to the accumulation of immune cells, specifically CD8⁺ T cells—but not inflammation. Further, wild-caught mice that are exposed to myriad microorganisms contain a significantly larger number of CD8⁺ T cells in the skin compared to pathogen-free, laboratory-raised mice.

In this study, the researchers isolated non-harmful strains of the bacteria *Staphylococcus epidermidis* (*S. epidermidis*) from the skin of healthy human volunteers, and found that some of these strains, when put on mice, had the ability to promote the accumulation of CD8⁺ T cells in the skin. This result suggests that CD8⁺ T cell buildup is a health-promoting response that occurs in the absence of inflammation or other disease state. Similar results were seen in the skin of non-human primates. Next, the researchers explored potential mechanisms whereby certain non-harmful microbes are capable of initiating this beneficial immune response in skin. They isolated CD8⁺ T cells from the skin of *S. epidermidis*-associated mice and determined that a molecule called MHC1b H2-M3 was required for alerting the immune system to the presence of bacteria, leading to an accumulation of CD8⁺ T cells at the skin surface. Having determined a mechanism for this immune response in a healthy state, they next compared gene profiles of *S. epidermidis*-induced CD8⁺ T cells to cells isolated from the skin in the context of infection. Strikingly, they found that,

compared to cells induced by infection, *S. epidermidis*-elicited CD8⁺ T cells had higher levels of genes associated with tissue repair and a range of molecules involved in wound healing. In a mouse model of skin-wounding, the researchers showed a remarkable accumulation of CD8⁺ T cells at the wound edge post-injury. After measuring the progression of healing, they found that cells induced by the beneficial *S. epidermidis* promoted accelerated tissue repair compared to cells not associated with these bacteria.

Taken together, these results identify an important role for non-harmful bacteria in driving a CD8⁺ T cell immune response to enhance tissue repair. These findings could have important clinical implications for wound healing following trauma, illness, or disease.

Linehan JL, Harrison OJ, Han SJ,...Belkaid Y. Non-classical immunity controls microbiota impact on skin immunity and tissue repair. Cell 172: 784-796, doi: 10.1016/j.cell.2017.12.033, 2018.

Novel Technique To Edit Immune Cell DNA Could Make New Treatments Possible: Scientists have discovered a new method for editing the DNA of T cells (a type of immune cell involved in pathogenesis of type 1 diabetes and other autoimmune disorders) that has significant advantages over existing techniques. The researchers demonstrated how this new tool could offer ways to treat certain autoimmune or genetic diseases or to target T cells to attack tumors, among other possible applications. This new genetic editing technique is based upon the existing CRISPR-Cas9 genetic targeting system. That system uses a protein “scissor” called Cas9, customized DNA templates that include instructions for the desired edits, and the cells’ own DNA repair machinery to latch onto, cut, and “edit” a cell’s DNA. Previously, genetic editing in T cells required specially designed viruses to deliver the editing system to the cells. Viral delivery systems, however, are costly and time-consuming to produce, and they can make genetic changes in unwanted places in the target DNA. Viral delivery systems were also the only option for applications requiring delivery of large DNA templates, as those large fragments were toxic to cells when delivered using previous non-viral delivery methods. To find an alternative that would avoid these limitations, researchers tested different ways to deliver the genetic

editing system to T cells via electroporation, a process that uses an electrical field to make cell membranes more permeable temporarily. They found that if a certain electrical field was used with specific ratios of T cells, DNA, and proteins, the genetic editing system efficiently entered the cells. This new technique resulted in precise genetic edits at the intended DNA target sites without the need for a virus. What DNA is targeted could also be easily changed by altering the DNA templates used, making this system flexible and suitable for a variety of possible uses, including those requiring large DNA templates.

To demonstrate the promise of this genetic editing system, researchers performed two demonstrations where editing genes in T cells could treat disease. In the first demonstration, they used their non-viral genetic editing system to correct a specific genetic defect (mutation) in the DNA of T cells that causes a rare, inherited autoimmune disease that is resistant to treatment. The scientists collected T cells donated by people with the disease and used their electroporation-based gene editing technique to correct the disease-causing mutation, thus restoring the T cell functions that were disrupted by the mutation. In a second demonstration, researchers used large DNA templates to reprogram T cells, which are usually tasked with attacking infections, to instead target a specific type of tumor cell. The reprogrammed T cells homed in on the target tumor cells both in a laboratory dish and in male mice carrying the tumors, attacked the tumor cells, and reduced tumor size.

Overall, these experiments showed that this novel non-viral genetic editing system allows rapid and economical production of genetically tailored T cells that could potentially be used for a variety of applications, including cancer immunotherapy, genetic disease therapies, and potentially for combating other diseases in which T cells play a role, like type 1 diabetes. Future research is required to determine whether this technology is safe for clinical applications, but this system provides a new tool that greatly expands the possibilities for using genetic editing to treat human disease.

Roth TL, Puig-Saus C, Yu R,...Marson A. Reprogramming human T cell function and specificity with non-viral genome targeting. Nature 559: 405-409, doi: 10.1038/s41586-018-0326-5, 2018.

Workshop Explores Role of the Autonomic Nervous System in Metabolic Health and Disease



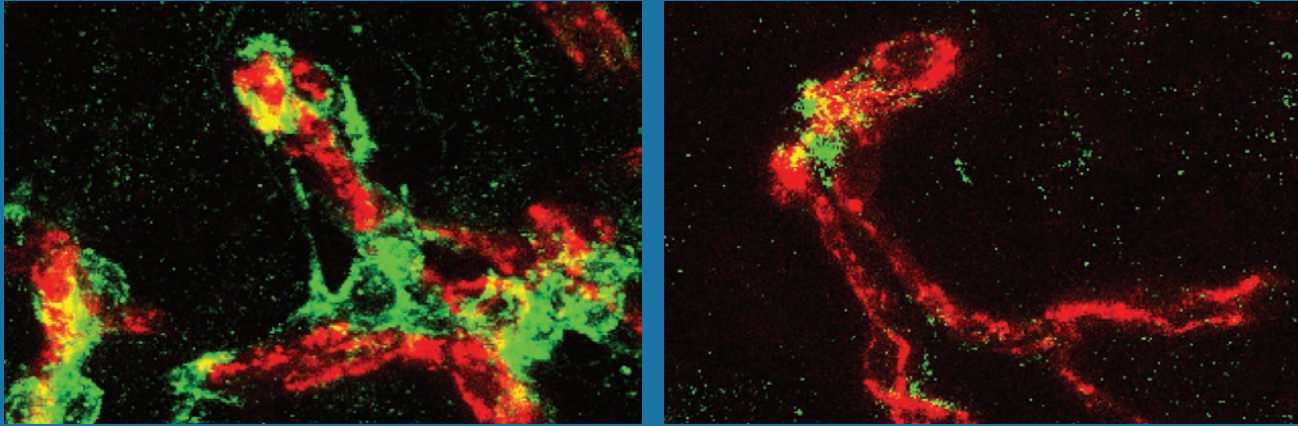
On September 20–21, 2018, the NIDDK sponsored a workshop in Bethesda, Maryland, focusing on the role of the autonomic nervous system (ANS)—nerves that transmit signals among the brain, spinal cord, and internal organs—in regulating healthy metabolism and how changes in nerve function may influence metabolic diseases such as obesity, diabetes, and fatty liver disease.

The ANS plays a key role in regulating physiological responses. Nerves running from peripheral organs to the central nervous system (spinal cord and brain) relay sensory and metabolic information, while nerves running in the opposite direction regulate metabolic processes, including the body's metabolism of glucose (sugar) and fat, as well as hormonal secretion in organs such as the liver, adipose (fat) tissue, intestine, and pancreas. New techniques in the fields of neuroscience and molecular genetics are being used to elucidate the structures, connections, and function of the ANS. The NIH Common Fund

program called Stimulating Peripheral Activity to Relieve Conditions, or “SPARC,” is supporting research to transform understanding of nerve-organ interactions controlling organ functions throughout the body and advance research geared toward more precisely treating diseases and conditions through neuromodulation. However, much remains unknown about ANS contributions to regulating glucose and fat metabolism, as well as metabolic disease processes.

This workshop brought together speakers and other participants with the goals of: 1) increasing foundational knowledge of the role of the ANS in regulating metabolism and metabolic disease; 2) fostering interactions between basic and clinical scientists with expertise in metabolism and neuroscience; 3) addressing limitations of current technologies and methods for measuring tissue-specific ANS activity and function in humans; 4) building on the NIH SPARC program to expand its focus to metabolic disease; and 5) identifying research gaps in basic and clinical science. Speakers presented on a range of topics related to the role of the ANS in regulating processes in the gastrointestinal tract, glucose metabolism and balance, liver metabolism and disease, metabolic diseases such as obesity and diabetes, and adipose tissue and fat metabolism.

The meeting organizers plan to develop a summary for publication in the scientific literature describing the workshop proceedings and highlighting current gaps in knowledge, which will inform future efforts to advance research on the ANS and its impacts on metabolic health and disease.



Pancreatic islets contain many different cell types, including β (beta) cells that release the hormone insulin into the bloodstream when glucose (sugar) levels are high. Effective insulin secretion from the pancreas depends on well-functioning islet blood vessels. Islets contain an extensive network of small blood vessels called capillaries, which are covered by cells called pericytes. Research described in this chapter shows that pericytes play an important role in regulating blood flow through pancreatic islets by controlling capillary diameter, and that β cells play an active role in controlling their own blood supply by regulating the pericytes. Additionally, as illustrated in the images above, the research shows that islets from a person with long-standing type 2 diabetes (right panel) have fewer pericytes (green) covering capillaries (red) compared to a person without diabetes (left panel). The loss of pericytes in type 2 diabetes suggests that β cells lose the ability to control their own blood supply, which could contribute to defects in insulin release associated with the disease.

Image courtesy of Joana Almac¸a, Ph.D. and Alejandro Caicedo, Ph.D., University of Miami Miller School of Medicine. Almac¸a J, Weitz J, Rodriguez-Diaz R, Pereira E, and Caicedo A. The pericyte of the pancreatic islet regulates capillary diameter and local blood flow. Cell Metabolism 27: 630-644, doi: 10.1016/j.cmet.2018.02.016, 2018. Reprinted with permission from Elsevier, copyright 2018.

Diabetes, Endocrinology, and Metabolic Diseases

NIDDK support of basic and clinical research in the areas of diabetes, endocrinology, and metabolic diseases spans a vast and diverse range of diseases and conditions, including diabetes, osteoporosis, cystic fibrosis, and obesity. Together, these diseases and conditions affect many millions of Americans and can profoundly decrease quality of life. Many of these diseases are complex—an interplay between genetic and environmental factors contributes to disease development.

Not only is diabetes chronic and relentless, but its slow accumulation of insults to the body can rob a person of the ability to see, hear, feel, think, and walk. In addition to increasing the risk for complications of vision loss, kidney failure, and amputation, diabetes doubles risk for heart disease, many forms of cancer, some forms of dementia, hearing loss, erectile dysfunction, urinary incontinence, and many other common diseases.¹ NIDDK is vigorously pursuing research to combat diabetes and its associated health consequences.

Diabetes is a debilitating disease that affects an estimated 30.3 million people in the United States—or 9.4 percent of the total population—and is the seventh leading cause of death.² Although rates of diabetes-related complications have declined substantially in the past two decades, disease burden remains significant as the number of people with diabetes is still very high.³ Diabetes can affect many parts of the body and is associated with serious complications, such as heart disease and stroke, blindness, kidney failure, and lower-limb amputation. In addition to these human costs, the estimated total financial cost for diagnosed diabetes in the United States in 2017—including costs of medical care, disability, and premature death—was \$327 billion.⁴ Effective therapy can prevent or delay diabetic complications, but nearly one-quarter of Americans with diabetes are undiagnosed and therefore not receiving therapy.²

Diabetes is characterized by the body's inability to produce and/or respond appropriately to insulin, a hormone that is necessary for the body to absorb and use glucose (sugar) as a cellular fuel. These defects

result in persistent elevation of blood glucose levels and other metabolic abnormalities, which in turn lead to the development of disease complications. The most common forms of diabetes are type 1 diabetes, in which the body loses its ability to produce insulin, and type 2 diabetes, in which the body becomes resistant to insulin signaling, with subsequent impaired insulin production. In addition, a significant proportion of pregnant women each year are diagnosed with gestational diabetes, a form of diabetes that is similar to type 2 diabetes but unique to pregnancy. Untreated, any form of diabetes during pregnancy increases the risk of serious complications for the

¹ *Diabetes in America*, 3rd ed. Cowie CC, et al., Eds. Bethesda, MD, National Institutes of Health, NIH Pub No. 17-1468, 2018.

² Centers for Disease Control and Prevention. *National Diabetes Statistics Report*, 2017. Atlanta, GA: Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, 2017.

³ Gregg EW, et al. *N Engl J Med* 370: 1514-1523, 2014.

⁴ American Diabetes Association. *Diabetes Care* 41: 917-928, 2018.

mother and baby before, during, and after delivery. Type 1 diabetes, formerly known as juvenile diabetes, affects approximately 5 percent of diagnosed diabetes cases in adults, and the majority of diagnosed cases in children and youth.² It most often develops during childhood but may appear at any age.

Type 1 diabetes is an autoimmune disease in which the immune system launches a misguided attack and destroys the insulin-producing β (beta) cells of the pancreas. If left untreated, type 1 diabetes results in death from starvation: without insulin, glucose is not transported from the bloodstream into the body's cells, where it is needed. Thus, people with type 1 diabetes require lifelong insulin administration—in the form of multiple daily injections or via an insulin pump—to regulate their blood glucose levels. The NIDDK's landmark Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) study demonstrated that keeping blood glucose levels as near to normal as safely possible reduced the risk of eye, kidney, nerve, and heart complications associated with type 1 diabetes. However, despite vigilance in disease management, with current technologies to test blood glucose levels and administer insulin, it is still not possible for people with type 1 diabetes to control blood glucose levels as well as functional pancreatic β cells do. Thus, researchers are actively seeking new methods to improve blood glucose monitoring and insulin delivery. In this regard, NIDDK-supported research has contributed to the development or testing of new diabetes management technologies recently approved by the U.S. Food and Drug Administration, including the first commercial “hybrid artificial pancreas” device that automatically links glucose monitoring and insulin delivery, and next-generation continuous glucose monitors, including the first fully implantable device. Researchers are also working to develop β cell replacement therapies, such as islet transplantation, to cure type 1 diabetes.

Type 2 diabetes is the most common form of the disease, accounting for about 90 to 95 percent of diagnosed diabetes cases in U.S. adults.² The risk for developing type 2 diabetes is associated with older age, obesity, family history of diabetes, history of gestational diabetes, impaired glucose metabolism, physical inactivity, and race/ethnicity.⁴ Type 2 diabetes occurs at higher rates among racial and

ethnic minority populations in the United States, including African Americans, Hispanic and Latino Americans, American Indians, some Asian Americans, and Native Hawaiians and Pacific Islanders.² Gestational diabetes is also a risk factor: about half of women with gestational diabetes will develop type 2 diabetes within 5 to 10 years after giving birth.⁵

In people with type 2 diabetes, cells in muscle, fat, and liver tissue do not properly respond to insulin. As a result, the pancreas initially produces more insulin to compensate. Gradually, however, the pancreatic β cells lose their ability to secrete enough insulin to restore balance, and the timing of insulin secretion becomes abnormal, causing blood glucose levels to rise. Treatment approaches for controlling glucose levels include diet, exercise, and oral and injected medications, with insulin often required as the disease progresses. There are also an estimated 84 million U.S. adults who have a condition called “prediabetes,” in which blood glucose levels are higher than normal but not as high as in diabetes.² This population is at elevated risk of developing type 2 diabetes. Fortunately, the NIDDK-supported Diabetes Prevention Program (DPP) clinical trial has shown that people with prediabetes can dramatically reduce their risk of developing type 2 diabetes with diet and exercise changes designed to achieve a 7 percent reduction in body weight. To a more limited degree, the safe and well-tolerated drug metformin can also help prevent or delay type 2 diabetes. Moreover, follow-up research has shown that the benefits of reduced diabetes risk from weight loss or metformin can persist for at least 15 years.

Type 2 diabetes was previously called “adult-onset” diabetes because it is predominantly diagnosed in older individuals. However, this form of diabetes is increasingly being diagnosed in children and adolescents, and it disproportionately affects youth from racial and ethnic minority populations in the United States. Believed to be related to increasing rates of pediatric obesity, this is an alarming trend for many reasons. For example, results from the NIDDK-supported Treatment Options for type 2 Diabetes in Adolescents and Youth (TODAY) clinical trial and the Restoring Insulin Secretion (RISE) Pediatric Medication Study showed that the disease

⁵ Kim C, et al. *Diabetes Care* 25: 1862-1868, 2002.

may be more aggressive and difficult to treat in youth compared to adults. This is worrisome because the onset and severity of disease complications correlate with both the duration of diabetes and control of blood glucose levels; thus, those with early disease onset are at especially high risk for developing complications. In addition, increasing rates of type 2 diabetes in girls may lead to more women who enter pregnancy with diabetes, and maternal diabetes during pregnancy—either onset of type 2 diabetes before pregnancy or the development of gestational diabetes during pregnancy—confers an increased risk of type 2 diabetes in offspring. Thus, the rising rates of diabetes and prediabetes in young women could contribute to a cycle of ever-growing rates of diabetes. Therefore, the advent of type 2 diabetes in youth has the potential to worsen the enormous health burden that diabetes already places on the United States.

The NIDDK is supporting research to better understand metabolism and the mechanisms that lead to the development and progression of diabetes and the many other endocrine and metabolic diseases within the NIDDK's mission; such research will ultimately spur the design of potential new intervention strategies. In parallel, based on knowledge from past scientific research investments, the NIDDK is vigorously pursuing studies of prevention and treatment approaches for these diseases.

PANCREATIC CELL BIOLOGY

Understanding How Cells in the Pancreas Change with Age: Researchers have used sophisticated technologies to examine individual cells from healthy human pancreata to understand how the cells change with age. As people and other organisms age, cells become damaged—e.g., they accumulate changes in their DNA sequence that could potentially be harmful. This damage eventually leads to reduced tissue and organ function, or could lead to diseases such as cancer. It is known that aging processes affect cells randomly, so it is possible that individual cells in an organ or tissue could be affected differently with age and that different organs or tissues may respond in varying ways to aging processes overall. However, changes at the level of a single cell have been difficult to tease out

because of experimental limitations. Now, scientists are using a technique called single-cell RNA sequencing (sc-RNAseq) to examine gene activity in individual cells. In new research, scientists used this technique to study the effects of aging on cells in the pancreas, an organ that is associated with age-related diseases, such as type 2 diabetes.

The scientists used sc-RNAseq to measure gene activity in 2,544 human pancreas cells from 8 female and male non-diabetic organ donors ranging in age from 1 month to 54 years old. They found increased “transcriptional noise” in endocrine cells—i.e., pancreatic cells that secrete hormones—from older donors compared to cells from young adults and children. Transcriptional noise refers to differences in gene activity among cells from the same individual. This suggests that aging is a process of random, rather than programmed, changes in gene activity, but the functional significance of the increased transcriptional noise remains unknown. Researchers also observed that the glucagon-producing pancreatic α (alpha) cells and insulin-producing pancreatic β (beta) cells from older people were more likely to produce both hormones simultaneously compared to cells from younger people. These so-called bi-hormonal cells have been observed previously, and have been linked to β -cell failure and type 2 diabetes. It is possible that the age-related increase in transcriptional noise could be driving this effect—i.e., the increased random variation in gene activity could give rise to bi-hormonal cells; this intriguing possibility remains to be tested. Finally, the scientists found a range in the number of genetic mutations in individual pancreatic cells, and, overall, discovered that the number of mutations increased with age. Further experiments suggested that oxidative stress, which can cause DNA damage, contributed to the age-related increase in the observed mutations. Oxidative stress has also been implicated in the development of type 2 diabetes.

The findings in this study are consistent with the notion that aging processes affect cells randomly, and provide an important look at how aging affects cells in the pancreas. They also show some surprising ways in which individual cells in a healthy pancreas change with age, stimulating questions about the factors driving these changes and the functional significance of them. Similar single-cell studies of pancreata from people with type 1 or type 2 diabetes could provide

key information about the development of these diseases.

Enge M, Arda HE, Mignardi M,...Quake SR. Single-cell analysis of human pancreas reveals transcriptional signatures of aging and somatic mutation patterns. *Cell* 171: 321-330, doi: 10.1016/j.cell.2017.09.004, 2017.

New Insights into the Regulation of Islet Blood Flow in Health and Diabetes: Researchers have discovered that cells called pericytes play an important role in regulating blood flow through pancreatic islets and may contribute to islet dysfunction in people with type 2 diabetes. Pancreatic islets contain many different cell types, including β (beta) cells that release the hormone insulin into the bloodstream when glucose (sugar) levels are high. It is known that effective insulin secretion from the pancreas depends on well-functioning islet blood vessels. As such, islets contain an extensive network of small blood vessels called capillaries, which are covered by pericytes—cells that have long, finger-like projections. Little is known about islet pericytes, but studies of pericytes in other parts of the body suggest that they are contractile cells that regulate the width (diameter) of capillaries. Much like hands squeezing a garden hose to slow the flow of water, when pericytes contract, the capillary tube narrows, and blood flow is restricted. Conversely, when pericytes relax, capillary diameter and blood flow increase. In new research, scientists set out to determine if pericytes play a similar role in controlling capillary diameter and blood flow in islets.

First, the researchers visually examined pericytes in healthy mouse and human (female and male) islets. They found that pericytes made up only about 3 percent of the islet cell population, but, because of their finger-like projections, covered about 40 percent of capillaries in both mouse and human islets. Additional experiments of mouse islets showed that about half of the pericytes had contractile properties, and that activating the pericytes to make them contract decreased capillary diameter and islet blood flow. These findings suggest that pericytes play an important role in controlling blood flow in the islet. Next, the researchers studied the effect of glucose on the islet pericytes. They found that when glucose levels were high—and β cells needed to secrete insulin into the bloodstream—mouse pericytes were inhibited and

relaxed, resulting in increased capillary diameter and islet blood flow. Other experiments suggested that under the high-glucose conditions, the β cells themselves secreted a molecule along with insulin that was involved in inhibiting the pericytes. These observations suggest that β cells play an active role in controlling their own blood supply. Interestingly, the researchers observed a large decrease in the number of pericytes in islets from people with type 2 diabetes. In fact, the longer a person had type 2 diabetes, the fewer islet pericytes were present. The loss of pericytes in type 2 diabetes suggests that β cells lose the ability to control their own blood supply, which the scientists suggest could potentially contribute to defects in insulin release associated with the disease, although more research is needed to delve further into the finding and examine that intriguing possibility. Additionally, future research to understand when and why pericytes are lost in type 2 diabetes could illuminate new therapeutic targets for maintaining a healthy islet blood supply and promoting efficient insulin secretion.

Almaça J, Weitz J, Rodriguez-Diaz R, Pereira E, and Caicedo A. The pericyte of the pancreatic islet regulates capillary diameter and local blood flow. *Cell Metab* 27: 630-644, doi: 10.1016/j.cmet.2018.02.016, 2018.

NOT SO SIMPLE SUGAR METABOLISM

A Tale of Two Sugars—Fructose and Glucose Cause Differing Metabolic Effects: Researchers have found that the sugars fructose and glucose cause different metabolic effects and health outcomes in mice, but only in animals eating a high-fat diet. Overconsumption of high-fat foods and sugar-sweetened beverages is a risk factor for developing obesity, type 2 diabetes, and nonalcoholic fatty liver disease. Most food and beverages are sweetened with table sugar and/or high-fructose corn syrup, both of which contain fructose and glucose. Though both sugars promote fat build-up in the liver, the liver metabolizes fructose and glucose differently. It is unclear whether those differences lead to different health outcomes, and there is scientific debate about whether one sugar or the other is less harmful to people's health. In a new study, researchers sought to tease out whether there are metabolic differences when mice consume similar caloric amounts of fructose and glucose.

To do this, researchers added fructose or glucose to the drinking water of male mice for 10 weeks, keeping their total caloric intake similar. There were no major health differences when the animals ate a standard, low-fat diet—both groups gained similar amounts of weight and had mild accumulation of fat in their livers compared to control mice drinking only water. However, the story was different in mice eating a high-fat diet. In those mice, fructose consumption caused them to have more obesity and other indicators of metabolic dysfunction (e.g., reduced tolerance to glucose, impaired insulin signaling) compared to mice drinking the same caloric levels of glucose. Surprisingly, glucose appeared to protect animals eating a high-fat diet: their glucose tolerance and sensitivity to insulin were similar to control mice eating a standard chow diet, and they did not gain more weight than animals eating a high-fat diet alone even though they were consuming extra calories from the glucose. In mice eating a high-fat diet, both fructose and glucose led to the accumulation of high levels of liver fat, but experiments suggested that the underlying mechanisms leading to fat accumulation differed between the two sugars. Because fructose was associated with poor metabolic outcomes as described above, the researchers next studied a protein called ketohexokinase (KHK) that is involved in the first step of fructose metabolism. They found that *Khk* gene activity in the liver was increased in mice consuming fructose compared to animals consuming glucose or water. Experimentally decreasing the activity of the *Khk* gene in the liver resulted in improved health outcomes in fructose-consuming mice eating a high-fat diet—e.g., they had less weight gain, improved glucose tolerance, and less fatty liver compared to animals with normal *Khk* gene activity eating the same diet. Extending their observations to people, the researchers found that *KHK* gene activity and protein levels were higher in liver biopsy samples from obese adolescents with more advanced fatty liver disease compared to adolescents with no or less severe fatty liver disease. Taken together, these findings suggest that KHK may be a target for treating fatty liver disease in people.

This research has found that, in mice eating a high-fat diet, fructose leads to poor metabolic outcomes, whereas glucose appears to be protective. Further research could determine whether the observed differences between the two sugars hold true in women and men, and if adjusting sweetener use could have beneficial health effects.

Softic S, Gupta MK, Wang GX,...Kahn CR. Divergent effects of glucose and fructose on hepatic lipogenesis and insulin signaling. *J Clin Invest* 128: 1199, doi: 10.1172/JCI 99009, 2018.

A Surprising Result Improves Understanding of Processes Controlling Blood Glucose Levels: An unexpected result has shed new light on pathways affecting blood glucose (sugar) levels, while suggesting a potential new therapeutic approach for people with type 2 diabetes. The body needs a certain level of glucose in the blood in order to sustain life, so during periods of fasting, the pancreatic hormone glucagon signals the liver, stimulating it to produce glucose for release into the blood. In people with type 2 diabetes, this pathway is often active even when not needed, with excess glucose production from the liver contributing to high blood glucose levels. Glucagon acts by binding to a receptor on liver cells, activating a protein in the cell called a G protein, which in turn sends molecular signals that lead to glucose production and secretion. This arrangement—a hormone outside the cell binding to a receptor that extends inside, where it is coupled to a G protein that in turn triggers the response to the hormone—is quite common. The G proteins come in several different types that have differing effects within the cell—sometimes completely opposite effects.

The glucagon receptor is typically associated with a G protein designated G_s , which when stimulated increases glucose production from the liver. Research on receptors in other parts of the body has shown that G_s -coupled receptors can be counteracted by the effects of other receptors bound to a different G protein designated G_i . Therefore, researchers thought it might be possible to counteract the glucagon pathway in people with type 2 diabetes by stimulating G_i signaling in the liver. To test the idea, they created mice that contain a special, G_i -linked receptor they could stimulate using a chemical that has no effect on ordinary mice. When they used the chemical to simulate G_i in male mice, however, they did not see the expected result: instead of going down, liver glucose production increased—significantly. In contrast, an experiment that effectively eliminated G_i in the livers of male mice greatly improved blood glucose control, even protecting the mice from the effects of a diet that otherwise would have induced them to develop type 2 diabetes. Experiments with human liver cells suggested that G_i signaling increases liver

glucose production in people as well. Thus, this new, if surprising, discovery in mice is encouraging, as a therapeutic that acts to reduce G_i signaling in the liver could turn out to be an attractive approach to help control blood glucose in people with type 2 diabetes. However, because G_i has important functions in many tissues other than the liver, time will tell whether a safe and effective liver-targeted approach can be found that avoids undesirable side effects.

Rossi M, Zhu L, McMillin SM,...Wess J. Hepatic G_i signaling regulates whole-body glucose homeostasis. *J Clin Invest* 128: 746-759, doi: 10.1172/JCI94505, 2018.

CLINICAL RESEARCH ON TYPE 1 DIABETES

Preserving Insulin Production in People with Newly Diagnosed Type 1 Diabetes: Researchers have discovered that treatment with a medicine that suppresses the immune system, called anti-thymocyte globulin (ATG), preserved insulin production and improved blood glucose (sugar) control for at least a year in people with newly diagnosed type 1 diabetes, as compared to placebo (no medicine). Type 1 diabetes is an autoimmune disease in which a person's immune system destroys β (beta) cells in the pancreas that make insulin. Previous research has shown that people whose bodies continue to produce some insulin had better blood glucose control, less hypoglycemia, and reduced rates of disease complications. Therefore, current research is examining ways to preserve more insulin production in people with type 1 diabetes. For example, a pilot study previously showed that treatment with ATG in combination with a modified protein called GCSF preserved insulin production for 1 year in people with established type 1 diabetes (duration of disease of 4 months to 2 years). ATG is a medicine used to prevent or treat immune system rejection of a transplanted organ; GCSF is used to increase white blood cell counts in people undergoing chemotherapy. Researchers in NIDDK's Type 1 Diabetes TrialNet built on the results of the pilot study to determine whether treatment with ATG alone or in combination with GCSF could preserve insulin production when used close to the initial diagnosis of type 1 diabetes.

To examine this question, researchers enrolled 89 female and male children and adults, ages 12 to 42 years, with newly diagnosed type 1 diabetes (less than 100 days since diagnosis) in a three-arm clinical trial. One group received a single course of ATG administered via intravenous infusions; another group received the single course of ATG followed by treatment with GCSF administered through an injection every 2 weeks for a total of 6 doses; and the control group received placebo. The study was blinded, meaning that all participants received the infusions and injections, but did not know whether they were getting medicine or placebo. After 1 year of follow-up, the researchers found that the group receiving ATG alone produced more C-peptide, a measure of insulin production, compared to the placebo group. However, C-peptide levels in the ATG/GCSF group were similar to the placebo group. People in both the ATG and ATG/GCSF groups had better average blood glucose control, as measured by hemoglobin A1c levels, than those in the placebo group. Trial participants continue to be followed to determine if the treatment effects persist for 2 years.

The findings show that a single course of ATG could preserve insulin production in people with newly diagnosed type 1 diabetes for at least 1 year, as compared to placebo, but that GCSF did not enhance benefit. These results differ from the pilot study that found benefit from ATG/GCSF combination therapy, although the pilot study did not examine ATG alone and those participants had more established type 1 diabetes. Like most drugs that target the immune system, ATG treatment has side effects of administration, and its therapeutic effects wane over time after treatment. TrialNet is continuing to follow the study participants, and the data from the 2 year follow-up will help researchers determine whether treatment with ATG alone or in combination with other agents should be pursued for preventing or delaying type 1 diabetes in individuals prior to clinical diagnosis.

Haller MJ, Schatz DA, Skyler JS,...Greenbaum CJ; Type 1 Diabetes TrialNet ATG-GCSF Study Group. Low-dose anti-thymocyte globulin (ATG) preserves β -cell function and improves HbA1c in new-onset type 1 diabetes. *Diabetes Care* 41: 1917-1925, doi: 10.2337/dc18-0494, 2018.

DIABETES TREATMENT AND VITAMIN D STATUS

Diabetes Drug Alters Vitamin D Levels, Possibly Explaining Increased Bone Fracture Risk: Scientists found that the diabetes drug canagliflozin reduces vitamin D levels and calcium uptake, which may explain why this drug can increase the risk of bone fractures. Canagliflozin is one of a class of drugs called sodium glucose cotransporter-2 (SGLT2) inhibitors, which lower blood glucose (sugar) levels by preventing glucose reabsorption from urine in the kidney. Several SGLT2 inhibitors are U.S. Food and Drug Administration-approved to treat type 2 diabetes, and they can also reduce kidney and heart complications, two major causes of death in people with diabetes. However, SGLT2 inhibitors such as canagliflozin can also have detrimental side effects, including an increased risk of bone fractures.

In a study conducted by intramural scientists and led by NIDDK, in conjunction with other NIH Institutes, scientists hypothesized that canagliflozin may affect bone health by reducing calcium uptake. In addition to its effects on glucose, the SGLT2 protein transports sodium into kidney cells. Inhibiting SGLT2 lets sodium build up outside the cells, causing another transporter to increase the cells' uptake of phosphate. Based on results from previous studies, the researchers suspected that this increase in phosphate could trigger body-wide signals that ultimately reduce calcium absorption in the gastrointestinal tract. To test this theory, 9 female and 16 male healthy, non-diabetic volunteers received courses of canagliflozin and a placebo for 5 days each, with participants blinded to which course they received first. The participants ate a standardized diet before and during the study, so the researchers could investigate how canagliflozin affects levels of nutrients like phosphate, sodium, vitamin D (which promotes calcium absorption in the gut), and calcium. Consistent with their prediction, canagliflozin rapidly increased phosphate uptake, resulting in signals that, on average, reduced vitamin D levels in the blood as well as calcium uptake from food. The magnitude of these reductions varied from person to person, however, and were generally small enough that they might only affect the health of certain people with already low vitamin D and/or calcium levels. Though the study was too brief to measure bone fracture risk, canagliflozin's effects on calcium uptake, a key contributor to bone health, provides

a likely explanation for the increased fracture risk with canagliflozin use observed previously.

The researchers noted that it is not yet known if all SGLT2 inhibitors affect vitamin D to the same extent. Future research will also be required to confirm whether baseline vitamin D levels affect fracture risk when taking SGLT2 inhibitors, and, if so, whether modifying vitamin D levels could help protect bone health. Overall, this research revealed new insights about a possible side effect of a commonly prescribed, heart-protective diabetes drug that could be crucial to people and their doctors seeking to personalize their diabetes treatment.

Blau JE, Bauman V, Conway EM,...Taylor SI. Canagliflozin triggers the FGF23/1,25-dihydroxyvitamin D/PTH axis in healthy volunteers in a randomized crossover study. JCI Insight 3: pii: 99123, doi: 10.1172/jci.insight.99123, 2018.

UNCOVERING CLUES TO METABOLIC CONTROL AND THE TREATMENT OF TYPE 2 DIABETES

Studies Show Difficulty in Treating Type 2 Diabetes in Youth and Highlight Unique Aspects of the Disease in Young People: New research reveals that two diabetes medications do not prevent rapid progression of prediabetes or recent onset type 2 diabetes in young people with the disease, even though these are the only two medications approved for pediatric type 2 diabetes. More encouragingly, however, analyses comparing metabolic tests of adults in a companion study and the youth study participants are helping scientists understand how the disease differs with age, which may one day lead to better treatment approaches. Resistance to the glucose-lowering effects of insulin is often associated with overweight, obesity, or advancing age. At first the pancreas can compensate for this problem by simply secreting more insulin, but type 2 diabetes results if the pancreas gradually loses its ability to do so. Although long thought of as a disease of middle-aged and older adults, type 2 diabetes has been appearing in teenagers in small but increasing numbers in recent years. This trend is alarming for many reasons—for example, because the complications of the disease, which can be both debilitating and life threatening, are more likely to develop the longer someone has diabetes.

Furthermore, the recent Treatment Options for type 2 Diabetes in Adolescents and Youth (TODAY) study found that type 2 diabetes was very difficult to treat when it occurred in younger people, and that the disease progressed much more rapidly than it did in those who develop it in middle age or later. TODAY compared metformin alone with metformin together with a lifestyle intervention, and with metformin plus another medication that was then commonly used to treat adults with diabetes; none of these approaches was found to be effective. One encouraging observation from that study was that participants who had been enrolled in the study earlier in the course of their disease fared better than those whose diabetes was already more advanced. This finding suggested that focusing on preventing disease progression in young people with prediabetes or recent onset type 2 diabetes might be beneficial.

Indeed, the idea for the Restoring Insulin Secretion (RISE) studies comes from earlier observations that use of the diabetes drug metformin and/or a long-acting form of insulin can improve the function of the insulin-producing β (beta) cells of the pancreas in adults with prediabetes or recent-onset type 2 diabetes. The theory is that by taking some of the burden off the pancreas through early treatment—giving it a “rest”—it may be possible to slow or even reverse the progressive loss of its capacity to respond to glucose by secreting insulin. RISE is actually a group of three clinical trials testing this idea in different ways. Two trials are testing approaches to restoring insulin secretion in adults with prediabetes or recent-onset type 2 diabetes, and one studied younger participants, who were between 10 and 19 years of age at study enrollment. This RISE Pediatric Medication Study focused on preventing progression of the disease by utilizing the two drugs approved for treating type 2 diabetes in children, metformin and insulin. The RISE study randomly assigned the 91 participants to take metformin for 1 year, or to take a long-acting form of insulin for 3 months, followed by metformin for 9 months. (Insulin had not been tested in the TODAY study.) Unfortunately, neither approach was successful, as the disease progressed markedly in both groups over the course of the study, underlining the great importance of finding better approaches to preventing and treating type 2 diabetes in young people.

To try to understand the physiological reasons why type 2 diabetes is so much harder to treat when it develops at a young age, researchers

also compared metabolic test results from participants in the pediatric RISE study with tests in adult participants in the other two RISE studies. Regardless of age, the RISE participants at entry into all three clinical trials had similar fasting blood glucose levels, and similar glucose levels 2 hours after ingesting a drink containing a standardized amount of sugar, referred to as an oral glucose tolerance test (OGTT). However, researchers examined the OGTT in greater detail, checking two measures of insulin levels before and after the glucose challenge and found a striking difference: at each time tested, the 10- to 19-year-old participants had markedly higher insulin levels than did the adults. This suggests that young people with prediabetes or early type 2 diabetes are different from older people with these conditions in that they retain a more robust capacity to produce insulin, but that this capacity is masked by a more severe degree of insulin resistance. Another analytic method supported these findings: researchers used an IV to increase blood glucose levels—holding them first at one level, and then at a higher level—and then measured how the participants’ pancreases and other tissues responded. Insulin levels rose significantly higher in the young RISE participants than in the adults, often beyond the amount of insulin that was needed or desirable. These results further confirm previous observations that puberty can aggravate insulin resistance, suggesting that improving insulin sensitivity may be a key therapeutic strategy; yet metformin—the only commonly used anti-diabetes drug that is known for improving insulin resistance—was ineffective for youth. These studies together underline the importance of finding new and better ways to treat and prevent type 2 diabetes in young people.

The RISE Consortium. Impact of insulin and metformin versus metformin alone on β -cell function in youth with impaired glucose tolerance or recently diagnosed type 2 diabetes. Diabetes Care 41: 1717-1725, doi: 10.2337/dc18-0787, 2018.

The RISE Consortium. Metabolic contrasts between youth and adults with impaired glucose tolerance or recently diagnosed type 2 diabetes: II. Observations using the oral glucose tolerance test. Diabetes Care 41: 1707-1716, doi: 10.2337/dc18-0243, 2018.

The RISE Consortium. Metabolic contrasts between youth and adults with impaired glucose tolerance or recently diagnosed type 2 diabetes: I. Observations using the hyperglycemic clamp. Diabetes Care 41: 1696-1706, doi: 10.2337/dc18-0244, 2018.

New Insights on Risk Factors for Severe Hypoglycemia in People with Type 2 Diabetes:

Results of NIDDK-supported research have improved understanding of factors that can help predict vulnerability to severe hypoglycemia in African American and White individuals with type 2 diabetes. Although untreated diabetes results in blood glucose (sugar) levels that are too high, accidental over-treatment can lead to blood glucose levels that are too low, a condition known as hypoglycemia. If glucose levels fall far enough, severe hypoglycemia can cause seizures, loss of consciousness, and even death. More mild instances of hypoglycemia can be disorienting, and may raise the odds of accidental injury. Hypoglycemia can occur not only when a person accidentally takes too much of a diabetes medication such as insulin or sulfonylurea, but also when a prescribed dose is accompanied by either more physical exercise or fewer calories consumed than usual, or when an unrelated illness such as an infection temporarily alters a person's metabolism. Importantly, while people with diabetes can live many years without a serious hypoglycemic episode, some people seem to be more susceptible to the problem.

To find ways to assess the risk of severe hypoglycemic episodes in people with diabetes, researchers examined outcomes from a well-characterized cohort of 1,206 African American and White women and men with diabetes who had participated in a prior study. The participants, who averaged 64 years of age at the beginning of the study, were followed for a median of about 15 years—that is, half of the study participants were followed for that long or longer, and half for that long or less. During the study, 185 participants were treated at least once for severe hypoglycemia in emergency rooms, via an ambulance call, and/or by hospitalization. The study confirmed some prior research that showed, for example, that risk of severe hypoglycemia increases with age, with poor cognitive function, and with poor kidney function, and is higher in African Americans than in Whites.

In addition, the study found some intriguing new risk factors. Not only is cognitive dysfunction a risk, but physical disability, assessed by the inability to perform activities of daily living, was found to be a new risk for hypoglycemia. Another demographic risk factor identified by this study is the use of Medicaid insurance, which may reflect socioeconomic disparities. Interestingly, researchers found that people with greater fluctuations in blood glucose

control—indicated by low blood levels of a compound called 1,5-anhydroglucitol (1,5-AG)—were at elevated risk for an episode of severe hypoglycemia. This means 1,5-AG may be a valuable new laboratory test of hypoglycemia risk, since HbA1c levels reflect a person's average blood glucose levels over several weeks, but indicate nothing about how much blood glucose has fluctuated around that average during that time period. Taken together, these results may help health care providers predict which people with type 2 diabetes may benefit from additional monitoring to prevent episodes of severe hypoglycemia.

Lee AK, Lee CJ, Huang ES, Sharrett AR, Coresh J, and Selvin E. Risk factors for severe hypoglycemia in Black and White adults with diabetes: The Atherosclerosis Risk in Communities (ARIC) Study. Diabetes Care 40: 1661-1667, doi: 10.2337/dc17-0819, 2017.

Treating Type 2 Diabetes in Adolescents with Severe Obesity:

In a new analysis comparing data from two different studies that evaluated treatments for adolescents with severe obesity and type 2 diabetes, researchers determined that bariatric surgery led to improved outcomes over treatment with medication. Type 2 diabetes is increasingly being diagnosed in youth, and it disproportionately affects youth from racial and ethnic minority populations in the United States. The Treatment Options for type 2 Diabetes in Adolescents and Youth (TODAY) clinical trial showed that the disease may be more aggressive and difficult to treat in youth compared to adults. Because the onset and severity of disease complications correlate with both the duration of diabetes and control of blood glucose (sugar) levels, those with early disease onset are at greater risk for complications than those who develop the disease later in life. Thus, it is imperative to find treatments that help this vulnerable population manage their disease and achieve better glucose control.

To glean more information to compare treatment options, researchers returned to the outcomes of two NIDDK-supported studies: TODAY and the Teen-Longitudinal Assessment of Bariatric Surgery (Teen-LABS). In the TODAY clinical trial, youth with type 2 diabetes received either the anti-diabetes medication metformin alone; metformin in combination with another medication, rosiglitazone; or metformin with an intensive lifestyle intervention. The trial showed that metformin alone and metformin with lifestyle intervention were insufficient to control blood glucose adequately in

about half of the participants. The trial also showed that, although the combination of metformin with rosiglitazone worked somewhat better than metformin alone, this drug combination failed to maintain adequate blood glucose control in a high proportion of participants. Teen-LABS is an observational study that has been evaluating health outcomes of adolescents with severe obesity who underwent bariatric surgery. Teen-LABS found that bariatric surgery resulted in major weight loss and improvement in overall health and quality of life. However, questions remained regarding treatment options for youth with severe obesity and type 2 diabetes.

Because some of the Teen-LABS and TODAY participants had both type 2 diabetes and severe obesity, researchers embarked on a new analysis to compare bariatric surgery and medication for treatment of these conditions in youth for the first time. They analyzed data for 30 adolescents with severe obesity and type 2 diabetes who underwent bariatric surgery in Teen-LABS in comparison with data from 63 adolescents with these conditions who received medication in the TODAY study. The researchers found that hemoglobin A1c levels (HbA1c, a measure of average blood glucose control) worsened over 2 years of follow-up in adolescents treated with medication (an average increase from an HbA1c of 6.4 percent to an HbA1c of 7.8 percent, with a higher value indicating worse control), whereas HbA1c levels improved in adolescents treated with bariatric surgery (an average decrease in HbA1c from 6.8 percent to 5.5 percent). Adolescents treated with medication were also more likely to gain weight (an average increase of nearly 13 pounds), have worse diabetes (as indicated by HbA1c and other measures), and show no improvement in blood pressure or kidney function. In contrast, adolescents treated with bariatric surgery showed weight reduction (an average loss of over 94 pounds), remission of their diabetes, and improvements in blood pressure and kidney function.

The striking improvements in those treated with bariatric surgery indicate that this could be a treatment option for adolescents with severe obesity and type 2 diabetes. Bariatric surgery, however, has serious surgical risks that need to be balanced with the potential benefits. For example, within 2 years of having bariatric surgery, 23 percent of adolescents with type 2 diabetes treated with

bariatric surgery required subsequent operation and/or hospitalization for conditions related to the bariatric surgical procedure. Further research is needed to understand the long-term outcomes of bariatric surgery in this population. These results also continue to highlight the pressing need for better treatments for youth with type 2 diabetes—and for better prevention of diabetes in youth.

*Inge TH, Laffel LM, Jenkins TM,... Zeitler PS; for the Teen-Longitudinal Assessment of Bariatric Surgery (Teen-LABS) and Treatment Options of Type 2 Diabetes in Adolescents and Youth (TODAY) Consortia. Comparison of surgical and medical therapy for type 2 diabetes in severely obese adolescents. *JAMA Pediatr* 172: 452-460, doi: 10.1001/jamapediatrics.2017.5763, 2018.*

NEW INSIGHTS ON THE COMPLICATIONS OF DIABETES

Immune Cells Convert into New Tissue To Close

Wounds: Scientists have shed light on a long-standing mystery: what happens to macrophages, a type of immune cell, during skin wound healing? A new study has found the answer: the macrophages change their properties to become part of healed skin, and this process is crucial for wound closure and may be impaired in diabetic wound healing. Previous work had suggested that wound inflammation can cause macrophages responding to the injury to “transdifferentiate,” or convert into another type of cell. However, what triggers transdifferentiation, what happens to macrophages after the wound heals, and what controls how quickly and how well wounds heal is still unknown.

To investigate these questions, researchers used microscopy to study macrophages in male and female mice’s skin wounds. They found that macrophages rushed to wound sites, then converted into cells similar to a type of connective tissue cell (fibroblasts) and eventually made up nearly two-thirds of new tissue formed to close the studied wounds. Researchers also found macrophage-derived fibroblast-like cells in healing chronic human skin wounds, suggesting a role for this process in human disease.

The scientists next investigated what was causing this transdifferentiation. Interestingly, they found that treating human macrophages with fluid from healing,

but not non-healing, wounds promoted conversion of the macrophages to fibroblast-like cells. This result suggested that a factor in the wound fluid played a role in macrophage transdifferentiation. Several experiments searching for this factor demonstrated that wounded human skin cells secreted more of a small molecule called miR-21 into the fluid of healing wounds than of non-healing wounds. Adding miR-21 to macrophages in the laboratory caused them to convert into fibroblast-like cells, and removing miR-21 from a new wound in mice impaired healing. These results indicated that miR-21 plays a critical role in signaling macrophages to become fibroblast-like cells and that this process is critical to wound healing. Additionally, researchers asked whether miR-21 and transdifferentiation have roles in chronic, slow-healing wounds, such as those seen in diabetes. They found that diabetic mice secreted less miR-21 than non-diabetic mice when wounded and that macrophage transdifferentiation and healing at the wound site was impaired. These differences were reduced if the mice's wounds were treated with miR-21.

These links between miR-21 and the transdifferentiation of macrophage cells into cells that become part of healed skin give new insight into how wound healing is impaired during diabetes. Further research is needed to test whether therapies targeting macrophage transdifferentiation could improve wound healing in humans.

Sinha M, Sen CK, Singh K,...Roy S. Direct conversion of injury-site myeloid cells to fibroblast-like cells of granulation tissue. Nat Commun 9: 936, doi: 10.1038/s41467-018-03208-w, 2018.

NEW TOOLS TO TREAT GENETIC DISEASES

Turning Pancreatic Alpha Cells into Insulin-Producing Beta-like Cells via Gene Therapy: Scientists have developed a new way to replace lost β (beta) cells in mouse models of type 1 diabetes. Type 1 diabetes stems from a misguided autoimmune attack destroying the pancreas' insulin-producing β cells, which reside in cell clusters called islets. Insulin therapy is a life-saving treatment for this disease, but it is difficult to keep blood glucose (sugar) levels within the recommended range with current therapies. Scientists are studying new ways to replace lost β cells, and one approach is

to “reprogram” other pancreatic cells—such as glucagon-producing α (alpha) cells, which are also in the islets—to take over β cells' function. Toward this end, one group of scientists has used a repurposed virus as a vehicle to deliver extra copies of two genes involved in β cell maturation and function (*Pdx1* and *Mafa*) into the mouse pancreas. They gave this gene therapy to mice who had diabetes because their β cells had been destroyed. After the therapy, the mice no longer had diabetes, and their blood glucose levels stayed in the normal range until the experiment's end 6 months later. Researchers found that the *Pdx1* and *Mafa* genes were delivered to pancreatic α cells, where the genes prompted the α cells to become β cell-like and produce insulin.

However, could this approach effectively treat diabetes in a mouse model mimicking the ongoing autoimmune attack seen in people with type 1 diabetes, or would this autoimmune response destroy the newly reprogrammed insulin-producing cells? To answer these questions, the researchers gave the *Pdx1* and *Mafa* gene therapy to mice made diabetic due to an ongoing autoimmune attack that destroyed their β cells. Mice that received the therapy soon after the onset of diabetes had increased insulin production and more β -like cells when compared to controls. Importantly, their blood glucose levels stayed in the normal range for about 4 months before returning to the diabetic range, indicating that the newly formed β -like cells could evade immune attack during that time. Also, the researchers were able to reprogram human α cells directly in cell cultures in the laboratory. Human pancreatic islets isolated from male and female deceased donors were experimentally treated to destroy their β cells and then treated with the *Pdx1* and *Mafa* gene therapy. When these reprogrammed islets were transplanted into an autoimmune diabetic mouse model, insulin production went up, and the mice had significantly better blood glucose control than similar mice given placebo-treated islets.

Overall, these results describe a new method to reprogram mouse pancreatic α cells to functionally replace destroyed β cells. The researchers cautioned that more research is needed to determine if this approach would work in people with type 1 diabetes, including whether human β -like cells would survive

for any length of time or if they would need extra protection against the ongoing autoimmune attack that occurs in type 1 diabetes.

Xiao X, Guo P, Shiota C,...Gittes GK. Endogenous reprogramming of alpha cells into beta cells, induced by viral gene therapy, reverses autoimmune diabetes. *Cell Stem Cell* 22: 78-90, doi: 10.1016/j.stem.2017.11.020, 2018.

Improved Treatment Targeting Root Cause of Cystic Fibrosis May Be on Horizon:

A clinical trial led by a pharmaceutical company with additional support from other organizations and from an NIDDK-supported Cystic Fibrosis Center that facilitated the conduct of the trial has shown that a combination of two medications provides significant clinical benefit in a subgroup of patients with cystic fibrosis (CF). CF occurs when people lack a functional copy of the *CFTR* gene, which encodes a protein required for transporting chloride, one of the two chemical components of salt, in and out of our cells. People with CF develop severe lung disease, and digestive problems, and commonly develop a form of diabetes once they reach adulthood; these complications significantly diminish both lifespan and quality of life. People have two copies of this gene, but in people with CF inactivating mutations affect both copies. While many *CFTR* mutations are known, the one that is the most common by far is designated Phe508del (also known as F508del or delta F508). This mutation not only dramatically reduces the amount of *CFTR* protein that reaches the cell surface where it is needed to function, but also practically eliminates the chloride transporting capability of the small amount of *CFTR* that does get there. The combination of these effects means people with two copies of Phe508del—about half of CF patients—have a very severe form of CF. About another 40 percent of people with CF have one copy of Phe508del paired with one of the rarer *CFTR* mutations. A few dozen of these rarer forms of the gene are termed “residual function mutations” as they encode forms of *CFTR* protein that retain a limited capacity to transport chloride. People with one copy of a residual-function mutation and one copy of Phe508del typically develop a less severe form of CF than those with two copies of Phe508del.

Using knowledge of the different *CFTR* mutations, scientists have developed candidate medicines that may help restore sufficient *CFTR* function

to partially alleviate CF symptoms. Already, a combination of ivacaftor, a compound that facilitates transport of chloride through mutant forms of the *CFTR* protein, plus lumacaftor, a compound that stabilizes *CFTR* proteins so they reach the cell surface more easily and in greater amounts, was approved by the U.S. Food and Drug Administration (FDA) in 2015 for use in CF patients with at least one copy of Phe508del. However, benefits are modest, and some patients experience significant side effects. The current study tested a combination of ivacaftor and a promising new (not yet FDA-approved) *CFTR*-stabilizing drug, tezacaftor, in people who have one copy of Phe508del and one copy of a residual-function *CFTR* mutation. The study had an unusual design that included two 8-week treatment periods, separated by 8 weeks in which the 248 male and female participants with CF (who were at least 12 years old) did not receive study medications. Each participant was randomly assigned to receive either combination therapy, ivacaftor alone, or a placebo during the first treatment period, and (after the break) to have one of the other two regimens during the second. As a result, all of the participants received at least one of the experimental therapies for at least one of the two treatment periods.

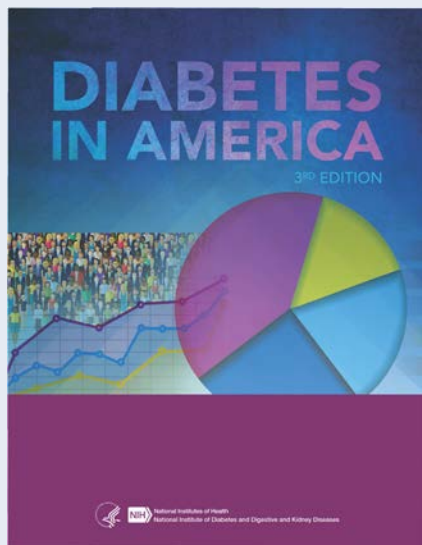
The researchers found that the amount of air that participants were able to exhale per second—a standard measure of lung function in people with CF—rose by an average 4.7 percentage points with ivacaftor treatment alone, and 6.8 percentage points with the combined ivacaftor-tezacaftor treatment, compared to a placebo. (That is, placebo-taking participants able to exhale 50 percent as much as expected for people without CF would be able to exhale an average of 54.7 percent with ivacaftor alone, or 56.8 percent with the combination therapy.) These improvements may seem small, but they suggest either therapeutic approach could confer clinical benefit. Indeed, participants—who did not know whether they were taking one of the medication regimens or placebo at any given time—reported significantly better measures of respiratory-related quality of life after taking ivacaftor or ivacaftor-tezacaftor than after taking placebo. Importantly, there were no differences in the adverse event frequency between placebo and drug treatments.

An important caveat is that the 8-week intervention periods of the study were too short to ascertain definitive effects on long-term clinical outcomes. However, all participants were enrolled in an ongoing follow-on study that should provide greater insights into the longer-term effects of the two treatments. Moreover, results from a different trial supported entirely by the same pharmaceutical company suggest that the drug combination may also benefit people with two copies of Phe508del. Together, these studies

may form the basis for eventual FDA approval of the tezacaftor-ivacaftor combination to treat the molecular cause of CF in a large proportion of people with the disease. If results from the two trials are borne out, this treatment approach may one day significantly improve the health and quality of life of many people with CF.

Rowe SM, Daines C, Ringshausen FC,...Davies JC. Tezacaftor-ivacaftor in residual-function heterozygotes with cystic fibrosis. *N Engl J Med* 377: 2024-2035, doi: 10.1056/NEJMoa1709847, 2017.

NIDDK's "Diabetes in America, 3rd Edition" Provides Preeminent Source for Scientific Information on Diabetes and Its Complications



Diabetes affects nearly everyone, from the more than 110 million Americans with or at risk for the disease to the many more people who care for them. It is chronic and relentless and increases the risk for many devastating conditions and diseases. To understand the burden of diabetes in the United States, in August 2018, the NIDDK completed the third edition of a resource designed to be a preeminent source for crucial scientific information on diabetes and its complications: *Diabetes in America, 3rd Edition*. Developed by researchers at NIDDK with contributions from leading diabetes experts from around the world, *Diabetes in America* is an assessment of epidemiologic, public health, clinical, and clinical trial data on diabetes and its complications in the United States. The resource covers the spectrum of diabetes, describing data and trends, complications of diabetes and related conditions, and prevention and medical care for diabetes, including outlining major diabetes research findings.

Diabetes in America is designed to be useful to a variety of audiences, presenting data patients can use to gain a fuller understanding of their condition, practitioners can use to determine the likelihood that their patients will develop diabetes or associated complications, health policymakers can use to help guide decision-making related to diabetes, and researchers can use to identify areas of needed research to advance care for people with or at risk for diabetes.

Diabetes in America also describes the effects the disease has on the entire body—from head to toe. Diabetes not only increases the risk for complications such as vision loss, kidney failure, nerve disease, and amputation, but also doubles the risk for heart disease, many forms of cancer, some forms of dementia, hearing loss, and urinary incontinence. *Diabetes in America* provides comprehensive information about the link between diabetes and those and other conditions, such as osteoarthritis, bone fractures, gum disease, and depression.

Diabetes in America shows that though much research progress has been made, there is still much work to be done to achieve the goal of good health for all people with or at risk of diabetes. The NIDDK hopes that this new edition of *Diabetes in America*, the first in more than 20 years, will help educate people about diabetes and its many complications, to lessen this burden for everyone.

Diabetes in America, 3rd Edition, is available, in individual chapters and online only, on the NIDDK website at: www.niddk.nih.gov/about-niddk/strategic-plans-reports/diabetes-in-america-3rd-edition

Workshop Highlights

State-of-the-Science in Diabetes Genetics

On April 23–24, 2018, the NIDDK hosted a workshop in Bethesda, Maryland, to accelerate the identification and characterization of genetic factors related to diabetes. This workshop—titled “Towards a Functional Understanding of the Diabetic Genome”—featured presentations by an international group of experts on recent findings and innovative approaches, as well as identification of knowledge gaps and future opportunities in the field.

Diabetes is a complex disease that can be affected by both genetic and environmental factors. In type 2 diabetes, the body does not make enough insulin or does not use insulin well, while in type 1 diabetes a misguided autoimmune attack destroys the insulin-producing cells in the pancreas. Understanding the genetic factors that affect diabetes development or progression could lead to new therapies and better disease outcomes. To find these factors, researchers have compared the genetic information of people with and without diabetes. These studies produced a wealth of data and identified many genetic regions related to the disease. It has been more difficult to connect all of these diabetes-associated genetic factors to their functions in the body, and this is an area ripe with opportunity. This knowledge could advance precision medicine approaches, allowing the tailoring of diabetes treatment plans to counter or take advantage of a person’s unique genetic makeup.

Workshop attendees presented their recent research progress using diverse approaches and multiple data types to “connect the dots” between genetic

factors and diabetes. Integrating all these data types with genetic association data will be important to build a complete picture of how particular genomic regions influence diabetes. One of the important themes that emerged from the workshop was that although the pancreas—the major organ responsible for controlling blood glucose (sugar) levels—plays an important role in diabetes development, other tissues such as fat, liver, skeletal muscle, and brain also play a part. Presentations highlighted new findings on diabetes disease mechanisms and on various ways that diabetes-associated genetic differences can control gene function. Workshop participants also shared information on cutting-edge genetic analysis techniques and valuable research model systems, as well as giving demonstrations of useful diabetes genetics resources such as the Type 2 Diabetes Knowledge Portal (www.type2diabetesgenetics.org; part of the Accelerating Medicines Partnership) and the Diabetes Epigenome Atlas (www.t2depigenome.org). Much of the workshop focused on type 2 diabetes, though the studies and resources discussed could eventually benefit all people with diabetes.

At the end of the workshop, attendees discussed how to fill research gaps, what resources are needed to build upon previous findings, and how to take advantage of opportunities in this developing field. This information is expected to inform NIDDK planning efforts to support diabetes genetics research and will help advance precision medicine approaches for predicting, preventing, and treating diabetes.

Diabetes Mellitus Interagency Coordinating Committee Meeting Focuses Attention on the Importance of Research in Older Americans with Diabetes Receiving Long-term Care

On May 29, 2018, the Diabetes Mellitus Interagency Coordinating Committee (DMICC) held a meeting about “Fostering Research on Older Adults with Diabetes Receiving Long-term Care.” U.S. adults over age 65 have a very high prevalence of elevated blood glucose (sugar) levels. In fact, nearly one-third have diabetes, and almost half have prediabetes (glucose levels higher than normal, but lower than in diabetes) according to recent Centers for Disease Control and Prevention (CDC) data. Over 61 percent of all health care expenditures attributed to diabetes in the United States are for health resources used by older adults with diabetes, yet many studies do not focus on this group, especially when they receive long-term care services rather than dwelling independently in the community. Critical gaps of knowledge on this understudied population were identified at this meeting, and ways to address these gaps were discussed.

There was a call for better understanding how different factors alone and in combination can alter an older adult’s ability to manage diabetes, including the presence of cognitive and functional impairments, numerous competing health issues referred to as “multimorbidity,” and complex medication regimens referred to as “polypharmacy.” Who assists with diabetes care and variations in the level of assistance in different settings can also impact how well glucose levels are controlled

for older adults with diabetes. Furthermore, it is critical not just to understand how older adults with diabetes can control elevated glucose levels, but also how they can do so while avoiding hypoglycemia (low blood glucose), which can increase the risk of falls, hospitalization, and worsening of other medical conditions.

Opportunities to address these issues were discussed at the meeting. For example, representatives from the Centers for Medicare & Medicaid Services, the Veterans Health Administration, the CDC, and the NIH’s National Institute on Aging presented information about sources of data that their agencies have assembled on large numbers of older adults with diabetes, particularly in long-term care settings. These presentations were paired with talks by researchers who have found innovative ways to mine these substantial quantities of data to yield key insights on how better to care for our large and growing older adult population, highlighting both the strengths and limitations of these data. Importantly, the Committee addressed how these sources of data may be utilized to fill gaps in our existing knowledge of diabetes treatment in older, managed care populations, so that we may one day achieve better long-term health outcomes for older adults with diabetes.

STORY OF DISCOVERY

Newborn Screening for Lysosomal Storage Diseases

For most people, having a baby is one of the greatest joys in life. When an infant is born with a rare genetic disorder, parental joy can be mixed with fear; but fortunately, recent decades have also brought good news for many families of children born with an inherited disease, as progress in the treatment of some of these disorders is improving health and quality of life for many affected infants.

In some cases, therapies achieve the best possible health outcomes if they are initiated before outward signs of the disorder develop. For this reason, it is critically important that advances in treatment for genetic disorders be matched with development of newborn screening programs that help quickly identify children who need them.

Newborn screening programs in most U.S. states test for dozens of genetic disorders, including several that are within the NIDDK's mission area. These include sickle cell anemia, cystic fibrosis, as well as some lysosomal storage diseases, a constellation of rare diseases whose detection in newborn screening has undergone a revolution in recent years.

WHAT ARE LYSOSOMAL STORAGE DISEASES?

The body's cells recycle many of the substances they no longer need by digesting them with proteins inside cellular compartments called lysosomes. The proteins that are missing or damaged in people with lysosomal storage diseases are considered "enzymes," meaning that each functions to promote the conversion of one chemical compound into another. Specifically, lysosomal enzymes convert toxic cellular waste products into materials that the cell can recycle or safely excrete. When one of

these enzymes is missing or inactive, toxic waste products are not properly degraded. Instead, they build up in the lysosomes where they can lead to severe organ damage. Diseases caused by such enzyme deficiencies—lysosomal storage diseases—are individually rare, but collectively affect about 1 in 7,700 infants born in the United States. Symptoms vary, and are often not apparent at birth; however, as the undigested materials accumulate, they can cause serious problems such as weakness, severe pain, brittle bones, intellectual disability, corneal clouding, organ failure, and death. Dozens of lysosomal storage diseases have been characterized, including Gaucher disease; Pompe disease; Fabry disease; and several forms of mucopolysaccharidosis (known as MPS I, MPS II, etc.), a subset of the lysosomal storage diseases resulting when someone lacks any of several enzymes needed to recycle one particular class of biological molecules.

Built on the discoveries of scientists supported by the NIDDK and other NIH Institutes and Centers and product development by pharmaceutical companies, lysosomal storage disease research is a classic story of translating remarkable findings from basic research into U.S. Food and Drug Administration (FDA)-approved treatments for many of these diseases. To take the greatest advantage of these developments, the NIDDK has also invested in developing improved methods for identifying infants who would benefit from these therapies.

BLOOD SPOT SCREENING: THE BEGINNING OF THE REVOLUTION

The first widely utilized newborn screen for a genetic disease did not detect a lysosomal storage disease; rather, it tested for phenylketonuria (PKU),

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a genetic disorder caused by the inability to break down an amino acid called phenylalanine. Amino acids are the building blocks of proteins. In PKU, phenylalanine can build up to harmful levels in the body, causing intellectual disability and other serious health problems. By the 1950s, physicians understood that the serious symptoms of PKU could be greatly reduced by carefully limiting the amount of phenylalanine in the diet of people with the disease. However, PKU can have serious, lasting effects on a child's development if the problem is not detected—and the dietary intervention initiated—early enough.

Thus, it was a landmark development when, in 1959, researchers developed a straightforward method to test for PKU. First, they saturated a small piece of paper with a drop of blood, usually collected by pricking the heel of an infant to be tested. After the spot had dried, they placed the paper onto a petri dish containing bacteria that can only grow in the presence of supplemental phenylalanine. Since healthy people store very little phenylalanine, and people with PKU accumulate significant amounts of the amino acid, this bacterial growth test made it much easier to identify babies with PKU during their first few days of life. First tested on a large scale in 1962 using blood spots from 400,000 babies, the approach was a tremendous success, allowing early dietary intervention that greatly improved outcomes for children with PKU. Within a few years, states throughout the Nation had adopted the PKU screen; a 1968 World Health Organization report led to screening in many foreign countries as well.

A METHOD TO IDENTIFY SEVERAL DISEASES AT ONCE

Similar tests using dried blood spots and bacterial growth soon followed for other genetic diseases, but there were limits to the number of such tests that could be performed with a single blood spot, and limits to the number of blood spots that could reasonably be collected from every infant. Researchers found a solution to this problem by utilizing “mass spectrometry,” a method for

simultaneously measuring the amounts of multiple chemical components from a small sample.

Early models of mass spectrometers, developed more than 120 years ago, relied on differences in the mass and charge of chemicals in a sample in order to separate them. Modern mass spectrometry methods take advantage of other chemical differences, and are powerful enough to separate and quantify thousands of molecules at once. This technology has been widely adopted by clinical laboratories to perform a wide variety of diagnostic tests. Importantly, these tests can include simultaneous screens for dozens of genetic diseases, provided that each of those diseases has a measurable effect on the chemical components of a dried blood spot or other easily obtained sample.

THE DEVELOPMENT OF TREATMENTS FOR LYSOSOMAL STORAGE DISEASES CREATES A NEED FOR EARLY DETECTION

Although mass spectrometry was utilized to detect a growing number of genetic diseases in newborns, for at least two reasons, lysosomal storage diseases were not initially among them. First, it turns out that the levels of the metabolites that accumulate in lysosomal storage diseases can be quite variable in both healthy babies and those with lysosomal storage diseases, which can lead to test inaccuracy. A second reason was that in the late 1990s, when mass spectrometry screening of dried blood spots was becoming more common, there were no effective treatments for lysosomal storage diseases. It is not considered useful or cost-effective to test for a condition if a positive result would only make parents worry that their children are likely to develop a disease when there is no treatment their pediatrician can suggest to keep them healthy.

Fortunately, research in the early 2000s—supported by the NIDDK, other components of NIH, and voluntary groups—led to significant improvements in the treatment of many lysosomal storage diseases. Scientists studying the basic biology of the

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lysosome discovered that healthy cells do not simply synthesize the enzymes and deposit them directly into their lysosomes. Rather, each enzyme is initially synthesized in an inactive, precursor form, which the cell excretes. Either the same cell or another cell nearby reabsorbs the precursor and directs it to a lysosome, where it is converted into its mature, active form. To treat a lysosomal storage disease, therefore, clinicians can periodically inject some of the needed enzyme in its precursor form, taking advantage of these final processing steps to deliver it to lysosomes and activate it.

While by no means a cure, “enzyme replacement therapy,” as the treatment approach is called, significantly improves health for people with a variety of lysosomal storage diseases. Several such therapies are FDA-approved, while still more are under consideration. Importantly, just as with PKU, best results are obtained when treatment begins early, before organ damage has occurred. So once treatments were available, there was a need for corresponding improvements in methods to screen for lysosomal storage diseases in newborns.

FLUORESCENCE TESTS FOR LYSOSOMAL STORAGE DISORDERS

Unlike with PKU, researchers found that the most reliable way to test for lysosomal storage diseases was to test for the activities of the lysosomal enzymes in a tissue sample from the person being tested. Early forms of such tests were both invasive—requiring a muscle or skin biopsy—and labor intensive, so they were not used unless there was a clear reason to suspect a child had a lysosomal storage disease (because he or she had developed the symptoms, or had an older sibling with the disease). Other tests were developed that measured the amount of the enzyme in the blood sample relative to the amount of other proteins, for example; but their usefulness was limited by the fact that some lysosomal storage disease-causing mutations do not eliminate the relevant enzyme, but rather make it unable to perform its chemical function.

A major step forward came in 2001, when researchers described a simple process whereby the activity of an enzyme called α -L-iduronidase (IDUA), which is missing or inactive in people with the lysosomal storage disease MPS-I, could be reconstituted from a dried blood spot. Just as importantly, they developed a straightforward test to check for that enzymatic activity. The test relied on a modified form of the chemical that IDUA normally acts on in the lysosome: IDUA splits the modified form of the chemical into two parts, one of which is highly fluorescent, and can be detected and quantified by a light sensor. The diagnostic test for MPS-I takes advantage of this fluorescent component—samples from people who lack normal levels of IDUA activity produce much less fluorescence than those from people without the disease. The principle utilized in the MPS-I test was extended to develop related fluorescence-based strategies for diagnosing Pompe, Gaucher, Fabry, and other lysosomal storage diseases.

Special tools allowed laboratories to perform these tests on blood spots from dozens of babies at once, suggesting that wide-scale screening might be possible. Therefore, other researchers conducted pilot studies in which they used these methods to screen for one or more lysosomal storage diseases in large numbers of children. One such study took place in Taiwan, where Pompe disease is more common than in the United States. Testing dried blood spots from over 200,000 newborns born in one region of the country, researchers diagnosed 6 babies with the disease. Compared to babies born in other regions of Taiwan at about the same time, those diagnosed earlier were also able to begin enzyme replacement therapy sooner and had better health outcomes. Taiwanese researchers later performed an analogous pilot study to diagnose Fabry disease with similar results. Other researchers supported these findings through pilot studies conducted in Europe, South America, and Missouri. The fluorescence assays were therefore the first practical approaches to screening for lysosomal storage diseases using dried blood spots from large numbers of newborns. The FDA has therefore

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approved tests using this technology for newborn screening to detect MPS-I, Pompe, Gaucher, and Fabry diseases.

THE NEXT GENERATION OF LYSOSOMAL STORAGE DISEASE TESTING METHODS

While these fluorescence tests were a critical stride toward allowing widespread newborn screening for lysosomal storage diseases, they are not perfect. For example, for reasons that are not fully understood, some people have low (but non-zero) IDUA activity, yet never develop MPS-I symptoms. And because the tests have a limited capacity to detect small differences in the enzyme activities, it was sometimes unclear whether babies with intermediate levels of IDUA activity were perfectly healthy or would go on to develop a mild form of MPS-I. A similar problem exists for the enzymes missing in some of the other lysosomal storage diseases. Another important limitation of the fluorescence approach is that all of the lysosomal storage disease tests utilize the same fluorescent product, meaning each must be performed separately.

Fortunately, researchers have identified a significantly more sensitive method for measuring the activities of lysosomal enzymes, and it allows testing for multiple lysosomal storage diseases at once. Their insight was to combine the enzyme reconstitution methods pioneered in the development of the fluorescence tests with use of mass spectrometry technologies to detect their activities—without the need for fluorescence. NIDDK-supported researchers first developed such an approach to diagnose a lysosomal storage disease known as Krabbe disease, in which people lack the enzyme galactosylceramidase (GALC). As described in a 2004 scientific publication, the scientists allowed enzymes extracted from a blood sample to mix with a normal, unmodified form of a chemical GALC splits into two products in lysosomes. With a few additional steps, they were able to use mass spectrometry to separate those products in the mixture, and measure how much of one of them

had been created, finding little or none if the sample was from someone with Krabbe disease.

Initially, the method relied on GALC extracted from a fresh blood sample, but later the researchers found they could reconstitute the enzyme from a dried blood spot, based on the same principles utilized for the fluorescence tests. And importantly, they also found that if they included chemicals acted on by IDUA and other enzymes missing in various lysosomal storage diseases, the mass spectrometry process could separate out each of the different reaction products. Thus, they were able to use just a small part of a single blood spot to check for at least six different lysosomal storage diseases simultaneously. In addition, the mass spectrometry screening methods are notably better than the fluorescence approach at reliably measuring small differences in the enzyme activities, making it easier for clinical testing labs to distinguish infants likely to develop lysosomal storage diseases from those with enough enzymatic activity to keep them healthy.

MOVING INNOVATIONS IN LYSOSOMAL STORAGE DISEASE TESTING INTO WIDESPREAD CLINICAL USE

Although there are tests for hundreds of other genetic diseases, in addition to the lysosomal storage diseases, it would be difficult and costly to screen every child for each of them. Some tests may be difficult, requiring a more invasive sample procedure than the use of a dried blood spot, or requiring expensive, unusual testing equipment, for example. Others may often yield ambiguous results, or cause needless worry among parents by misdiagnosing a disease in infants who are actually healthy. Even if the test is accurate and simple to perform, if there is no way to treat for the disease, or if it can be treated effectively even after symptoms develop, routine screening of most newborns would not be useful.

For these reasons, the U.S. Health Resources and Services Administration assembled a group of experts in diagnostic testing called the Advisory Committee on Heritable Disorders in Newborns

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and Children, which carefully considers evidence from clinical research to determine whether states throughout the Nation should routinely screen newborns for various genetic diseases. The Committee publishes and periodically updates a list, the Recommended Uniform Screening Panel, that now includes tests for MPS-I and Pompe disease among the dozens of diseases infants should be screened for. The bar is high for adding additional diseases, given limited resources, but the availability of reliable tests and treatments for several other lysosomal storage diseases suggest they may be added in the future. Indeed, many states require testing for lysosomal storage diseases not currently listed on the Uniform Panel.

THE FUTURE OF RESEARCH AND TREATMENT FOR LYSOSOMAL STORAGE DISEASES

In 2018, the FDA approved a simultaneous mass spectrometry test for Gaucher, Niemann-Pick A/B, Pompe, Krabbe, Fabry, and MPS I diseases. The availability of reliable testing means many children with lysosomal storage diseases may soon

be diagnosed earlier, and more accurately. This fact, combined with the therapeutic options now available, is reducing the burden of these serious diseases for affected children and families. Earlier diagnosis not only allows therapy to begin before irreversible organ damage may have occurred, it also potentially helps avoid costly diagnostic efforts to determine the cause of a child's health issues, as well as potentially ineffective therapies that might precede an eventual correct diagnosis. While lysosomal storage disease therapeutics currently in clinical use are not perfect and well short of a cure, further research to develop improved methods to treat these diseases is currently in development.

In addition, researchers have developed and are testing diagnostic methods for many more lysosomal storage diseases, including other forms of MPS (such as types II, IIIA, IIIB, IVA, and VI). Such developments would provide the opportunity to screen for many more lysosomal storage diseases, administer available therapy early, and greatly improve the lives of those affected by these disorders.

SCIENTIFIC PRESENTATION

Dr. Maike Sander—Harnessing Insights into Beta Cell Plasticity for Regenerative Therapies

Dr. Maike Sander is a Professor of Pediatrics and of Cellular and Molecular Medicine at the University of California San Diego (UCSD). She is also the Director of the Pediatric Diabetes Research Center at UCSD and is part of the Stanford Consortium for Regenerative Medicine. An expert in pancreatic stem cell biology with over 20 years of experience in medicine and diabetes research, Dr. Sander is an elected member of the American Society for Clinical Investigation, a member of the NIDDK-supported Human Islet Research Network, and the recipient of JDRF's Gerold and Kayla Grodsky Basic Research Scientist Award.

At the May 2018 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Sander presented an overview of selected regenerative medicine approaches to diabetes treatment that are currently under investigation, including some of her own laboratory's work. The following summarizes the research highlights of her presentation.

HOW CAN REGENERATIVE MEDICINE BE APPLIED TO DIABETES?

Pancreatic β (beta) cells are crucial to the body's ability to manage blood glucose (sugar) levels. The insulin these cells secrete helps keep the body's constantly-cycling blood glucose levels within a healthy range. Loss of β cells (such as that caused by the misguided autoimmune attack that causes type 1 diabetes) or loss of the β cells' ability to produce enough insulin for the body's needs (as seen in type 2 diabetes) can lead to abnormally high blood glucose levels. These high blood glucose levels can, in turn, affect the eyes, nerves, heart, kidneys, and other organs, causing many of the long-term complications associated with diabetes.

Despite the current tools and therapies available, keeping blood glucose levels within a healthy range is still challenging, and better therapies are needed.

The field of regenerative medicine offers a promising avenue to these new therapies. Regenerative medicine is the process of creating living, functional tissues to repair or replace tissue or organ function lost due to disease, damage, or other dysfunction. In the case of diabetes, regenerative medicine approaches usually focus on restoring or replacing the function of β cells. Many of these approaches capitalize on what is known as "cell plasticity," or the ability of one cell type to convert into another cell type with different functions. Some of these approaches include: stimulating stem cells in the laboratory to become insulin-producing cells for β cell replacement transplants, restoring existing β cells' ability to replicate, and reprogramming other pancreatic cell types to take over β cells' functions.

In her talk, Dr. Sander gave a broad review of the diabetes regenerative medicine field and then focused on two approaches that her lab is pursuing: β cell replacement and β cell replication.

INSIGHTS INTO BETA CELL GENETICS

One way to replace β cells lost to disease is by transplanting insulin-producing cells into the body. One challenge for this strategy, however, is procuring the large quantity of transplantable cells needed, since the supply of donated β cells, mostly derived from the pancreases of deceased donors, is limited.

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A promising strategy to overcome this shortage is to make insulin-producing cells in the laboratory from pluripotent stem cells (cells that are able to become any type of cell in the body). For decades, scientists—including those in the Beta Cell Biology Consortium (funded by the NIDDK and Special Statutory Funding Program for Type 1 Diabetes Research)—meticulously identified the factors needed to coax lab-grown stem cells to become insulin-producing, β -like cells. This work has yielded the recent ability to produce large quantities of insulin-producing cells in the laboratory and also resulted in a model to further study what genes control the stem cell plasticity that allows it to become a β cell.

Dr. Sander described how researchers, including herself and her colleagues, are studying these stem cell-derived, insulin-producing cells to identify exactly what genetic and cellular changes occur as they are made. For instance, what genes are “turned on” or “turned off” in cells that are more stem cell-like, compared with cells that are more β cell-like? And what cellular pathways and proteins are affected by those changes? By comparing the genes that are turned off/on in the β cells of people with type 1 and type 2 diabetes to those of people without diabetes, researchers are learning more about diabetes-associated genetic changes in β cells. Dr. Sander expressed hope that studies such as these will shed light on how diabetes occurs and reveal new targets for diabetes therapies.

INSIGHTS INTO IMPROVED BETA CELL MODELS

Insights from the genetic studies highlighted above are also helping scientists build more realistic laboratory models for studying β cells. Insulin-producing cells made from stem cells may have many of the characteristics of β cells, but they are still different in key ways from the β cells found in the human pancreas. These differences may limit how useful these lab-grown cells would be for testing new diabetes therapies, for instance, or for studying how β cells in the pancreas behave. Therefore, there is an ongoing need for better β cell models in the laboratory, especially those that realistically mimic the pancreatic islet, the complex pancreatic structure where β cells and other hormone-producing cells normally are found.

Dr. Sander and her colleagues are working to develop such a model by creating an artificial islet system. Their goal is to model, in the laboratory, the islet's complicated contents and support structure, including not only the β cells and other hormone-producing cells, but also blood vessels, surrounding structural cells, and other components that keep the artificial islet supplied with fluid and nutrients. They have had early success in constructing devices that can support primary (directly from the body) human pancreatic islets, and researchers hope that in the future this system will be useful for studying lab-grown insulin-producing cells, as well.

INSIGHTS INTO BETA CELL REPLICATION

Dr. Sander also discussed another promising regenerative medicine approach to treat diabetes: encouraging functioning β cells to replicate. Other types of cells in the body may constantly replicate to renew themselves, but β cells only seem to replicate under certain circumstances, such as during development or pregnancy. Younger β cells also appear to replicate more than older β cells, for reasons that are not well understood. Overall, much is still unknown about what triggers or prevents β cell replication.

Studying replicating β cells in the body, however, can be very difficult, as they are very rare. In the past, studying β cells required pooling data on many cells, which did not allow researchers to look specifically at replicating β cells. Now, however, cutting-edge technologies have allowed Dr. Sander and her colleagues to study individual β cells.

These single-cell technologies allow researchers to specifically identify and compare replicating β cells and their non-replicating counterparts. Dr. Sander and her colleagues are interested in differences between the RNA in replicating and non-replicating cells. The RNA molecules she studies act as messengers, carrying the cell's instructions for making proteins from the genes to the protein-making machinery, much like an order being sent from a customer's table to a restaurant kitchen. Many proteins are being made every second, and looking at a cell's RNA at a specific moment in time

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can give a photo-like snapshot of what proteins a cell is making and thus what the cell is doing. Dr. Sander and her colleagues have analyzed these RNA snapshots in an effort to identify proteins that control the switch from a replicating to a non-replicating state.

They found significant variety in the RNA found in different β cells, even when those cells were the same age. To make sense of this information, they used computer algorithms to construct a timeline of β cell maturation from least mature (replicating most) to most mature (replicating least). Then, comparing cells at differing levels of maturity, they identified RNAs that corresponded with each state. As expected, some of the RNAs that differed between these two types of cells carried instructions to make proteins involved in replication and insulin secretion. However, they also found that as a cell lost its ability to replicate, it also contained fewer RNAs coding for proteins involved in a metabolic chemical process called oxidative phosphorylation. These findings provided an interesting new clue to β cells' inner workings.

INSIGHTS INTO BETA CELL METABOLISM

Oxidative phosphorylation is part of the process that creates reactive oxygen species (ROS), a type of metabolic byproduct. Dr. Sander's group hypothesized that replicating β cells might be more metabolically active. If this was true, they asked, then could the ROS itself be a trigger for β cell replication? Upon further investigation, they found that this was the case: replicating β cells had higher ROS levels than non-replicating β cells, and inhibiting ROS in mice resulted in those mice having fewer β cells. These results suggested that a β cell's metabolism regulated its ability to replicate. This finding was corroborated by the proteins found in β cells, as well. When the researchers compared snapshots of the proteins found in young, replicating β cells to those found in older, non-replicating β cells in mice, proteins involved in cellular metabolism were reduced in the older cells.

Dr. Sander and other researchers continue to perform targeted experiments to determine what aspects of metabolism are affected by β cell aging. For example,

what specific chemical reactions in the β cells are affected by ROS, and how do they change the β cells' function? And how does aging regulate metabolism, insulin secretion, and blood glucose levels? Answering these questions could be the key to future regenerative medicine approaches. Being able to manipulate β cell replication could lead to, for instance, increased production of β cells for transplantation therapies, or even to being able to induce β cell growth in people with diabetes.

INSIGHTS INTO THE FUTURE OF REGENERATIVE MEDICINE IN DIABETES

Having the ability to replace lost β cells would be a tremendous breakthrough in diabetes treatment. As regenerative medicine studies in diabetes continue, scientists are continuously discovering more about β cell biology and developing new technologies. These advances could lead, in the future, to safe, effective, long-lasting β cell transplantation therapies, or to identification of molecular triggers that could "turn back the clock" and allow older β cells to replicate again.

Future research will also be needed to surmount the challenges these strategies face in the clinic. For instance, therapies that replace or cause existing β cells to replicate may require a way to protect these new insulin-producing cells from the immune response. This will be particularly important in the case of those with type 1 diabetes, which is caused by a misguided autoimmune attack on the β cells that might also attack the new cells. Recent research demonstrating that some β cells can survive the ongoing autoimmune attack in type 1 diabetes offers hope that β cells can be protected from this attack, and research into this area is still ongoing. Solutions to these and other challenges in diabetes regenerative medicine will rely heavily on collaborative research. Researchers from different specialties, such as bioengineering and β cell biology, continue to work together to find ways to overcome these technical hurdles. Success could lead to revolutionary new treatments for diabetes and perhaps, in the future, a cure.

PATIENT PROFILE

Mike: Participating in a Long-term Type 1 Diabetes Research Study To Stay Healthy While Helping Others



Mike

Mike has been physically active his whole life, participating in sports like skiing, racquetball, baseball, and football in high school and college. Now, at age 58, outside of his work in the mortgage industry, he participates in competitive ski racing in his home state of Ohio and in places like New York, Vermont, Colorado, and Canada. “It’s a lot of fun—a lot of exercise,” Mike states. He’s even had success on the race course: “I actually won a pair of racing skis.” He also enjoys doing other outdoor activities like hiking, roller blading, and biking in his home city of Cleveland. “I try to stay as active as possible,” Mike says, “as healthy as possible.”

Contributing to his good health is a long-term, landmark NIDDK-supported research study that Mike has been participating in for the last 35 years: the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) study. Mike, like other DCCT/EDIC participants, has type 1 diabetes. He credits his participation in DCCT/EDIC with helping him learn how to manage his type 1 diabetes on a day-to-day basis while living a full, active life.

A DIAGNOSIS OF TYPE 1 DIABETES

Mike remembers when he first had symptoms of type 1 diabetes. He was 16 years old, and his family was driving from Cleveland to a much-anticipated beach vacation in South Carolina. During the car trip, Mike was very thirsty and, as he recalls, “I was drinking what seemed like a gallon of iced tea, lemonade.... I’m just slugging it down and then within short order having to go to the bathroom.” His father was not too thrilled about having to make so many bathroom stops, and one stop was particularly memorable: “I came out of the bathroom and the family car was gone,” Mike says with a laugh. Turns out that after Mike’s mother and sister got into the car, his father took off without realizing they had left Mike behind. Fortunately, about 10 minutes later, the car pulled up, Mike got in, and the family made their way to the beach.

However, it wasn’t a great trip for Mike. “I just had no energy. There were days when I just sat around and didn’t feel like doing anything,” he recollects. Although he had no family history of type 1 diabetes,

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his mother was a nurse, so she recognized that his symptoms of excessive thirst, fatigue, and weight loss were hallmarks of the disease. He went to a general practitioner back in Cleveland who diagnosed him with type 1 diabetes—an autoimmune disease in which a person’s immune system destroys the cells that make the hormone insulin, which promotes the transport of sugar (glucose) from the bloodstream into the body’s cells where it is used as fuel. Over time, people with the disease can develop severe and often life-threatening complications, like eye, kidney, nerve, and heart disease.

Mike remembers when he first had symptoms of type 1 diabetes... “I just had no energy. There were days when I just sat around and didn’t feel like doing anything,” he recollects.

Mike didn’t get much information about how to manage his type 1 diabetes when he was first diagnosed because of the lack of tools and knowledge available in the mid- to late-1970s. Back then, there was debate about whether keeping blood sugar levels as low as safely possible would prevent the long-term disease complications. The debate didn’t matter much from a practical standpoint, though, because there were no available tools to help people achieve near-normal blood sugar levels—for example, blood sugar monitoring did not yet exist. Instead, people like Mike monitored their body’s sugar levels with urine tests, which had limited utility: they recognized high but not dangerously low sugar levels and reflected past, not current, sugar levels. Thus, at first, Mike was just given advice about managing his diabetes with diet and exercise, being told to watch what he ate and avoid foods with sugar. Then he started taking one or two shots of insulin a day.

That management regimen did not work well for him. Without a way to monitor his blood sugar levels, judging his body’s needs could be difficult. He recalls a bad experience at a high school marching band summer camp, where the extra exercise made it

extremely challenging for him to manage his diabetes: “I must have lost 10 to 15 pounds in that week from physical activity,” he recalls. On top of the physical challenges, Mike also remembers feeling different from his friends because of his type 1 diabetes. For instance, because of the required medical forms, he had to delay getting his driver’s license, when his other driving-age friends were getting theirs. At that time, Mike didn’t know that things would eventually change for the better because of a new research study.

JOINING THE DCCT

Mike calls himself fortunate: “One of my family members worked with one of the doctors, Dr. Saul Genuth, who was the lead research doctor for the DCCT in Cleveland. We were at a family event [and I found] out that this doctor may be willing to see me.” With that, Mike was introduced to the DCCT and ultimately became one of its 1,441 volunteer participants.

The DCCT began in 1983 to address the debate about the importance of controlling blood sugar levels to prevent long-term complications in people with type 1 diabetes. Importantly, in the late 1970s and early 1980s, there was significant progress in developing new tools and tests needed for blood sugar control—such as meters for self-monitoring of blood sugar and insulin pumps—enabling a trial like the DCCT to be conducted.

Specifically, the DCCT compared the effects of “intensive” versus conventional treatment of blood sugar levels on the development of eye, kidney, and nerve disease. Participants in the intensive treatment group followed a regimen that included self-monitoring of blood sugar at least four times per day and at least three insulin injections per day or use of an insulin pump. The goal was to keep their blood sugar levels and hemoglobin A1c levels (HbA1c, a measure of average blood sugar levels over the previous 3 months) as close to normal as safely possible. Conventional treatment—which was the standard treatment at the time and similar to the regimen that Mike was using before he joined the DCCT—consisted of one or two insulin injections per day, with once-a-day urine or blood sugar testing.

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When asked if it was a hard decision for him to enroll in the trial, Mike responds, “No, it wasn’t really hard because [of] the way I was feeling physically and knowing that things weren’t the way they should be.” Not knowing at that time whether one or the other treatment would have better outcomes, he also didn’t have much of a reaction when he found out that he was assigned—by chance—to the intensive treatment arm of the trial. “It was like, you’re either in Group A or Group B,” he says, meaning that neither group had much significance to him at the time.

Mike describes some of his early memories of being involved in the trial. Because he was in the intensive treatment arm, he says that the study “was strict on multiple testing and multiple injections, and constant follow-up.” As part of that follow-up, people in the intensive treatment arm visited the study center each month and were contacted even more often by phone to go over and adjust their treatment regimens. Mike remembers the DCCT study team being very accommodating of his busy schedule and making him feel like he was a partner in the research. “There were times I was on the phone with them in the evening,” he says. “They were great on just engaging and making you feel that you were part of things.”

Because the DCCT was determining if intensive blood sugar control could prevent the development of complications, Mike says that the researchers took many measurements at the start of the study so that they could then detect changes over time: “[They] were checking all kinds of things, from nerves to kidney function, eyesight...you name it and they were testing it.”

Mike states that even with implementing the more intensive treatment approach as part of the DCCT, his overall health didn’t improve immediately—it took him a year or two to feel like his diabetes was under good control. He explains that part of that was dealing with the mental aspect of having a chronic disease. “It’s not easy to say I’ve got this permanent [disease]—it’s never going to go away... and to be separate from the rest of the crowd.” For example, he said it was difficult being told around the time of his type 1 diabetes diagnosis to avoid

foods with sugar, even though his peers had no such restrictions, so he would sometimes sneak a candy bar. “I’m thinking I’m fooling everybody,” he says, “and it was kind of satisfying, but then my blood sugar is up and I’m feeling lethargic.”

While in the trial, Mike experienced acute episodes of hypoglycemia, or dangerously low blood sugar, which is a limitation of implementing intensive insulin therapy. He recalls times when he would take insulin and then decide to do an outdoor activity like skiing or sled riding, which would lower his blood sugar levels even more and sometimes cause them to go too low. He depended on his friends to recognize the signs of hypoglycemia (e.g., being confused, dizzy, jittery), and give him a sugary drink to bring his blood sugar levels back up; sometimes he had to manage the hypoglycemia on his own. “That was the difficult part,” he states.

Why does Mike continue to participate in the DCCT/EDIC research study after all these years?
“Honestly,” he says, “what motivates me the most is the avoidance of [diabetes] complications.”

DCCT RESULTS

The DCCT ended after 10 years in 1993—a year earlier than planned—when the study proved that participants in the intensive treatment arm, like Mike, who kept their blood sugar levels close to normal, greatly lowered their chances of having eye, kidney, and nerve disease compared to people in the conventional treatment arm. These landmark findings changed the way type 1 diabetes is treated worldwide, making intensive blood sugar control early in the course of the disease the standard treatment.

For Mike personally, the importance of these impressive results didn’t really hit him until later in life. At the time, he was happy that his tests through the DCCT indicated that he didn’t have complications, but it didn’t change the fact that he was still living with a chronic disease. “It’s the

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look-back part that puts it in perspective,” he explains. Now with the wisdom of hindsight, he recalls the time before he joined the trial when his diabetes wasn’t in good control, like when he was in marching band camp, and sees how things could have gone quite differently. He now realizes that if he hadn’t started implementing intensive glucose control, “I could have been one of those people that started to have complications,” he states.

CONTINUING IN THE EDIC STUDY

When the DCCT ended, participants who had received conventional treatment were taught the intensive treatment regimen, and all were encouraged to use it. Nearly every DCCT participant, including Mike, volunteered for the follow-on EDIC study, which began in 1994 and is ongoing. EDIC was established to determine the long-term outcomes of reducing exposure of the body’s tissues and organs to high blood sugar levels. EDIC is an observational study, so participants independently see their own health care team and participate in annual follow-up visits with EDIC.

The annual follow-up visits allow EDIC investigators to collect information about Mike’s health. He explains that each year, the research team may do different measurements: “[One year], it’s testing kidney function.... The next year it may be nerve testing.” He says that sometimes the tests could take a lot of time and not be too much fun. What makes it easier, though, he says, is that these important tests continue to indicate that he is free of complications.

Because of Mike and the other dedicated participants, there continues to be a wealth of important information emanating from EDIC. EDIC has shown that, compared to people formerly in the DCCT’s conventional treatment arm, people in the former intensive treatment arm have a reduced risk of cardiovascular disease (such as heart attack and stroke), eye disease and related eye surgery, kidney disease and kidney failure, and nerve disease. They also have been living longer. These findings underscore current clinical practice guidelines recommending that people with type 1 diabetes

practice early and intensive blood sugar control to improve their long-term health.

In addition to helping Mike stave off diabetes complications, his continuing participation in EDIC had an unexpected benefit related to finding a major health problem that likely resulted from a bacterial infection he had as a teenager before he developed type 1 diabetes. About 6 or 7 years ago, both the DCCT/EDIC study team and his personal endocrinologist “were hearing stuff within my heart and telling me to go to a cardiologist, and I ignored them for a couple of years,” Mike admits. Then, at one of his annual EDIC visits, the study team told him that the noise was getting worse. As a New Year’s resolution, he went to the cardiologist who broke the bad news: Mike had a damaged aortic valve. He was quickly scheduled for open heart surgery to replace it. “He [the cardiologist] said if I had waited probably 6 more months, I’d be having emergency surgery,” Mike recalls. The doctor told him that the damaged valve was probably caused by the bacterial infection he had as a teenager, and thus was unrelated to his diabetes. “So, I’m a survivor today of that [open heart surgery] and the longevity of diabetes,” he states.

When asked what it means to him to be part of a study that has changed so many lives for the better, Mike replies: “I feel good about it.... I want to continue because if there is anything more that can be learned or gained, I want to be able to be part of that, whether that be as a collective group or individually.”

Mike feels that so many years of intensively managing his type 1 diabetes during DCCT/EDIC, as well as leading a healthy, active lifestyle, helped him get through the surgery. “I actually skied 6 months after surgery,” he exclaims. A month after that, “My son and I drove out to the Grand Canyon and we hiked it. I was kind of proud of that.” The experience again made him realize what the DCCT has meant to his long-term health—he feels that if he had not started intensive control, his health

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today could be much worse, especially now knowing that he had underlying heart damage. Instead, he says, “I have more energy now than ever.”

PARTICIPATING IN A LONG-TERM RESEARCH STUDY

It’s been 35 years since the DCCT began in 1983, and over 90 percent of living DCCT/EDIC participants, including Mike, are still enrolled in the study. Why does Mike continue to participate in the DCCT/EDIC research study after all these years? “Honestly,” he says, “what motivates me the most is the avoidance of complications. [Losing] my eyesight is the number one thing that I worry about.” Thus, it’s a huge relief every time he gets a good bill of health from his eye tests done through EDIC.

“I’d encourage people that if they have a chance to do something research-wise to do it.”

Mike says that one of the biggest benefits of being in DCCT/EDIC was learning how to manage his type 1 diabetes daily. Now, instead of sneaking candy bars like he did as a teenager, he eats and does the activities that he wants, but knows he must be vigilant about checking his blood sugar levels. In other words, “I know how to adjust for some of the more day-to-day-stuff. I didn’t know that at the beginning [of the DCCT]. I have a better sense of it now—it’s only taken 30 years to figure it out,” he says with a laugh. Today, he continues to manage his type 1 diabetes with frequent blood sugar testing and insulin administration that he learned as part of the DCCT and has been able to keep his HbA1c levels below recommended levels, which has been shown by DCCT/EDIC to reduce his risk of long-term complications.

Mike’s personal experiences illustrate another important finding from DCCT/EDIC: overall, participants in the former intensive treatment arm have maintained a lower risk of complications for 25 years, even though after DCCT ended their blood sugar control gradually became indistinguishable from

that of the participants in the former conventional treatment arm. (After the DCCT ended, blood sugar control in participants from the former intensive treatment group was not as good as it was during the trial, when they had the advantages of the clinical trial setting—although they still had better control than before the trial. At the same time, those in the former conventional treatment group improved their blood sugar control when they began implementing more intensive therapy after the DCCT.) This long-term benefit of a period of intensive blood sugar control has been termed “metabolic memory.” These findings emphasize the importance of implementing intensive blood sugar control from the earliest stages of diabetes. They have also spurred NIDDK-supported research to develop new tools and technologies to help people with type 1 diabetes achieve recommended levels of blood sugar control, like artificial pancreas technologies that aim to automate insulin delivery in response to blood sugar levels. Mike is fortunate to have enrolled in the DCCT/EDIC when he did—at a young age when he could learn about adjusting for “some of the more day-to-day stuff,” as he says, and incorporate intensive blood sugar control as routine practice.

When asked what it means to him to be part of a study that has changed so many lives for the better, Mike replies: “I feel good about it.... I want to continue because if there is anything more that can be learned or gained, I want to be able to be part of that, whether that be as a collective group or individually.” He also has a message for others: “I’d encourage people that if they have a chance to do something research-wise to do it.”

Finally, Mike has advice for people facing a recent type 1 diabetes diagnosis: “Don’t give up and put aside what it is that you like.... You can manage it [the disease] and manage to continue to live the way you want to.” For Mike, that means continuing to participate in ski racing and other outdoor activities. At the same time, he and the other DCCT/EDIC participants can take pride in knowing that their remarkable and continuing dedication to research is a key reason why people with type 1 diabetes are living longer, healthier lives than ever before.

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Angela: RISE-ing Above Diabetes



Angela

In 2015, Angela had just obtained health insurance, so she saw her doctor for an overdue physical examination. A massage therapist in her fifties who sees her clients at their homes and businesses, she is dependent on her health to be able to carry her equipment to appointments and stay on her feet to work. In general, her health was good, but her health care provider did identify one area of concern: elevated blood sugar (glucose) levels—termed prediabetes—that suggested she was at risk to develop type 2 diabetes. “When I was diagnosed as prediabetic, that made me very nervous,” says Angela, who was aware that her family history of type 2 diabetes also increased her risk of developing the disease.

A DISEASE THAT RUNS IN THE FAMILY

Born in Trinidad to a family of South Asian descent, Angela immigrated to the United States as a child and grew up in Miami with five of her siblings—three

sisters and two brothers. (Another sister moved from Trinidad to England.) Angela knew that her mother, who recently passed away, had type 2 diabetes for the last 2 decades of her life. The family history runs deeper, however. “My father died at 75,” Angela relates, and she and her siblings “didn’t know this [at the time]—but he also had diabetes.” The complications of the disease had taken their toll: her father developed kidney failure and was on dialysis for 10 years, before dying from cardiovascular complications of the disease. In fact, the family’s history “stems way back,” Angela says, as her mother’s father had the disease as well.

Two in her generation—Angela and one of her sisters—have developed prediabetes. Angela is concerned about her and her siblings’ health, but also is thinking of the next generation. “So far, none of my [three] kids have” developed diabetes or prediabetes, Angela notes, gratefully. “Thank you, God, for that.” But Angela isn’t taking her future health—or theirs—for granted. She agreed to participate in a clinical trial, she says, “for my years of life and my children’s, and the generations to come!”

DIABETES AND PREDIABETES

When we eat, sugars and other nutrients enter our bodies through the digestive process. The pancreas responds by producing the hormone insulin, which signals cells throughout the body to absorb sugar. However, while the body needs blood sugar levels to remain above a certain level to maintain brain and other functions, higher levels lead to organ damage. Thus, a healthy pancreas helps keep blood sugar levels within a narrow, optimal range.

Unfortunately, some people develop a condition called insulin resistance, in which their cells no longer respond as strongly to insulin. Aging and excess weight—as well as genetics and other

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factors—can increase risk for insulin resistance. At first, the pancreas compensates for this by simply producing more insulin, but gradually it may lose the ability to do so. At that point, blood sugar levels begin to rise, often reaching levels in excess of the diagnostic threshold for type 2 diabetes. Although this form of diabetes can occur in anyone, people of African, Hispanic, Native American, or Asian descent (like Angela) are at particularly high risk.

THE RESTORING INSULIN SECRETION CLINICAL STUDIES

In 2013, the NIDDK-supported Restoring Insulin Secretion (RISE) consortium formed to conduct three related clinical studies. Each stems from an important observation: previous research suggesting that lowering blood glucose levels with certain diabetes drugs during prediabetes or early in the course of type 2 diabetes may slow or perhaps even reverse the loss of pancreatic function. Although it is not clear why this works, possibilities include that such treatments may limit the exposure of the pancreas to damage from chronically high levels of sugar and other nutrients, or that they may simply relieve the burden on a person's pancreas.

The RISE studies, two in adults, and one in youth, were therefore designed to test the potential for different methods to improve and preserve the production of insulin in people with prediabetes or recently diagnosed type 2 diabetes. “I hope that is accomplished,” Angela says fervently. Fortuitously, her prediabetes diagnosis coincided with a timely offer to participate in one of the three RISE clinical trials. “I was very fortunate that RISE sent me a letter in the mail,” Angela relates, “and I said well, you know, there’s no harm in trying ... to see if they can do something about it.” She was quick to call and was soon enrolled in a RISE study. As she says, “I am very happy to participate in the RISE study ... to help [people with or at-risk for diabetes] maintain function [and] a healthy life.”

PARTICIPATING IN THE RISE ADULT MEDICATION STUDY

The RISE clinical trial Angela is participating in—the RISE Adult Medication Study—is comparing the capability of various blood glucose-lowering medication regimens to preserve or restore the ability of the pancreas to secrete insulin. The four approaches being compared are: 1) early intensive insulin treatment with a long-acting form of insulin called “glargine,” followed by the first-line type 2 diabetes drug, metformin; 2) an injected diabetes medication called liraglutide delivered along with metformin; 3) metformin by itself; and 4) placebo. Each participant was randomly assigned to receive one of the four treatment approaches for 12 months. Just before starting treatment, as well as periodically during and afterward, the participants received a variety of tests to ascertain how well their bodies responded to insulin, and to test the ability of their bodies to produce and release their own insulin in response to glucose in a drink or delivered intravenously. The participants also received cognitive testing to determine whether any of the treatments had beneficial effects on mental function.

Angela agreed to participate in a clinical trial, she says, “for my years of life and my children’s, and the generations to come!”

In addition, the RISE studies have enabled an important substudy having to do with obstructive sleep apnea (OSA)—pauses of breathing due to closing of the airway during sleep due to relaxation of the muscles in the throat. OSA can cause excessive daytime sleepiness, and severe cases are associated with high blood pressure, a risk factor for cardiovascular disease. There is now evidence that OSA may increase the risks of type 2 diabetes, and vice versa. To improve our understanding of the relationship between apnea and diabetes, researchers enrolled a subset of RISE participants—

PATIENT PROFILE

including Angela—in a RISE sleep substudy. In this way, Angela learned that she has a mild case of OSA, primarily when she slept on her back. As a result, she now makes an effort to ensure that she sleeps on her side.

Angela was randomized to receive either metformin or placebo. Although she didn't know which she was taking, she didn't mind. "You know I just kind of left it up to them.... Sometimes I thought I was taking placebo, and other times I'm like, maybe I *am* taking metformin." In any case, she was pleased with the result. As she says, "maybe my mind was just playing games with me, but I lost 10 pounds during the whole treatment."

And that wasn't the only benefit of participating in the trial: Angela says the study also helped her track her own health. "They were very efficient at giving me a list of all my test results," noting they also sent the results to her doctor. "They kept me informed of what's going on, and ... I felt good about that." This meant that if a major health issue had arisen, whether or not it was related to diabetes, it might have been caught early. For example, Angela recalls, "At one point they said I had a little bit low blood count ... and I spoke to the doctor about it." Fortunately, it turned out to be nothing serious.

Angela has high praise for RISE study and its staff: "The bunch of people that I came in contact with, you know, I love them, and I would love to continue to be a participant, because I feel I could trust them. They showed me a great amount of kindness, and it's very supportive as well."

Angela speaks glowingly of her experience in the trial, especially of the staff with whom she worked. "It's just amazing the amount of testing they were doing, and they were very safe—I felt safe.... Everybody was friendly—super friendly—and I felt like I can trust them."

Regarding the metabolic testing, she notes, "I was totally feeling good when they were doing all that." She has more mixed feelings about the cognitive testing: "Every time I went in and did a cognitive test ... that was hard, but it was a great challenge!"

"They were very efficient at giving me a list of all my test results," she said, noting they also sent the results to her doctor. "They kept me informed of what's going on, and... I felt good about that."

TAKING CHARGE OF HER HEALTH

After she was diagnosed with prediabetes, Angela worked on improving her diet and exercising more. She joined a gym, which she confesses she doesn't like, but she enjoys going out walking. She even participated in a 3-day, 60-mile walk for breast cancer research and prevention, which not only reaped rewards for the breast cancer community but also personal ones for Angela. "I forced myself to go to the gym and get in shape for it, and it was awesome! I got to meet a lot of ladies that had had breast cancer and victory over it, and there were some that were ... [still] in the midst of it all. And I heard a lot of testimonies, which was awesome.... It was fun, too!"

Angela credits participating in the RISE Adult Medication Study with helping change her outlook. "It has definitely shown me the importance of taking care of yourself," she shares. She also has high praise for RISE and its staff: "The bunch of people that I came in contact with ... I love them, and I would love to continue to be a participant, because I feel I could trust them. They showed me a great amount of kindness, and it's very supportive as well.... A lot of care and love," she says, was put into the RISE study.

Of the RISE staff who worked with her, Angela continues, "they're wonderful. And I wouldn't mind

PATIENT PROFILE

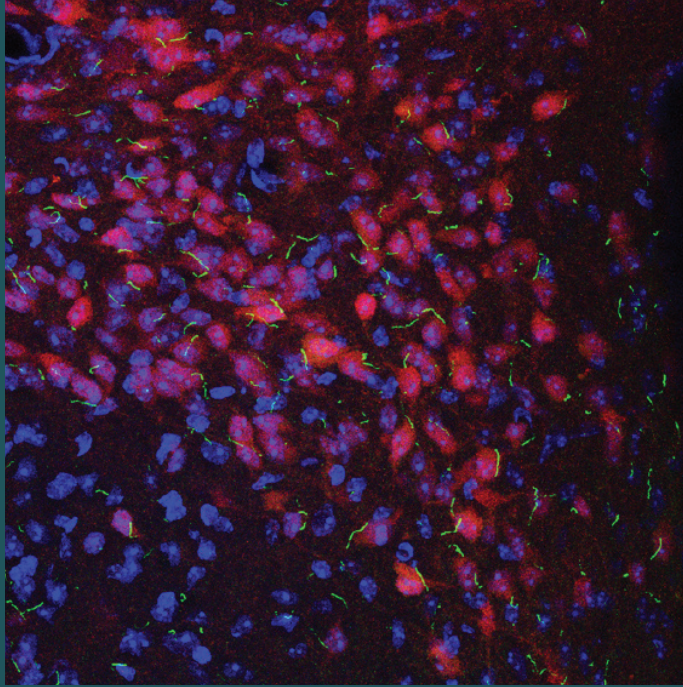
doing another study again so I can see them again, and just be willing to do something for the good of others. Or even for myself to benefit from it.” She adds that “I’ve learned more, come to appreciate my health more. I’m realizing that the more you know, the more you take care of yourself!”

“I thought that the RISE Study is a great study,” Angela says, and she adds “they are doing a tremendous good.”

At the end of the intervention period of the trial, the staff went over Angela’s test results with her, giving her and her health care provider information to help keep Angela as healthy as possible. “They said I’m

doing really good.... They said my results turned out to be really good, and they also gave me a rundown on my good cholesterol and my bad cholesterol, and they said that it’s normal,” although “the bad is a little bit high.” In view of this, they reminded her of the importance of keeping a healthy diet.

Angela can’t say enough good things about her experience with RISE. “I thought that the RISE Study is a great study, and ... they are doing a tremendous good,” she says. She notes that she is looking forward to finding out about results, as well: “When everybody that has participated [has] completed the study, they’re going to have a big party, and then all the participants will get the inside scoop: the results of what we were taking, and so forth, and it’s going to be exciting!”



Obesity is a multifaceted condition that involves the interplay of diet, genetics, and many other factors, even the microbiome. In a recent study in mice, highlighted in this chapter, researchers found that small, antenna-like projections called primary cilia (shown here in green) on brain cells (shown here in purple) have a critical role in a known “hunger circuit,” which receives signals from the body to control appetite. Changes in these antenna-like structures could produce a “short circuit” leading to overeating. The researchers provide evidence showing that two proteins work together specifically in primary cilia of brain cells to regulate appetite and body weight and provide new insight into the role of primary cilia in obesity. This recent discovery could lead to new approaches to treating and preventing obesity.

Image courtesy of Christian Vaisse, M.D., Ph.D., University of California San Francisco.

Obesity

Obesity has risen to epidemic levels in the United States. Individuals who have obesity may suffer devastating health problems, face reduced life expectancy, and experience stigma and discrimination. Obesity is a strong risk factor for type 2 diabetes, fatty liver disease, and many other diseases and disorders within the NIDDK's mission. Nearly 40 percent of U.S. adults are considered to have obesity based on body mass index (BMI), a measure of weight relative to height.¹ More than 18 percent of children and adolescents also have obesity, and thus are at increased risk for developing serious diseases both during their youth and later in adulthood.^{1,2} Obesity disproportionately affects people from certain racial and ethnic groups and those who are socioeconomically disadvantaged.

The high prevalence of obesity in the United States is thought to result from the interaction of genetic susceptibility with behaviors and factors in the environment that promote increased caloric intake and sedentary lifestyles. Diet, activity, and aspects of our environment may also modify biologic factors in ways that promote obesity. Research is providing the foundation for actions to address this major public health problem by illuminating the causes and consequences of obesity, evaluating potential prevention and treatment strategies, and providing an evidence base to inform policy decisions.

The NIDDK supports a multi-dimensional research portfolio on obesity, spanning basic, clinical, and translational research. NIDDK-funded studies investigate a variety of approaches for preventing and treating obesity. These span behavioral and environmental interventions in families and in health care and other settings, using a variety of approaches and technologies; surgical interventions; and combinations of strategies. In parallel, Institute-supported investigations into the biologic processes associated with body weight have continued to spark new ideas for intervention approaches. To help bring research results to those affected by obesity and their families, health professionals, and the general public, the Institute sponsors health information programs.³

The NIDDK also continues to play a leading role in the NIH Obesity Research Task Force. The NIDDK Director co-chairs the Task Force along with the

Directors of the National Heart, Lung, and Blood Institute and the Eunice Kennedy Shriver National Institute of Child Health and Human Development. The Task Force includes representatives from these and numerous other NIH Institutes, Centers, and Offices.

Highlights of recent advances from NIDDK-supported research on obesity are provided in this chapter.

THE BRAIN'S REGULATION OF APPETITE, ENERGY BALANCE, AND OBESITY

"Sensing" a Genetic Predisposition to Obesity: New research suggests a mechanism by which antenna-like sensory projections, called primary cilia, located on brain cells play a role in the genetic predisposition to and development of obesity. Primary cilia are found on many types of cells, and defects in these structures contribute to a wide range of human diseases collectively called ciliopathies. For reasons that have

¹ Hales CM, et al. 2017. CDC. National Center for Health Statistics Data Brief No. 288.

² For children and adolescents, obesity refers to a BMI at or greater than the 95th percentile on growth charts (which are based on previous national surveys).

³ NIDDK Weight-control Information Network, <http://win.niddk.nih.gov/index.htm>

remained unclear, certain ciliopathies typically result in obesity. Mutations in some genes that produce proteins located in primary cilia cause obesity in both mice and humans, and recent studies have suggested a role in human obesity for the protein ADCY3, which is specifically found in primary cilia of brain cells.

In this study, scientists investigated whether another protein, MC4R, which is found on the surface of brain cells and transmits appetite-regulating information, could be involved in a primary cilia signaling pathway. Several known mutations in the gene encoding MC4R cause severe obesity in people; similarly, in mice, deletion of the gene that produces MC4R induces severe obesity. For the first time, the researchers were able to visualize the precise cellular location of MC4R in the brains of male and female mice by using a fluorescent tag along with imaging technology. The scientists discovered that, in a group of brain cells whose job it is to communicate information about appetite to the rest of the brain, MC4R is located in close proximity to ADCY3 on the primary cilia. Furthermore, when they introduced a known human obesity-associated mutation into the gene encoding MC4R in mice, MC4R no longer traveled to the primary cilia with ADCY3, suggesting that the mutation may cause obesity by disrupting the protein's localization to cilia. To determine if MC4R and ADCY3 work together at primary cilia to regulate body weight, the researchers blocked the function of ADCY3 specifically in MC4R-containing brain cells in mice. The mice increased their food intake and gained significantly more weight than mice with functional ADCY3.

Taken together, these results suggest that ADCY3 and MC4R work together specifically in primary cilia of brain cells to regulate appetite and body weight, and provide new insight into the role of primary cilia in obesity. Further research could determine if additional proteins have a role in the brain's primary cilia and may suggest new approaches to treating or preventing obesity.

Siljee JE, Wang Y, Bernard AA,...Vaisse C. Subcellular localization of MC4R with ADCY3 at neuronal primary cilia underlies a common pathway for genetic predisposition to obesity. *Nat Genet* 50: 180-185, doi: 10.1038/s41588-017-0020-9, 2018.

Your Brain on Food—How Genetic Variants Increase Obesity Risk Through Impaired Brain Signaling and Augmented Appetite: In the largest study of its kind to date, researchers used imaging technology to show that certain DNA sequence variants in a gene called *FTO* (fat mass and obesity-associated) raise obesity risk in humans by disrupting brain processing, leading to a weakened sense of fullness and subsequent overeating. It is well-known that DNA variants in a particular region of the *FTO* gene increase obesity risk, and people with “high-risk” variants prefer high-fat foods and tend to eat more. However, a direct link had not been established between these genetic variants and how the brain processes rewarding aspects of food and feeling full after a meal.

The team of scientists recruited both men and women with high-risk and lower-risk variants of the *FTO* gene. While the participants had a range of body mass indexes (a measure of weight relative to height), the majority had obesity. The investigators compared subjective reporting of appetite between the groups with high-risk and lower-risk *FTO* gene variants, in addition to comparing appetite-regulating hormones, food intake, and brain response to visual food cues before and after a meal. They found that the group with the high-risk *FTO* gene variant consistently reported feeling less full than their counterparts with lower-risk *FTO* variants. Compared with the lower-risk group, high-risk individuals rated calorically dense, “fattening” foods as more appealing. Moreover, when presented with a buffet meal, those with the high-risk *FTO* variant consumed approximately 350 more calories compared to individuals with a lower-risk genetic variant. Using brain imaging technology, the researchers found that the high-risk group experienced greater activation in certain brain regions with known roles in food reward and food motivation when they were presented with images of fattening foods both before and after a meal; this activation was directly linked to greater food intake. In contrast, participants in the lower-risk group experienced greater brain activation when presented with images of low-calorie food, especially after a meal. Blood sample analysis provided no evidence that appetite-regulating hormones were responsible for differences in brain processing.

Taken together, these findings point toward a brain processing mechanism associated with certain *FTO* gene variants driving decreased satisfaction after a meal, which can lead to overconsumption of food in individuals with these high-risk genetic variants. However, the researchers point out that it is also possible that genes other than *FTO* that predispose for obesity act upon the same brain pathways and contribute toward the overall effect. In addition, it is possible that individuals' dietary habits prior to this study influenced results, based on prior research showing that access to a high-fat diet in rodents increases activity of *FTO* in certain brain regions. Additional studies could provide greater insight into the role of *FTO* genetics, brain processing, and obesity.

Melhorn SJ, Askren MK, Chung WK,...Schur EA. *FTO* genotype impacts food intake and corticolimbic activation. *Am J Clin Nutr* 107: 145-154, doi: 10.1093/ajcn/nqx029, 2018.

From Body to Brain and Back Again—How the Hormone Leptin Utilizes Brain Cell Circuits To Regulate Appetite, Calorie Burning, and Glucose Levels: Scientists have used a new genetic tool in mice to map out the cellular brain circuits used by the hormone leptin to control energy balance (calories consumed versus calories burned) and blood glucose (sugar) levels. Leptin, which is produced in fat tissue, acts in the brain to regulate food intake, promote calorie-burning, and control blood glucose levels following a meal. Leptin deficiency or dysfunction of leptin receptors (molecules on the cell surface with which leptin interacts) results in severe obesity and diabetes in humans. It has been widely suggested that certain types of brain cells called “AGRP” and “POMC” cells, which contain leptin receptors, are essential to carrying out leptin's effects. However, selectively deleting leptin receptors in these cells in mice using traditional genetic manipulation methods has failed to reproduce the obesity and diabetes seen in mice completely lacking leptin receptors, suggesting that other cells may play a key role.

To identify leptin's primary target, researchers used a chemical agent to induce diabetes in male mice, which subsequently leads to leptin deficiency. They then determined the brain region primarily affected by the loss of leptin by measuring brain activity: during leptin deficiency,

one brain region became intensely active, and when they administered leptin, the activity subsided. This finding led the researchers to investigate if this area contained AGRP cells because it is known that leptin suppresses AGRP cell activity. After administering an agent that selectively suppresses AGRP cell activity in diabetic, leptin-deficient mice, the mice experienced significantly reduced blood glucose levels, indicating that AGRP cells play a primary role in leptin responsiveness and blood glucose control. But, why had previous studies deleting leptin receptors in AGRP cells failed to induce diabetes in mice? The answer could lie in the limitations of older, traditional methods.

To explore this further, the researchers used a powerful, new genetic engineering tool—one that had not been used in the previous studies—to selectively delete leptin receptors in AGRP cells of non-diabetic male and female mice. This deletion induced severe obesity and diabetes, increased food intake, and reduced calorie burning. These results suggest that leptin is acting through its receptors on the surface of AGRP cells to induce widespread metabolic consequences. Interestingly, when they performed a similar experiment by selectively deleting leptin receptors in POMC cells, they observed no effect on body weight or blood glucose, indicating that leptin acts primarily on AGRP cells to maintain blood glucose control and prevent weight gain and diabetes. They further investigated the mechanism by which leptin suppresses activity of AGRP cells and found that by acting through its receptor, leptin modifies the function of nearby proteins on the surface of AGRP cells to reduce the cells' activity and modulate communication between cells.

Taken together, these findings identify the critical elements of a brain cell circuit through which leptin governs energy balance and blood glucose levels. Importantly, this study shines a light on the disparity in experimental results that can occur when different gene-altering methodologies are used and suggests a critical need to reexamine previous conclusions drawn from past studies.

Xu J, Bartolome CL, Low CS,...Kong D. Genetic identification of leptin neural circuits in energy and glucose homeostases. *Nature* 556: 505-509, doi: 10.1038/s41586-018-0049-7, 2018.

NEW INSIGHTS INTO THE MOLECULAR REGULATION OF BODY WEIGHT

Genes Newly Associated with Body Weight:

Scientists' recent analysis of hundreds of thousands of human genomes has identified new links between certain gene sequence variations and body mass index (BMI), a measure of weight relative to height. Obesity is a complex condition that can be caused by multiple factors, including a person's genes. Identifying the genes (and the specific natural variations in those genes) that contribute to body weight regulation can provide new information on what causes obesity and how best to treat it. Genome-wide association studies (GWAS) have been used to rapidly scan the DNA of large groups of study participants, with the aim of finding genetic variations linked to differences in BMI. Previously, analyses of common variations identified through GWAS have led to identification of some of the mechanisms involved in regulating body weight. It has been challenging, though, to identify what specific genetic variants govern this regulation, as identified variants were sometimes outside the "exome"—regions of the genome that code for proteins—making it unclear how those variants affect cell function. Further, most GWAS to date have identified fairly common variations, and less common variations may also shed light on the biology of obesity.

To identify more BMI-specific genes and gene variants, researchers scanned only the exomes of over 700,000 individuals who participated in 125 clinical studies, looking for uncommon or rare gene variants that correlated with higher or lower BMI. The study identified 14 uncommon gene variants in 13 genes, with one of the variants only being associated with BMI in females. Eight of these genes had never before been implicated in human obesity. Further investigation of these variants showed that the four that were least common affected BMI on average about 10 times more strongly than more common, previously identified obesity-related variants. Researchers found that many of these newly BMI-associated rare gene variants coded for proteins that are enriched in the brain, and further research is needed to fully understand their role in body weight. None of the identified variants contributed significantly to BMI variation in the overall population, but they may substantially affect the weight of individual

people. Overall, these findings provide new insight into the causes of obesity, which could lead to new therapeutic targets and more personalized obesity treatments.

Turcot V, Lu Y, Highland HM,...Loos RJF. Protein-altering variants associated with body mass index implicate pathways that control energy intake and expenditure in obesity. Nat Genet 50: 26-41, doi: 10.1038/s41588-017-0011-x, 2018.

Examining the Effects of Weight Gain and Loss—

Multiple Molecules at a Time: In a controlled study of weight gain and loss, researchers have assembled a comprehensive molecular profile of dramatic changes that occur in humans during short periods of weight fluctuation. By using a variety of different analytical methods on a large scale, they collected more than 2 million measurements that provide a window into the dynamic nature of the molecular, metabolic, and gut bacterial changes in study participants during weight gain and loss.

The team of scientists selected 23 men and women who volunteered for the study and were overweight or had moderate obesity. While none of the participants had diabetes, they differed in their levels of insulin sensitivity or insulin resistance. People who are insulin-resistant (IR) require greater amounts of insulin, a hormone made by the pancreas, to maintain blood glucose (sugar) levels than people who are insulin-sensitive (IS), and they are more likely to develop type 2 diabetes and associated complications later in life. Therefore, the researchers performed a weight gain/loss intervention on weight-matched individuals with different insulin-sensitivity profiles to identify specific molecules and signaling pathways that could be involved in weight-related insulin resistance. At the study's onset, the investigators analyzed participants' biological samples (blood and stool) and found that differences in protein levels and gut microbial populations already existed between IR and IS individuals. For example, IR individuals exhibited molecular markers for inflammation in their blood. Because people with type 2 diabetes are known to have inflammation, this could potentially be an early warning sign for future disease. All participants then went on a controlled, high-calorie diet for 30 days, during which time each individual consumed approximately 880 excess calories daily and gained an average of 6 pounds. With just this modest amount of weight gain, molecular markers for fat metabolism, inflammation, and heart disease

increased in both IR and IS participants, potentially providing a biological explanation for the link between weight gain and heart failure. In addition, the researchers observed a dramatic increase in numbers of a type of gut bacteria known to protect against insulin resistance in response to weight gain in animal models, but this increase only occurred in IS individuals. The weight gain period was followed by a 60-day period of calorie restriction designed for participants to return to their original weight. Notably, most biomolecules, pathways, and microbes that were disrupted by weight gain returned to normal levels with weight loss, suggesting that deleterious effects of short-term, modest weight gain can be mitigated with dietary intervention. Some effects, however, continued post-weight loss, indicating some longer-lasting consequences of even a brief period carrying extra pounds.

Taken together, these results provide a dynamic picture of the human body's molecular response to weight fluctuation and illustrate how even short-term periods of modest weight gain can affect metabolism, the gut microbiome, and heart health. Moreover, this research highlights the fact that individuals are unique at the molecular level and emphasizes the need for personalized analysis in medicine. Further studies with the publicly available data generated by this study could lead to personalized, predictive molecular signatures for type 2 diabetes and other weight-related conditions long before a disease manifests.

Piening BD, Zhou W, Contrepois K,...Snyder MP. Integrative personal omics profiles during periods of weight gain and loss. *Cell Syst* 6: 157-170, doi: 10.1016/j.cels.2017.12.013, 2018.

Restricted Feeding Leads to Metabolic Benefits

in Mice: Scientists found that feeding mice twice a day, with complete food restriction in between, improved metabolism and prevented age- and obesity-associated metabolic defects compared to allowing them 24-hour access to food. Previous research demonstrated that fasting can lead to improved metabolic health and extended life. Fasting has been shown to activate a cellular process called “autophagy” that removes damaged parts of cells, and this process decreases with obesity and/or aging. If autophagy is responsible for the metabolic improvements associated with caloric restriction the scientists hypothesized that a feeding strategy that induced robust autophagy—without needing to

restrict calories or alter the type of food consumed—might lead to metabolic benefits.

To test this hypothesis, the researchers developed a twice-a-day (TAD) feeding model where mice were fed only during two intervals—one early and one late in a 24-hour period. TAD mice were compared to a group that had 24-hour access to food, but were otherwise identical. Importantly, both groups ate the same amount of food. The scientists found no difference in body weight between the two groups of mice fed a healthful diet after 1 year, but observed that the TAD mice had less body fat and an increase in muscle mass. Additionally, TAD mice fed a diet that typically causes weight gain and metabolic problems were protected from these. TAD mice also showed lower blood glucose (sugar) and lipid (fat) levels and an increase in energy expenditure. By comparing young TAD mice to old TAD mice, the scientists observed that the TAD feeding prevented metabolic defects associated with aging. Studying the impact of the TAD feeding in multiple tissues—liver, fat, muscle, and brain—the scientists found that the diverse metabolic benefits of TAD feeding were linked to changes in daily cycles of autophagy. The results raise the possibility that twice-a-day feeding could have similar benefits in humans, although it remains to be tested; and many other factors, such as genetic makeup, may influence the benefits in people.

Martinez-Lopez N, Tarabra E, Toledo M,...Singh R. System-wide benefits of intermeal fasting by autophagy. *Cell Metab* 26: 856-871., doi: 10.1016/j.cmet.2017.09.020, 2017.

CIRCADIAN RHYTHMS AND OBESITY

The Timing Is Everything—Understanding How Obesity Alters the Liver's Circadian Clock:

Researchers have gained new insights into how obesity influences the “circadian” activity of genes in mouse liver, and found that administering medicine at a certain time of day—to align with that circadian activity—results in better metabolic outcomes. It is known that in animals and humans, biological “circadian clocks” regulate behavior and bodily processes, harmonizing them with daily, rhythmic changes in the environment, most notably day/night cycles. In humans, altering normal circadian rhythms with changes in the

timing of behaviors such as sleep and eating—for example, during night shift work—increases susceptibility to diabetes, obesity, and other metabolic disorders. In new research, scientists studied the underlying mechanisms by which obesity affects circadian rhythms in the liver, an organ that plays a key role in metabolism.

First, the researchers examined global gene activity in the livers of male mice throughout the day and night, comparing obese mice eating a high-fat, high-sugar diet with control animals eating a normal chow diet. They discovered that global circadian gene activity was significantly altered in the obese mice compared to the control mice. Next, they studied the new circadian gene activity patterns in the obese mice, making the surprising finding that genes involved in the opposing metabolic processes of producing and burning fatty acids (key components of both solid fat and fat in the blood) developed a circadian rhythm that was not present in control mice. Further experiments allowed them to “zero in” on proteins that were playing important roles in these processes—SREBP and PPAR α . Both proteins are transcription factors, which control whether genes are “turned on” or “turned off.” Previous research had shown that SREBP is an important regulator of cellular processes related to fatty acid production, while PPAR α plays a key role in fatty acid burning. In fact, PPAR α is targeted and activated by drugs used clinically to lower fat levels in the blood. In the study, the scientists showed that the levels of SREBP and PPAR α varied much more throughout the day and night in obese mice compared to control mice. Based on this observation, the scientists questioned whether giving obese mice a PPAR α -activating medicine would be more effective when PPAR α was at its highest level. Indeed, administering the drug at the time when PPAR α levels were at their peak resulted in improved metabolic outcomes in mice after 3 weeks, including reducing fat in the animals’ liver and blood, compared to animals getting the drug at a time of day when PPAR α levels were lower.

These results show that obesity causes major changes to circadian gene activity in mouse liver, and these changes influence fatty acid production and burning. They also suggest that the timing of administering some drugs that lower fat in the

blood may be an important factor to consider related to maximizing their effectiveness. Further research could help determine if the results hold true in people, toward finding novel ways to combat obesity-associated conditions.

Guan D, Xiong Y, Borck PC,...Lazar MA. Diet-induced circadian enhancer remodeling synchronizes opposing hepatic lipid metabolic processes. *Cell* 174: 831-842, doi: 10.1016/j.cell.2018.06.031, 2018.

RESEARCH TOWARD IMPROVING HEALTH IN PREGNANCY

Understanding Calorie Burning in Early Pregnancy—Moving Toward Improving Health for Mothers and Children: New research is shedding light on calorie consumption and calorie burning in early pregnancy in women with obesity, which could inform strategies to promote healthy gestational weight gain and reduce racial disparities in pregnancy outcomes. Because of increasing rates of obesity, larger numbers of women than ever before are entering pregnancy with obesity. Pregnant women with obesity are more likely to have excess gestational weight gain. They are also at higher risk of developing other complications, such as gestational diabetes, disorders related to high blood pressure (e.g., preeclampsia) and having a baby with high birth weight. Racial disparities during pregnancy also exist. For example, pregnant African American women are more likely than White women to have complications such as gestational diabetes, preeclampsia, stillbirth, preterm birth, and weight retention after giving birth. In new research, scientists examined calorie (energy) intake and energy expenditure (calorie burning) in early pregnancy in women with obesity to identify factors that could promote healthy pregnancies and reduce racial health disparities.

Researchers studied healthy, pregnant women with obesity from different racial/ethnic backgrounds at 13 to 16 weeks of gestation. In their first analysis, which included data from 72 women, the researchers sought to identify factors that may influence excess gestational weight gain. They found that 88 percent of participants were sedentary, 12 percent were moderately active, and none were highly active. Furthermore, they found that energy expended

during sedentary activities (e.g., rest and sleep) accounted for nearly 70 percent of the women's daily total energy expenditure, and that energy expenditure from activity (e.g., exercise) was low. The researchers also discovered variability in the rate at which the participants burned calories regardless of their activity level: over a quarter of them had a low metabolic rate, which means they burned fewer calories than women with average or high metabolic rates and thus may have been more susceptible to excess gestational weight gain. Based on the energy expenditure measurements from their experiments, the researchers determined that the number of calories that the women should be consuming to maintain appropriate gestational weight gain was lower than what is estimated based on currently used models; those models were developed based on data primarily from women without obesity. Together, these findings identify three factors that could be contributing to excess gestational weight gain in women with obesity: current recommendations related to caloric intake during pregnancy may overestimate the needs of women with obesity, pregnant women with obesity have low activity levels, and some women have a low metabolic rate.

In their second analysis, which included data from 66 African American and White women, the researchers sought to identify biological factors that predispose African American women to worse pregnancy outcomes. They found that African American women consumed fewer calories than did White women, but there was no racial difference in physical activity levels, which were low in all women. However, after

adjusting for individual differences in body weight and body composition (proportions of fat and lean mass in the body), African American women were found to burn significantly fewer calories than White women. Based on that finding and similar to results described in the first analysis, the researchers calculated that current recommendations for caloric intake during pregnancy, which do not differ by race, may overestimate the needs of African American women with obesity. Although studies have shown that African American women do not have higher rates of excess gestational weight gain than White women, it is possible that the lower energy expenditure in African American women may contribute to their higher risk of excess weight retention after giving birth—making it more likely that they will have higher levels of obesity in subsequent pregnancies. Further research to develop interventions to address the newly identified factors associated with energy expenditure during pregnancy, such as personalizing guidelines related to caloric intake and developing strategies to promote physical activity during pregnancy, could help support healthier pregnancies, reduce racial disparities, and improve health outcomes for mothers with obesity and their children.

Most J, Vallo PM, Gilmore LA,...Redman LM. *Energy expenditure in pregnant women with obesity does not support energy intake recommendations.* *Obesity* 26: 992-999, doi: 10.1002/oby.22194, 2018.

Most J, Gilmore LA, Altazan AD,...Redman LM. *Propensity for adverse pregnancy outcomes in African-American women may be explained by low energy expenditure in early pregnancy.* *Am J Clin Nutr* 107: 957-964, doi: 10.1093/ajcn/nqy053, 2018.

NIH Obesity Research Task Force Seminar Series: Moving Toward Precision Obesity Treatment



A significant challenge in developing better obesity treatment strategies is the large amount of individual variability in treatment response, and it has become clear that a one-size-fits-all treatment plan is not the most effective approach. Precision or personalized medicine is defined as medical care that is designed to optimize therapeutic benefits by targeting the needs of the individual based on differences in people's genes, environments, and lifestyles. Four leading scientists—Mr. Eric Dishman and Drs. Michael Snyder, Elizabeth Speliotes, and Nancy Sherwood—highlighted their research on precision medicine in general and on precision obesity treatments at a September 2018 seminar at the NIH in Bethesda, Maryland. Dr. Penny Gordon-Larsen moderated a panel discussion following the presentations. The research presented was supported by several NIH Institutes, including NIDDK. The seminar was organized as part of the NIH Obesity Research Task Force seminar series.

As the Director of the *All of Us* Research Program at the NIH, Mr. Dishman leads the agency's efforts to build a national cohort of one million or more U.S. participants to advance precision

medicine. But, it is his personal journey that set him out on this path. After battling a rare form of kidney cancer for more than two decades, Mr. Dishman became cancer-free thanks to early access to precision medicine that clarified the right treatment plan for him. But, not everyone has such access to state-of-the-art medical care. Therefore, a major goal of *All of Us* is to focus on health disparities, enrolling volunteers from communities that have been historically underrepresented in research to make the program the largest, most diverse resource of its kind. The *All of Us* Research Program launched nationally in May and currently has more than 100,000 participants enrolled from all 50 states. As the program continues to grow, Mr. Dishman hopes the study will accelerate the path from bench research to bedside medicine for many conditions, including obesity, ultimately getting the right treatment to the right person at the right time. People interested in signing up for the *All of Us* Research Program can find more information at www.joinallofus.org/en

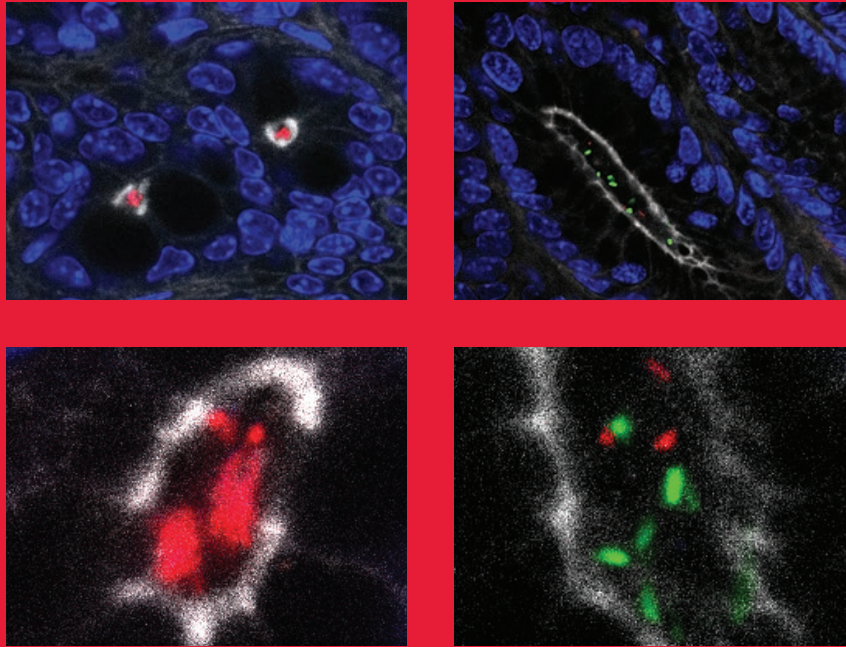
Dr. Snyder's research is centered around the "omics" revolution in which entire complements of molecules such as genes and proteins (genomes and proteomes, respectively) in an organism can be readily characterized. Using a variety of approaches to "omics" profiling, Dr. Snyder and his group are increasing understanding of variation in individual human health that could ultimately lead to more effective ways of predicting disease risk and managing personalized treatment plans. For example, in a recent study, Dr. Snyder's group provided a dynamic picture of the human body's molecular response to weight fluctuation and illustrated how short-term periods of modest weight gain can affect metabolism, the gut microbiome, and heart health. This work could lead to predictive molecular signatures for weight-related conditions, such as type 2 diabetes, long before a disease sets in. A complete write-up of this study can be found earlier in this chapter.

As obesity has reached epidemic proportions in the United States, Dr. Speliotes has focused her research on why some people do not develop obesity in an obesity-promoting environment and why some people with obesity do not develop its complications, including type 2 diabetes, cardiovascular disease, and non-alcoholic fatty liver disease. Shining a light on variations in human disease processes is critical for precision care. To that end, Dr. Speliotes and her team have carried out large-scale analyses in humans to identify genetic variants that are associated with body fat distribution. Because different regions of body fat associate with different obesity complications, identifying genetic variants associated with body fat deposition can potentially lead to new therapeutic targets.

Standard behavioral weight loss programs can help people achieve meaningful weight loss. However,

only about half of participants typically achieve that goal, illustrating once again that a “one-size-fits-all” intervention is not the best strategy. To increase the amount of people who experience significant weight loss, Dr. Sherwood and her colleagues are conducting Sequential Multiple Assignment Randomized Trials (SMART), which are clinical trials that include adaptive interventions that allow the investigators to alter treatment type throughout the trial. In other words, these trials seek to find which intervention being tested works best for each individual participant. Personalized adaptive interventions have the potential to increase weight loss and improve health outcomes for people with obesity.

Continued research in this important area of precision medicine can potentially reveal better ways to personalize obesity treatment plans and deliver the right treatment to the right person at the right time.



The gut is home to trillions of bacteria that play many roles in human health and disease. One goal of digestive disease research is to devise ways to “reprogram” this diverse microbial community by incorporating more bacterial species that are beneficial to their human hosts. Research described in this chapter has identified several factors, including the availability of nutrients, that determine how successfully an introduced bacterial species can displace native bacteria in the gut. The above pictures show a microscopic view of a strain of the “friendly” gut bacterial species *Bacteroides thetaiotaomicron* (green) that was engineered by scientists to metabolize porphyran, a nutrient derived from a type of seaweed. The engineered strain was introduced into the gastrointestinal tract (blue and white) of mice that already harbored a normal strain of *Bacteroides thetaiotaomicron* (red) that could not metabolize porphyran. The engineered strain failed to colonize the intestine when the mice were fed a diet that lacked porphyran (left panels, higher magnification on bottom). However, when porphyran was added to the mice’s diet, the engineered strain displaced the normal strain from the intestine (right panels), suggesting that nutrient availability—along with the ability to utilize that nutrient—can determine the success of bacterial colonization. These results point to ways to selectively colonize the gut with specific, beneficial bacterial species, which could help guide the design of new, tailored probiotic therapies.

Image courtesy of Justin Sonnenburg, Ph.D., Stanford University. Shepherd ES, DeLoache WC, Pruss KM, Whitaker WR, and Sonnenburg JL. An exclusive metabolic niche enables strain engraftment in the gut microbiota. Nature 557: 434-438, doi: 10.1038/s41586-018-0092-4, 2018. Reprinted with permission from Springer Nature, copyright 2018.

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.¹ In addition, 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 were reported.¹ 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 costs totaling \$27.9 billion in 2007.²

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 better understand 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

¹Everhart JE and Ruhl CE. *Gastroenterol* 136: 376-386, 2009.

²Myer PA, et al. *Am J Gastroenterol* 108: 1496-1507, 2013.

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. Scientists are working to understand the causes of functional bowel disorders, which will lead to improvements in diagnosis and management for patients with these conditions. Fecal incontinence, or impaired bowel control, is a 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.

Gastroparesis, another type of functional bowel disorder, is characterized by delayed emptying of food from the stomach, resulting in nausea, vomiting, and abdominal discomfort. Most cases of gastroparesis are of unknown origin, which makes it difficult to treat. Most current therapies are directed toward helping people manage this chronic condition so they can be as comfortable and active as possible. The NIDDK's Gastroparesis Clinical Research Consortium is fueling research on the causes and progression of gastroparesis and exploring new approaches to treat the disorder.

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 ingestion of gluten—a protein 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 strict gluten-free diet, which is difficult for many people. Recent and continued research advances in the understanding of genes and environmental

triggers that are involved in the development of celiac disease may contribute to improved diagnosis and new ways to treat this condition in the future.

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. Disruptions in this microbial ecosystem are associated with diseases such as IBD or infections by the harmful bacterium *Clostridium difficile*. 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 because pancreatitis is difficult to detect in its early stages, many cases are advanced by the time they are diagnosed. 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, bile duct obstruction, 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, sometimes a healthy living person will donate part of his or her liver, most often to a family member who is recommended for a liver transplant. The living donor's liver eventually regenerates and grows back to normal size, as does the part of the liver that is donated. 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, behavioral, 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. Investigators also conduct basic, clinical, and translational research on the requirements, bioavailability, and metabolism of nutrients and

other dietary components in order to understand dietary needs in health and disease. The NIDDK's Office of Nutrition Research spearheaded a process to develop the first NIH-wide strategic plan for nutrition research by the NIH Nutrition Research Task Force that is chaired by the NIDDK Director and co-chaired by Directors of the NHLBI, NCI, and NICHD. Staff from these Institutes and a number of other NIH components have participated in the Task Force's plan development.

GUT MICROBIOME IN HEALTH AND DISEASE

Finding a New Home—How Good (and Bad)

Bacteria Colonize the Gut: Three studies have revealed details of what happens when a community of bacteria inhabiting the gut is disrupted and then rebuilt by a change in diet or by adding bacteria from a healthy donor, providing valuable insights that could help with the design of microbe-based therapies for the treatment of gastrointestinal infections and other digestive diseases. The digestive tract provides a home to the gut microbiome (also called gut microbiota), which includes trillions of bacteria that aid digestion and help to prevent disease by acting as a competitive barrier to pathogenic microbes. For example, a healthy microbiome can effectively prevent infections by *Clostridium difficile* (*C. difficile*), an opportunistic bacterial species that can thrive in the gut when the microbiome is disrupted, such as after antibiotic-based treatments. *C. difficile* bacteria produce a toxin that causes inflammation in the intestinal wall, creating an even more welcoming home for themselves but severe diarrhea and pain for their human host. One treatment option in use for this infection is fecal microbiota transplantation (FMT), whereby a sample containing gut bacteria from a healthy donor is introduced to help re-establish a functional gut microbiome. Although there is accumulating evidence for the effectiveness of this therapy in people with recurrent *C. difficile* infections, significant roadblocks remain, such as how to predict which bacteria will settle the gut and what factors influence their colonization.

In the first study, researchers sought to develop a method to predict which bacteria will colonize the gut following FMT. To do so, they monitored the

microbiomes of 19 people with recurrent *C. difficile* infections after they underwent FMT from one of four healthy donors. Stool samples were collected from the donors and the *C. difficile* patients before the procedure and from the patients in follow-up visits afterward to track which types of bacteria from the donors colonized and persisted in the patients' guts. While previous studies were limited to cataloging gut bacteria at the species level, the researchers developed a more precise method, called "Strain Finder," to deduce specific strains within the species. Applying Strain Finder to the data garnered from the samples, the researchers found that the degree of colonization was determined by the species of bacteria, along with how much of that species was in the donor's sample. They also found that only a fraction of bacterial species colonized the gut, and the strains within those species colonized in an all-or-nothing fashion—generally, either all strains from a species colonized the gut, or none did. Based on these results, the researchers were able to create a mathematical formula that they could use to predict which species and strains of bacteria from an FMT donor will successfully colonize a recipient's gut. To test their formula, the researchers applied it to analyze studies of groups of people who were treated with FMT for *C. difficile* infections or, in research at an earlier stage of exploration, people who were given FMT to see whether it might affect metabolic syndrome (a condition characterized by a set of risk factors for cardiovascular disease and diabetes). The model's further evaluation for patients with a condition besides *C. difficile* infection was particularly important because the *C. difficile* patients received antibiotics to attempt to treat the infection prior to FMT—potentially affecting colonization of incoming bacteria—while other patients did not. Overall, the results of this study will help to optimize FMT and to develop the composition of specific and effective microbiome-targeted treatments. However, even though these results can be used to predict which bacterial strains will colonize the gut after FMT, they raise the question of why these strains will colonize while others will not.

In the second study, another group of researchers attempted to answer this question by using a mouse model to test whether access to specific nutrients would affect the ability of bacterial strains to settle in the gut. They engineered a strain of gut-friendly *Bacteroides* bacteria to enable it to metabolize porphyrin, a complex carbohydrate found in a species of seaweed. They added porphyrin to the diet of

both male and female mice, which harbored either a conventional mouse microbiome or a microbiome from a human fecal sample, to determine whether the porphyrin would allow the engineered bacteria to colonize the mice's guts. The researchers found that not only did the engineered strain readily colonize the mice that were fed porphyrin, but it also displaced native *Bacteroides* strains that were unable to metabolize this nutrient. In fact, the researchers were able to calibrate the number of engineered bacteria that settled in the guts by varying the amount of porphyrin in the mice's diets. These results show that nutrient availability can be an important factor in determining whether a bacterial strain will successfully colonize the gut and that the gut's environment can potentially be manipulated to favor colonization by a select bacterial strain. This knowledge could help in the design of microbiome-based therapies to enable the introduction of specific desirable bacterial strains into the gut.

The third study examined how changes in diet could bolster the microbiome's ability to keep *C. difficile* infections at bay. The researchers focused on microbiota-accessible carbohydrates (MACs)—carbohydrates in fiber from plant-based foods that are resistant to digestion and therefore available for metabolism by beneficial bacteria in the lower intestine. They had previously shown that a diet low in MACs could stoke inflammation in the gut, so they decided to test whether such a diet could also exacerbate *C. difficile* infections, presumably by disrupting the microbiome. The researchers infected female and male mice harboring human gut microbiota with *C. difficile* and fed the mice diets that were either deficient or rich in MACs. While the MAC-deficient mice maintained persistent *C. difficile* infections, the mice fed the MAC-rich diets were able to clear the infection. These mice also had more diverse communities of bacteria in their microbiomes, but that seemed unrelated to the ability to clear *C. difficile* infections because mice fed only a specific kind of MAC called inulin also cleared the infections even though inulin did not increase microbiota diversity. Rather, the effects of the MAC-rich diet appeared to be caused by an increase in some products of bacterial metabolism, called short-chain fatty acids, that suppress *C. difficile* growth. The researchers also found that the amount of *C. difficile* toxin in the infected mice initially increased under a MAC-rich diet, even as the number of *C. difficile* bacteria decreased, suggesting that the

bacteria respond to a MAC-rich diet by ramping up toxin production in an effort to maintain an inflammatory environment. The overall level of toxin declined after a few days, however, as the *C. difficile* population decreased. These results point to diet-induced changes in the microbiome as a valuable means of overcoming gastrointestinal infections.

Although it remains to be seen whether the results of these studies in mice can be translated into humans, they offer significant progress in the understanding of the relationships among diet, the intestinal environment, the microbiome, and bacterial infections, including interactions among specific types of bacteria. More importantly, they also provide insight into how future therapies could be designed to treat or prevent disease by shaping the gut microbiome.

Smillie CS, Sauk J, Gevers D,...Alm EJ. Strain tracking reveals the determinants of bacterial engraftment in the human gut following fecal microbiota transplantation. *Cell Host Microbe* 23: 229-240, doi: 10.1016/j.chom.2018.01.003, 2018.

Shepherd ES, DeLoache WC, Pruss KM, Whitaker WR, and Sonnenburg JL. An exclusive metabolic niche enables strain engraftment in the gut microbiota. *Nature* 557: 434-438, doi: 10.1038/s41586-018-0092-4, 2018.

Hryckowian AJ, Van Treuren W, Smits SA,...Sonnenburg JL. Microbiota-accessible carbohydrates suppress *Clostridium difficile* infection in a murine model. *Nat Microbiol* 3: 662-669, doi: 10.1038/s41564-018-0150-6, 2018.

How the Body Brings About Tolerance to a Gut Microbe: A recent study in mice suggests that the body's ability to "tolerate" the presence of a specific gut microbe is mediated by the interplay of two different types of immune system cells, and that defects in this interaction can contribute to intestinal disease. Humans host trillions of microbes in the gut, including bacteria and viruses and other microorganisms, referred to collectively as the "gut microbiota" or "gut microbiome." These diverse intestinal inhabitants process dietary components and contribute to nutrient absorption and metabolism, interact with the immune system, and produce molecular signals that affect not just the intestines but also other organs throughout the body. However, while normally tolerating the microbiome as a whole, the immune system can react inappropriately to components of the

microbiome, launching a long-lasting attack that damages the intestinal lining and results in diseases such as inflammatory bowel disease.

In the new report, scientists describe how the immune system can establish a condition of tolerance in the gut. In a mouse model, a type of bacteria called *Helicobacter hepaticus* (*H. hepaticus*) was used to investigate the response of the host immune system. *H. hepaticus* is known to play a role in gut inflammation and cancer in immunocompromised mouse strains, yet can coexist without harm in other, "healthy" strains. Colonization of *H. hepaticus* in the large intestine of both female and male healthy mice resulted in an increase in immune system white blood cells called T_H cells, specifically a subset called T_H17 cells. T_H17 cells have been previously shown to be key players in host defense as they produce interleukin 17 (IL-17), a protein that recruits other immune cells to fight an invading microbe. Additional experiments showed that, following *H. hepaticus* colonization, the immune system also produces a second type of immune system cell, called iT_{Reg} cells, to selectively restrain the activity of T_H17 cells—thereby inducing tolerance to this bacterial species. Using genetically modified mice, the researchers then found that iT_{Reg} cells were dependent on a protein called c-MAF for their ability to induce tolerance. Specifically, in the presence of *H. hepaticus*, iT_{Reg} cells lacking c-MAF resulted in the accumulation of T_H17 cells and intestinal inflammation.

The results of these experiments in mouse model systems suggest that when iT_{Reg} cells are missing or defective (e.g., lacking c-MAF), certain bacteria may circumvent tolerance and contribute to the development of immune system-mediated disease, such as inflammatory bowel disease. These results point to potential therapeutic approaches that bolster the iT_{Reg} cell population to help prevent the immune system from launching improper attacks on the gut microbiome.

Xu M, Pokrovskii M, Ding Y,...Littman DR. c-MAF-dependent regulatory T cells mediate immunological tolerance to a gut pathobiont. *Nature* 554: 373-377, doi: 10.1038/nature25500, 2018.

Gut Microbe Helps Program Immune Cells via Dietary Metabolites: Scientists have identified an important role for a gut bacterial species and its metabolites in the maturation of a key immune

cell. Many cells of the immune system rely on the presence of specific gut bacteria to be programmed into mature, functioning immune cells. In fact, these immune programming processes are absent in animals lacking gut bacteria, such as mice raised in germ-free conditions.

Researchers endeavored to find the gut bacteria responsible for promoting maturation of a recently discovered gut immune cell called the double-positive intraepithelial lymphocyte (DP IEL). DP IELs are formed in the intestine from another immune cell type—the CD4⁺T cell—upon activation of a specific molecular program known to be influenced by the presence of gut microbes. The DP IEL performs a wide range of important functions, including ensuring the immune system's tolerance of substances in the gastrointestinal (GI) tract, such as food components and microbes, that might otherwise provoke a negative reaction and inflammation. Their first clue to identifying the gut microbe(s) important for DP IEL formation came from the observation of a wide variation in levels of these immune cells between mice of the same genetic strain obtained from two different companies. When the mice from different sources were co-housed, those animals initially lacking the immune cells went on to develop them, indicating that some transmissible factor—such as a microbe—was at work. The researchers were able to identify the gut bacteria present in the mice by sequencing genetic material from the animals' intestines. Homing in on a bacterial species called *Lactobacillus reuteri* (*L. reuteri*) present in the mice with high DP IELs, the scientists tested two *L. reuteri* strains and found they were able to induce DP IEL formation when given to mice lacking these immune cells. Next, scientists wanted to uncover the mechanism by which *L. reuteri* bacteria accomplish this feat. Some bacteria exert powerful effects on human cells through metabolism of dietary components to create bioactive metabolites, such as short-chain fatty acids. Likewise, *L. reuteri* bacteria grown in culture in the presence of tryptophan—an amino acid present in high-protein foods—were found to produce a class of metabolites called indole derivatives. When added to CD4⁺T cells in culture, these metabolites set off a cascade of molecular signals to transform the cells into the DP IEL cell type. Additionally, mice harboring *L. reuteri* and other bacteria in their guts had elevated DP IELs when given a high-tryptophan diet compared to animals eating a standard chow or low-tryptophan diet. Yet, *L. reuteri* bacteria do not appear to act alone—female germ-free

mice colonized solely with one *L. reuteri* bacterial strain and given a high-tryptophan diet did not form DP IELs at all. Additionally, as observed in one of the earlier experiments, germ-free mice colonized only with the other *L. reuteri* bacterial strain and fed a regular diet experienced only a slight increase in DP IELs. In other words, the larger microbial context also plays a role in this immune cell programming process.

Future studies will be needed to explore potential applications for these findings that *L. reuteri* prompts formation of this key intestinal immune cell, and whether this is also the case in humans. One possible use for the bacteria could be as a probiotic, along with tryptophan-rich foods, for digestive diseases in which the immune system is overly active, such as inflammatory bowel disease.

Cervantes-Barragan L, Chai JN, Tianero MD,...Colonna M. *Lactobacillus reuteri* induces gut intraepithelial CD4⁺CD8α⁺ T cells. *Science* 357: 806-810, doi: 10.1126/science.aah5825, 2017.

MICROBIAL FACTORS IN INFLAMMATORY BOWEL DISEASE

Antibiotic-altered Gut Microbial Communities May Increase Risk of Inflammatory Bowel Disease Across Generations: Antibiotics can adversely alter gut microbial communities, and scientists recently discovered that these alterations can pass from pregnant mice to their offspring and increase the offspring's risk for intestinal inflammation similar to human inflammatory bowel disease (IBD). Microbes in the gut (also called the gut microbiota), as well as their collective genetic material (microbiome) are passed down from mothers to their offspring at birth and play an essential role in the development of a healthy immune system, which can protect against diseases such as IBD. Antibiotics can be life-saving for mother and child when prescribed appropriately, and they are routinely prescribed during pregnancy and for young children. However, they are sometimes overprescribed when not warranted, and they can disrupt the gut microbiome and are a known risk factor for increasing susceptibility to IBD. Further research is needed to inform health care decisions weighing the benefits and risks of antibiotic use in these populations.

Researchers were curious to find out the impact of antibiotic exposure during pregnancy on offspring's risk for later developing IBD. They also aimed to tease apart the effects of the antibiotic-altered set of microbes themselves from any other effects of antibiotic treatment. They designed their study so that pregnant mice raised under sterile conditions free of microbes were colonized a week before giving birth with microbial samples taken from other female mice given a course of low-dose antibiotics, or nothing, in their drinking water. Some of the pregnant mice that received microbes from antibiotic-treated or untreated donors lacked a key immune molecule called IL-10 as a result of a genetic variant; this deficiency increases IBD susceptibility. Fecal samples taken from mothers and their male and female pups revealed that the microbes were passed down effectively between generations, with some pups "inheriting" a normal microbiota and others inheriting an antibiotic-altered microbiota. These differences in the offspring's gut microbes persisted even at 5 months of age, which is nearing adulthood for mice. Pups who inherited the antibiotic-exposed microbes had gut microbial communities that were less diverse and more unstable than animals whose mothers passed on the untreated gut microbes. Furthermore, the pups that lacked IL-10 and also inherited antibiotic-exposed microbes had a dramatically higher risk of developing a severe form of IBD than the pups who inherited an untreated gut microbial community. By sequencing bacterial genetic material from fecal samples in the experimental groups, researchers were able to sketch a rough outline of the key microbial species altered in the mice with elevated IBD due to the presence of both antibiotic-exposed microbes and IL-10 deficiency.

These findings show how antibiotic exposure alters not only gut microbiota, but also the IBD susceptibility of future generations. Further research will be required to identify which specific microbial species increase risk for IBD and which may protect against this disease, and whether transplanting a healthy mix of gut microbes into mice with the antibiotic-altered microbiota and genetic risk can save these mice from developing severe IBD. It also remains to be seen whether similar results are observed in humans. Nevertheless, this research raises new

questions as to how much of the inherited IBD risk, previously thought to have only a genetic basis, might be due to the gut microbiota passed down from mothers to offspring.

Schulfer AF, Battaglia T, Alvarez Y,...Blaser MJ. Intergenerational transfer of antibiotic-perturbed microbiota enhances colitis in susceptible mice. *Nat Microbiol* 3: 234-242, doi: 10.1038/s41564-017-0075-5, 2018.

Becoming Unglued: How a Genetic Variant May Affect the Gut Barrier and Contribute to Inflammatory Bowel Disease: Researchers have found that a genetic variant may impart risk for inflammatory bowel disease (IBD) by disrupting the cellular "glue" that keeps the gut's lining intact. People with IBD suffer from chronic inflammation in the gut, resulting in symptoms such as diarrhea, cramping, and weight loss. Scientists have been sorting through the complicated mix of factors that contribute to IBD, including numerous possible genetic components that are important for maintaining effective physical and immunological barriers to the multitude of bacteria that inhabit the gut. The International IBD Genetics Consortium, of which the NIDDK-supported IBD Genetics Consortium is a member, has identified over 200 regions of the human genome that are associated with IBD. Scientists are now combing through these regions to identify genes—and variants of those genes—that are involved in the disease.

One of the genetic variations that consortium scientists had identified as a risk factor for IBD was in a gene called *C1orf106*; however, until recently it was not clear exactly how variants of this gene might lead to disease. Researchers attempting to uncover the function of "normal" *C1orf106* found that laboratory-grown gut cells produced high amounts of the *C1orf106*-encoded protein when they were in close contact with each other. This suggested that *C1orf106* may contribute to cellular junctions—the "glue" that cobbles gut cells together to create a continuous, sheet-like barrier. Another hint was uncovered when the researchers found that the *C1orf106* protein interacts with cytohesin-1, a protein that disrupts cellular junctions by activating a molecular switch called ARF6. Functional *C1orf106* in cells caused degradation of cytohesin-1 and lower ARF6 activity, stabilizing cellular junctions. These signs pointed to a role for *C1orf106* in maintaining the intestinal barrier by keeping cytohesin-1 levels in intestinal cells relatively low.

Likewise, male and female mice engineered to lack *C1orf106* had higher levels of cytohesin-1 than mice whose genes were unaltered. These mice also showed greater intestinal damage after they were infected by a bacterial pathogen, supporting the idea that *C1orf106* is important for maintaining a barricade against gut pathogens. However, some variants of the *C1orf106* gene may not be as effective as others. In fact, when the scientists replaced *C1orf106* in cells with the specific variant of the gene that is associated with human IBD, the cells were unable to make enough of the *C1orf106* protein to form proper junctions. These studies strongly imply that defects in *C1orf106* contribute to IBD by failing to maintain an adequate intestinal barrier. This information could help to guide the development of improved therapy for people with this genetic variant, although more work is needed to determine if the observations from the mouse model hold true in humans.

Mohanan V, Nakata T, Desch AN,...Xavier RJ. *C1orf106 is a colitis risk gene that regulates stability of epithelial adherens junctions. Science* 359: 1161-1166, doi: 10.1126/science.aan0814, 2018.

Bacterial Enzyme Implicated in Inflammatory Bowel Disease:

Using human samples and animal models, researchers have found that an enzyme produced by certain bacteria could disrupt the gut microbiome, potentially playing a significant role in the development of inflammatory bowel disease. People with inflammatory bowel diseases such as Crohn's disease and ulcerative colitis suffer from symptoms, including diarrhea, cramping, and unintended weight loss. Among other factors, inflammatory bowel disease is associated with changes in the makeup of the microbiome, including the trillions of bacteria living in the gut. Like other organisms, these bacteria thrive by breaking down nutrients to produce components that are critical for growth and survival. However, it is not clear how these bacterial products, or "metabolites," affect the microbiome and disease development.

In a recent study, researchers attempted to determine whether specific bacterial metabolites could be altering the gut microbiome in people with Crohn's disease. They analyzed fecal samples from 90 individuals younger than 22 years old with Crohn's disease and compared them to samples from healthy persons of similar age to identify metabolites associated with disease. Most of the metabolites that were found at higher levels in the samples from people with Crohn's disease were amino acids, which are the building blocks

of proteins. Knowing that many types of bacteria will produce amino acids by breaking down a nitrogen-rich compound called urea, the scientists focused on an enzyme—urease—that is critical for this process. The researchers first sought to establish mouse models that harbored bacteria with high urease levels to determine if bacterial urease might be important for the development of Crohn's disease. To do this, they treated mice with antibiotics and a gut-purging agent to deplete their microbiomes, clearing the way for the establishment of new microbiomes in the guts of these mice. They next inoculated the mice with either bacteria lacking urease (Ure⁻ bacteria) or bacteria engineered to produce urease (Ure⁺ bacteria) and allowed the mice to naturally re-establish their microbiomes over the next month. The scientists found that the two groups of mice developed significantly different microbiomes: the mice initially inoculated with the Ure⁺ bacteria were more likely to develop microbiomes that contained relatively greater numbers of bacteria that are associated with poor health. In fact, when this experiment was repeated in mice that were engineered to develop a form of inflammatory bowel disease, the mice that were inoculated with Ure⁺ bacteria showed a more aggressive form of the disease. These results suggest that urease may play a role in exacerbating inflammation in the gut by disrupting the microbiome. This research also points to urease as a possible target for therapy, either by direct inhibition or by manipulating the microbiome's bacterial components to decrease the amount of urease in the gut. The findings also indicate that a therapeutic strategy to rehabilitate the gut microbiome may require first removing some of the existing bacteria before administering a probiotic with a more healthy bacterial mix. However, more research is needed to determine whether these results seen in mice translate to humans.

Ni J, Shen TD, Chen EZ,...Wu GD. *A role for bacterial urease in gut dysbiosis and Crohn's disease. Sci Transl Med* 9: pii: eaah6888, doi: 10.1126/scitranslmed.aah6888, 2017.

INTESTINAL REGENERATION

A "Support System" for Intestinal Stem Cells

Renewing the Gut Lining: Researchers have uncovered a key role for a unique cell type, called a telocyte, in supporting proliferation and maturation of the nearby intestinal stem cells that perpetually

replenish the inner lining of the gut. The telocytes accomplish this feat by regulating important growth signals sent to the stem cells.

The intestinal lining is continually turning over and replacing cells, regenerating itself in humans about once every week. This high turnover is needed to make up for cells lost in the course of performing the intestinal lining's many functions, including nutrient absorption, waste elimination, and protection against potential pathogens. The intestinal stem cells are highly concentrated in deep pits called crypts located at the bottom of hair-like projections called villi that line the inside of the intestine, effectively increasing its surface area and absorptive power. Scientists have been interested in finding out how the local "neighborhood" of cell types and signals surrounding the intestinal stem cells might contribute to this regenerative process.

A group of researchers focused on an important growth signal called FOXL1, emanating from a layer containing the relatively rare telocytes located just beneath the intestinal lining. For the experiments, they genetically engineered male mice so that their intestinal telocytes would emit a fluorescent green light upon producing Foxl1, the mouse version of the protein. They mapped telocytes using the glowing green marker, showing how the cells formed a network in close proximity to cells lining the intestine, including stem cells. They found these telocytes also produced a potent mix of factors that regulate intestinal stem cells, including a class of essential proteins for stem cell proliferation called Wnts. Surprisingly, in addition to Wnts themselves, the telocytes also produced factors to both stimulate and inhibit Wnt activity. Exploring this further, the scientists discovered that the telocytes finely calibrated production of Wnts, Wnt activators, and Wnt inhibitors depending on their location, with cells closest to the stem cell-rich crypts producing more pro-growth signals. Additional evidence confirming the telocytes' critical role in intestinal stem cell growth came from experiments in male mice genetically altered to inhibit Wnt production from telocytes when given a particular drug. The mice lacking telocyte-derived Wnts showed dramatically reduced populations of stem cells in their small and large intestines and reduced capacity to renew the intestinal lining.

This research illustrates the importance of telocytes in supporting continual replenishment of the intestinal stem cells, the source of the regenerative capacity in the intestinal lining that performs so many vital functions. Future studies can build on this work to further explorations into the nature of these telocytes and their role in the intestine.

Shoshkes-Carmel M, Wang YJ, Wangenstein KJ,...Kaestner KH. Subepithelial telocytes are an important source of Wnts that supports intestinal crypts. Nature 557: 242-246, doi: 10.1038/s41586-018-0084-4, 2018.

IRRITABLE BOWEL SYNDROME RESEARCH

Primarily Home-based Cognitive Behavior Therapy as Effective as Standard Therapy for Treatment of Irritable Bowel Syndrome: New research has shown that a mainly home-based behavior therapy regimen to treat irritable bowel syndrome (IBS) is just as effective as a similar, more expensive, strictly clinic-based therapy, and is more effective than an education-only approach. IBS is marked by a group of symptoms that occur together, including recurring pain in the abdomen and bowel movement issues, such as diarrhea, constipation, or both. Although the cause of IBS is not clear, its symptoms are often triggered by stress, so a significant portion of IBS research has focused on the gut's intimate connection with the brain. This has led to interest in the use of a type of evidence-based, structured psychological treatment called cognitive behavior therapy (CBT), which typically consists of regular counseling sessions with a licensed psychotherapist. Although CBT has been shown to be an effective treatment for IBS, several barriers have prevented it from being widely adopted, including cost, time, therapist availability, and stigmas associated with IBS and psychosocial therapies.

To make CBT more accessible and less costly, a group of researchers recently developed a modified form of CBT for IBS that is primarily home-based, consisting of only four clinic visits over 10 weeks and relying more on home-study materials to strengthen skills that had been introduced in the clinical sessions. To test the new therapy's effectiveness, 436 people with IBS underwent standard, clinic-based CBT (10 weekly counseling sessions),

primarily home-based CBT (four weekly counseling sessions with home-study materials), or four education-only sessions that provided information on the roles of diet, stress, and exercise in IBS. In both the mainly home-based and standard CBT groups, counseling sessions involved education on the relationship between stress and IBS, along with coaching on relaxation exercises, methods for relieving stress and pain associated with the syndrome, and certain problem-solving strategies. About 80 percent of the participants were women, reflecting the fact that women are more likely than men to develop IBS. By the end of the treatments, 61 percent of the people who participated in the mainly home-based CBT reported moderate to substantial improvements in their symptoms, compared to about 55 percent for clinic-based CBT participants, suggesting that home-based CBT is at least as effective as standard CBT. In participants who received only educational sessions, about 44 percent reported symptom improvements. Assessment of participants by gastroenterologists after completion of study therapies supported these findings, and the general differences in symptom improvement scores among the three treatments persisted for at least 6 months. These results suggest that the primarily home-based CBT, with more limited therapist contact than standard CBT, is an effective method to treat IBS, providing a more low-cost, time-efficient, and accessible alternative to the standard clinic-based therapy.

Lackner JM, Jaccard J, Keefer L,...Sitrin MD. Improvement in gastrointestinal symptoms after cognitive behavior therapy for refractory irritable bowel syndrome. *Gastroenterology* 155: 47-57, doi: 10.1053/j.gastro.2018.03.063, 2018.

PEDIATRIC LIVER DISEASE RESEARCH

New Biomarker To Diagnose Life-threatening Liver Disease in Children: Researchers have identified a protein present at high levels in blood from infants with biliary atresia that may enable early and accurate detection of this potentially deadly disease. Biliary atresia is a serious liver disease that occurs during the first few months of life. In this disease, bile ducts that drain from the liver, delivering bile acids to the intestine, become inflamed and scarred, leading to a back-up of bile into the liver. This

back-up can result in liver damage, as evidenced by jaundice, or the yellowing of the skin and eyes. If not treated with surgery or liver transplantation, biliary atresia can lead to liver failure and is ultimately fatal in these infants. Although a rare disease, biliary atresia remains the most common form of severe liver disease in children and the leading cause for pediatric liver transplantation. While its causes are not fully understood, both inherited and environmental factors appear to play a role in disease development. Early diagnosis and treatment are critically important for ensuring the best outcomes for infants with biliary atresia, but often diagnosis is delayed because jaundice in infants is fairly common, resulting from a number of conditions that affect bile flow.

Researchers set forth to identify a clinically useful biomarker for diagnosing biliary atresia by analyzing proteins present in samples from infants enrolled in the NIDDK-supported Childhood Liver Disease Research Network (ChILDRen). By comparing blood samples from infants with biliary atresia to those from infants with other forms of impaired bile flow, they found that a number of proteins were elevated in infants with biliary atresia. One of these proteins in particular, called matrix metalloproteinase-7 (MMP-7), was able to best distinguish the biliary atresia samples from the others, especially when combined with another marker of impaired bile flow called γ -glutamyltranspeptidase. Exploring MMP-7's potential role in biliary atresia development, they found it is produced by the cells lining the bile ducts outside the liver. While MMP-7 levels were not associated with the degree of liver damage in infants with biliary atresia, tests in a mouse model of biliary atresia showed that the protein was released into the blood upon bile duct injury. The investigators also found that MMP-7 inhibitors protected against bile duct obstruction and liver damage in the mouse biliary atresia model.

These findings suggest that MMP-7 is not only a promising candidate as a much-needed biomarker for improving early diagnosis of biliary atresia, but also likely plays a role in disease development and may be a therapeutic target. Future studies will be required to confirm MMP-7's utility as a diagnostic tool, to test its usefulness for monitoring disease progression long-term—after interventions in infancy—and to pave the way for possible treatment trials of MMP-7 inhibitors in humans.

Lertudomphonwanit C, Mourya R, Fei L,...Bezerra JA. Large-scale proteomics identifies MMP-7 as a sentinel of epithelial injury and of biliary atresia. *Sci Transl Med* 9, pii: eaan8462, doi: 10.1126/scitranslmed.aan8462, 2017.

Genetic Risk Factors for Obesity and Insulin Resistance Identified in Hispanic Boys with Nonalcoholic Fatty Liver Disease: Researchers have identified novel genetic variants that are associated with obesity and insulin resistance in Hispanic boys with nonalcoholic fatty liver disease (NAFLD). A form of chronic liver disease marked by excess fat stored in the liver, NAFLD has affected an increasing number of people in the United States and around the world in recent years, including children, in whom it is now the most common form of liver disease. Its more severe form, called nonalcoholic steatohepatitis (NASH), can lead to cirrhosis, liver failure, and liver cancer. NAFLD is often associated with obesity and insulin resistance, as well as with a cluster of conditions known as “metabolic syndrome” that is linked to increased risk of heart disease, stroke, and diabetes. People of some racial/ethnic backgrounds, such as individuals of Hispanic descent, are also at increased risk of developing NAFLD. Although peoples’ genetic backgrounds appear to affect risk of the disease, little is known about the genes that drive NAFLD susceptibility in children.

As part of the NASH Clinical Research Network, scientists conducted a genetic study at 12 clinical centers located throughout the United States, which recruited 234 Hispanic boys who had been diagnosed

with NAFLD or NASH based on liver biopsy. The study participants were also obese or overweight with signs of insulin resistance. Investigators analyzed DNA from the participants’ blood samples, leading to the identification of 10 genetic variants associated with overweight/obesity and nine associated with insulin resistance. The variants associated with elevated fat mass implicate specific elements of the metabolic machinery, such as a protein involved in glucose formation in the liver.

These findings highlight some potential genetic factors in Hispanic boys with NAFLD that influence development of obesity and insulin resistance in this population. This analysis can help improve understanding of the underlying mechanisms driving NAFLD and some of its associated metabolic conditions. Further studies will be required to test if these genetic associations are relevant to other populations, such as Hispanic girls with NAFLD or young people of other racial/ethnic groups.

Rausch JC, Lavine JE, Chalasani N,...Rotter JI; for the NASH Clinical Research Network. Genetic variants associated with obesity and insulin resistance in Hispanic boys with nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr* 66: 789-796, doi: 10.1097/MPG.0000000000001926, 2018.

New Strategic Plan for NIH Nutrition Research

Recently, the NIH developed its first agency-wide strategic plan for nutrition research. The *Strategic Plan for NIH Nutrition Research* emphasizes cross-cutting, innovative opportunities for advancing NIH-supported nutrition research across a wide range of areas—including basic, translational, and clinical research, as well as research training activities—over the next decade. The *Strategic Plan* also highlights ways to enhance new and ongoing research efforts across NIH to improve health and prevent or combat diseases and conditions affected by nutrition.

The *Strategic Plan* was developed by the NIH Nutrition Research Task Force with crucial and extensive input from researchers and others external to NIH. The Task Force was established in 2016 by the NIH Director, Dr. Francis Collins, and chaired by Dr. Griffin Rodgers, NIDDK Director, with Co-chairs Dr. Gary Gibbons, Director of the National Heart, Lung, and Blood Institute; Dr. Norman Sharpless, Director of the National Cancer Institute; and Dr. Diana Bianchi, Director of the Eunice Kennedy Shriver National Institute of Child Health and Human Development. The NIDDK Office of Nutrition Research, which coordinates nutrition research across NIDDK and the NIH, spearheaded the NIH-wide nutrition research strategic planning process, with the Office's Director, Dr. Christopher Lynch, serving as Executive Secretary of the Task Force. The Task Force was also assisted in this effort by representatives from across the NIH's many Institutes, Centers, and Offices participating in its Working Group and Senior Leadership Group, as well as external experts serving on its Thought Leaders Panel and others.

Throughout its development, the *Strategic Plan* was informed by broad stakeholder input. The Task Force started by collecting information on the landscape of existing nutrition research plans and recommendations from the research community, including professional societies, academic researchers, nonprofit organizations, and federal agencies. In spring of 2017, the Task Force set up a “crowdsourcing” website to solicit ideas for the

nutrition research planning effort from a broad swath of researchers and members of the public. The Task Force then convened a Thought Leaders Panel of federal and external nutrition experts for a 2-day series of meetings in June 2017 to provide their recommendations on research priorities. The diverse input received from these sources informed the Task Force's Working Group and Senior Leadership Group in their work developing a draft of the *Strategic Plan* in spring of 2018, which was circulated for comment within NIH and then posted for public comment on the NIH website, prior to its revision and finalization. Throughout the planning process, the Task Force convened regularly to discuss priorities and progress.

The *Strategic Plan* identifies a number of cross-cutting research areas—such as addressing minority health/health disparities, women's health, and other critical areas—that will underpin successful future activities across the field of nutrition research. The *Strategic Plan* is structured around major scientific themes, each of which is linked to a list of associated research priorities and examples of potential future research activities.

In the years ahead, the Task Force plans to implement the recommendations made in the *Strategic Plan* to advance NIH-sponsored nutrition research in a way that is responsive to emerging opportunities and the changing scientific landscape.

For additional information on the *Strategic Plan for NIH Nutrition Research*, please visit the NIH Nutrition Research Task Force website at: www.niddk.nih.gov/about-niddk/advisory-coordinating-committees/nih-nutrition-research-task-force

Workshop Explores Ways To Accelerate Pancreatitis Treatment Development



On July 25, 2018, the NIDDK, with support from the National Pancreas Foundation, sponsored a workshop to identify research gaps and opportunities in the development of new drugs and clinical testing approaches to managing acute and chronic forms of pancreatitis. The workshop was organized and led by staff from the NIDDK's Division of Digestive Diseases and Nutrition and took place in conjunction with an annual meeting of pancreatitis researchers called PancreasFest in Pittsburgh, Pennsylvania.

In addition to producing the hormone insulin and other hormones, the pancreas produces a fluid containing both enzymes and bicarbonate that is released into ducts leading to the intestine, where the enzymes are activated to digest food. Pancreatitis is a condition caused by the activation of these digestive enzymes while they are still inside the pancreas, resulting in damage and inflammation. Acute and chronic pancreatitis are leading causes of emergency room visits and hospitalizations from gastrointestinal conditions. In individuals with acute pancreatitis, some experience recurring episodes—termed acute recurrent pancreatitis; acute forms of pancreatitis can develop into chronic pancreatitis, which carries an increased risk of pancreatic cancer.

Currently, there are no effective drugs specifically for pancreatitis that halt progression of this potentially debilitating disease or reverse the disease process. Available treatments are limited to supportive therapy for pain and also surgical procedures. Although there are potential treatments in the pre-clinical stage of the drug pipeline, challenges exist in moving these compounds

into clinical trials. For example, there are no guidelines available from the U.S. Food and Drug Administration (FDA) for researchers to use in designing trials to test new therapies targeting pancreatitis.

The workshop objectives included: 1) identifying requirements, obstacles and opportunities to enhance the development and clinical testing of agents for the treatment of acute pancreatitis, recurrent acute pancreatitis, and chronic pancreatitis; 2) identifying research gaps that currently inhibit drug development and clinical testing; and 3) engaging representatives from the FDA to assist with the development of guidance documents that will help investigators in studies of the treatment of these diseases.

The workshop brought together presenters and participants from the NIH, FDA, researchers throughout the Nation and from other countries, health care organizations, pharmaceutical industry representatives, and patient advocacy groups with a goal of sparking research to enhance development of new treatments and testing methods for pancreatitis. During the workshop, working groups focused on challenges specific to drug trials for acute pancreatitis, recurrent acute pancreatitis, and chronic pancreatitis. Presentation topics included past successes in drug development for other diseases, as well as research on children and adults with pancreatitis conducted through the Consortium for the Study of Pancreatitis, Diabetes, and Pancreatic Cancer, which is supported by the NIDDK and the National Cancer Institute.

The meeting organizers and members of its three working groups have developed multiple manuscripts describing the workshop proceedings.¹ Recommendations from the workshop will inform future NIDDK efforts—in partnership with FDA, patients, researchers, and others—to advance research accelerating the development of new pancreatitis therapies.

¹ Uc A, et al. *Pancreas* 47: 1180-1184, 2018; Abu-El-Hajja M, et al. *Pancreas* 47: 1185-1192, 2018; Lowe ME, et al. *Pancreas* 47: 1193-1199, 2018; Forsmark CE, et al. *Pancreas* 47: 1200-1207, 2018.

Workshop Explores Approaches and Challenges to Dietary Biomarker Discovery



The NIDDK hosted a workshop on September 26–27, 2018, to explore approaches to dietary biomarkers research.

Our health is influenced by what we eat. While the primary goal of nutrition research is to optimize health through diet, this requires establishing firm associations between nutrient intake and disease outcomes. For example, it would be extremely valuable to know which foods might increase risks for certain cancers—and which foods might help to prevent them. However, accurate measurement of dietary intake is a major challenge in nutrition research. Collecting data on what people eat usually relies on self-reporting, which is limited by their memories of what and how much food they ate. Precise measurement of the effects of diet on health will require an independent, unbiased approach to detecting dietary components in the body following a meal.

One potential approach would be to use dietary biomarkers, which are detectable molecules in the body (such as in the blood) that serve as indicators for which nutrients had been absorbed from food. Biomarkers would be extremely helpful to track nutrient intake in studies that are probing links between diet and disease. Several dietary biomarkers are already in use by clinicians, who frequently measure protein intake by analyzing the amount of nitrogen in urine, for example. However,

there is a critical need to identify more biomarkers that are specific for certain foods and that could be utilized to improve assessments of dietary intake.

The NIDDK’s workshop explored approaches for identifying and using such biomarkers. Among the topics discussed were tools to assist in the discovery and validation of new biomarkers, optimal strategies for designing biomarker studies, and what statistical approaches should be used to accurately analyze data. A particular focus was placed on identifying new biomarkers using “omics” methods to examine diet-related changes in the body, such as proteomics (analyzing changes in bodily proteins) and metabolomics (analyzing molecular products of human metabolism), and how different types of these omics approaches might be integrated to enhance biomarker discovery. The workshop culminated in discussions of topics such as challenges to biomarker discovery, new tools for biomarker identification and validation, platforms for sharing data, and new opportunities to use biomarkers in nutrition research.

Recommendations from the workshop will be made available to the scientific community through a publication in a scientific journal. These recommendations will help improve nutrition research by highlighting new ways to examine the link between dietary components and health outcomes.

STORY OF DISCOVERY

Discoveries Moving Towards Understanding and Personalized Treatment of Inflammatory Bowel Disease

After leaving the stomach, ingested food travels through approximately 25 feet of small and large intestines. The innermost part of the intestinal lining that comes in direct contact with food—called the intestinal epithelium—is composed of a diverse mix of cells that perform critical roles for digestion, including absorption of nutrients and secretion of substances that aid in the sensing and movement of intestinal contents. Surrounding the epithelium is a conglomeration of blood vessels, nerves, and muscle cells. Also within the intestinal lining are immune cells that act as sentinels ready to defend the body against gastrointestinal pathogens. These immune cells are overly active in people with inflammatory bowel disease (IBD), leading to painful inflammation that can devastate the intricate intestinal lining. The accompanying symptoms can be crippling. For example, diarrhea results if inflammation shuts down absorption in the intestine. In severe cases, lesions develop, accompanied by intestinal bleeding. IBD can also lead to malnutrition when nutrients from food cannot be absorbed, a condition that is especially harmful to children because it can stunt growth.

The development of effective IBD treatments has been inhibited by a lack of understanding of the causes of the underlying inflammatory process. But in recent years, researchers have discovered ways in which the bacteria and other microbes that reside in the gut—the gut microbial community or microbiome—can affect risk of IBD, and efforts such as the NIDDK's IBD Genetics Consortium are

shedding new light on the genetic foundations of the disease. With its Intestinal Stem Cell Consortium and clinical research efforts, the NIDDK is already leveraging those insights to test new approaches to therapy for IBD. The story of IBD is one of increasing complexity, but also the discovery of new immunologic, genetic, and microbial links to the disease, and the potential for novel, more effective therapies.

AN EARLY PICTURE OF IBD: CROHN'S DISEASE AND ULCERATIVE COLITIS

Inflammation is normally a part of the immune system's response to fighting infection, but in the latter half of the nineteenth century clinicians described cases of intestinal inflammation that did not seem to have an infectious origin. By the 1930s, it had become apparent that most of these cases could be segregated into two separate diseases: ulcerative colitis and Crohn's disease. These two diseases would eventually be grouped under the umbrella term "IBD."

There are fundamental differences between Crohn's disease and ulcerative colitis. In ulcerative colitis, the inflammation encompasses only the colon (large intestine) and is generally limited to the innermost layers that make up the intestinal wall. In Crohn's disease, which is not as common as ulcerative colitis, the inflammation may involve any part of the gastrointestinal tract (although it usually manifests

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in the small intestine and the beginning of the colon) and is not continuous, resulting in patchy lesions. These lesions also tend to be deeper than in ulcerative colitis, producing strictures (places where the intestines narrow) and fistulas (abnormal passageways between areas of the intestine).

Early treatments for IBD were limited to surgical removal of the affected area—a strategy that is still in practice today for people who have severe inflammation that does not respond to other therapies. For people with ulcerative colitis, this usually means removal of the entire colon. For Crohn's disease, surgery is limited to smaller affected areas. However, removal of a lesion does not cure Crohn's disease, and it is possible that additional lesions will appear elsewhere.

The recognition that IBD encompasses two distinct but related entities was a major step toward understanding these diseases, but researchers sought to understand the excessive inflammation underlying both forms. They would eventually discover that the intestinal inflammation results from multiple interplaying factors, including the immune system, genetics, and the environment.

SUPPRESSING THE IMMUNE RESPONSE

The knowledge that inflammation was the driving force behind the symptoms of IBD led naturally to the first useful, non-surgical therapeutic approach: reducing inflammation. Since the 1950s, for example, clinicians have prescribed anti-inflammatory drugs such as corticosteroids, which are fast-acting and powerful enough to suppress flareups (the sudden worsening of symptoms) but cannot be used as a long-term treatment because of potentially serious side-effects. Aminosalicylates, such as mesalamine-based drugs, are another class of anti-inflammatory medications. While they are generally well-tolerated, they may not be effective for people with severe IBD symptoms, so their use is generally limited to people with mild to moderate cases.

Other medications reduce the inflammatory response by hampering the immune processes that drive it. These therapies, called immunomodulators, have been used to treat people with IBD since the 1960s. Although they can be an effective treatment for those who do not respond to (or cannot tolerate) corticosteroids or aminosalicylates, immunomodulator-based treatments are not without risks. They could produce potentially severe side-effects, including an increased risk of infections because the drugs reduce the activity of the body's immune system.

Despite the long history of using immunomodulators to treat IBD, the risk-benefit profile of one such medication has only recently been thoroughly ascertained: methotrexate, an inexpensive yet potentially toxic immunomodulator that is prescribed for adult Crohn's disease patients in whom established therapies have failed. The NIDDK funded the Methotrexate Response in Treatment of Ulcerative Colitis (MERIT-UC) study to determine its risk-benefit profile in the more common form of IBD. Recent results from this study showed that methotrexate did not improve ulcerative colitis symptoms compared to a placebo, suggesting that this drug, which could lead to chronic liver disease and liver fibrosis, is not beneficial for colitis patients.

Other means of modulating the inflammatory response in IBD have been suggested by advances in basic research on the immune system. For example, immunologists have identified several key molecular signals that trigger inflammation. Among these are molecules called cytokines, which are secreted by immune cells and play important roles in driving the inflammation in the gut. The pro-inflammatory cytokines found to be involved in IBD include tumor necrosis factor alpha (TNF α) and interleukin-12/interleukin-23 (IL-12/IL-23), which play major roles in other inflammatory diseases such as arthritis and psoriasis. Researchers found that blocking the activity of these cytokines can suppress inflammation in the gut. There are numerous drugs that have been developed to target TNF α and IL-12/IL-23, or the cellular signals

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that are elicited by these cytokines. Other drugs have been developed to block molecules, called integrins, that immune cells use to stick to the walls of blood vessels as they move out of the blood and into the tissue where they contribute to inflammation. Not every person with IBD responds to these anti-cytokine and anti-integrin treatments, however, and these drugs too can cause serious side-effects, including a heightened risk of infections. Also, most of these drugs are proteins and would be digested and broken down before they reach the site of inflammation if taken orally. Therefore, they must be administered through regular injections.

DIGGING THROUGH THE GENETICS OF IBD

By the end of the twentieth century, with evidence mounting that IBD varies significantly from person to person, it was apparent that developing safer, more effective therapies would likely depend on determining the factors that contribute to the inflammation in ulcerative colitis and Crohn's disease.

IBD tends to run in families, so scientists strongly suspected that genetic factors were important. Identifying the genes involved in the disease could reveal new targets for therapy. A better understanding of IBD genetics might also enable clinicians to screen for people at risk for IBD—and early detection might make it possible to prevent the inflammation before it starts and becomes difficult to restrain. Genetic studies might even lead to personalized therapy by predicting which individuals would respond best to different types of IBD treatment.

Therefore, through grants to researchers at academic medical research institutions across the United States, the NIDDK established the IBD Genetics Consortium (IBDGC) 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 complex and individual nature of the disease. In one IBDGC study, for example, researchers analyzed data from over 29,000 men and women with IBD and found that, in Crohn's disease, inflammation in the ileum of the small intestine (near the junction with the colon) has distinct genetic causes from forms of Crohn's in which inflammation occurs in the colon. In other words, there are actually at least three distinct types of IBD—ulcerative colitis and two forms of Crohn's disease—which could help guide targeted treatments in the future. Other advances from the IBDGC have identified genetic variants that affect the microbiome (the diverse community of bacteria and viruses living in the gut), the immune system, or the integrity of the intestinal epithelium—all of which play important roles in IBD. 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 precisely identified. A goal of the current phase of the IBDGC, which was renewed in 2017, is not only to continue identifying regions of the genome associated with genetic risk for IBD, but also to identify the specific genes and genetic variants within these regions that influence IBD susceptibility. Consortium members are also exploring the role of epigenetics—how genes are turned on and off independently of their DNA sequences—and how genetic factors influence the development of IBD by investigating the functions of candidate genes.

Exploring the genetics of IBD has provided insight into its origins, along with an abundance of possible molecular targets for screening or therapy. It also has cemented the understanding of IBD as a disease that could vary greatly from person to person, depending on genetic backgrounds, highlighting the need for more personalized approaches to treatment and prevention.

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THE ROLE OF THE MICROBIOME

IBD was rarely diagnosed before the twentieth century, and today it is more common in industrialized regions of the world. Although this may stem in part from greater awareness of the disease, it also suggests that while genetics are an important contributor to IBD susceptibility, environmental factors—including the microbiome or factors that affect the microbiome—also likely play a role. Scientists thinking along these lines considered the possibility that an abnormal immune reaction to components of the microbiome might provide the long-sought explanation for the inflammation that leads to IBD. In fact, numerous studies have shown correlations between IBD and changes either in the microbiome or in how the body reacts to it.

Early support for this idea came from a study in a rat model that found a link between the composition of gut microbes and colitis. Scientists also showed that feeding a diet high in saturated fats from milk to mice with a genetic susceptibility to intestinal inflammation altered their intestinal microbial communities and the composition of their bile acids, induced changes in their immune function, and increased intestinal inflammation. 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.

Important insights are also coming from the NIH's Human Microbiome Project (HMP), which was launched in 2007 to characterize the community of microbes present in humans. As part of the HMP, the NIDDK co-funded and managed a study designed to understand how the gut microbiome is altered in IBD. This study integrated many different types of measurements of gut microbes as they change within IBD patients, including both children and adults, over time. The researchers found that the microbiomes of people with IBD were more volatile and fluctuated to a

greater extent than those of healthy people, providing still more evidence that changes in the microbiome are closely linked to the disease.

Future research will seek to elucidate the specific ways in which components of the microbiome may promote IBD—or protect from the disease. Microbiome analyses in individuals with IBD may one day even help personalize treatment of their disease.

THE FUTURE OF IBD THERAPY: PERSONALIZED MEDICINE

Armed with what they have learned about the many factors affecting susceptibility to IBD, researchers are now seeking to develop personalized therapeutic approaches that consider the complex interactions among the immune system, genetics, and the environment driving development of the disease in any given individual.

It is now becoming possible to correlate specific genetic variants and other clinical test results with disease severity and responsiveness to therapies. For example, the NIDDK-sponsored Predicting Response to Standardized Pediatric Colitis Therapy (PROTECT) study has been evaluating whether a combination of clinical, genetic, and immunologic tests can be used to predict response to standard medical therapy for children newly diagnosed with ulcerative colitis. Recent results from the PROTECT study found that higher amounts of an immunologic biomarker called pANCA in the blood correlates with disease severity, suggesting that this biomarker, which also may be associated with resistance to standardized therapy, could potentially be used as a diagnostic tool to help plan individualized treatments for children with the disease.

Another personalized approach that holds promise is the manipulation of the microbiome to maintain it in a healthy state. This could be accomplished by restoring a health-promoting profile of bacteria in the gut of a person with IBD using tailored probiotics to reintroduce underabundant bacterial species, or by fecal microbiota transplant whereby

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the gut bacteria from a healthy donor would be introduced into a person with IBD.

An additional therapeutic approach would be to use cellular models of the gut to develop new personalized treatments in the laboratory and to possibly replace the damaged tissue in a person with IBD. Toward this goal, members of the NIDDK-supported Intestinal Stem Cell Consortium (ISCC) are developing methods to generate small conglomerations of cells that look and behave like a miniature portion of a human intestine. The ISCC has demonstrated that these “mini-intestines” can be generated using adult cells as starting material, suggesting that a patient’s own cells could be used to screen for drugs that would be effective for that individual’s disease. Personalized mini-intestines could also be potentially invaluable research models to study the genetic roots of IBD and how the intestine heals itself when injured. Additionally, mini-intestines may one day be implanted to repair or replace areas of the gut that have been damaged by inflammation, and because they could be derived from a patient’s own cells, they would less likely be rejected by the immune system. ISCC scientists

have already used these mini-intestines to generate a functional enteric nervous system—the mesh-like arrangement of nerves that governs the function of the gastrointestinal tract—demonstrating their potential as models for *bona fide* intestines. They also generated mini-intestines that resemble different parts of the intestine, such as the ileum (the lower end of the small intestine) and duodenum (the section of the small intestine closest to the stomach). This is important from a replacement therapy standpoint because distinct regions of the intestine have different functional roles in digestion. Although research into regenerative medicine-based approaches is still in its early stages, these advances hint that the coming years may eventually see intestinal stem cell-based therapies for IBD.

IBD is indeed a complicated disease with many players. But for every cytokine, gene, or bacterial species linked to IBD, a new path opens for the development of a method to detect, prevent, or treat the disease. With more research into how combinations of factors drive the disease, and how they vary from person to person, we come closer to a future of far better health outcomes for people with IBD.

SCIENTIFIC PRESENTATION

Dr. Ramesh Shivdasani— How Discoveries in the Gut Redefined Stem Cell Biology

Dr. Shivdasani is a Professor of Medicine at Harvard Medical School and a Distinguished Physician at the Dana-Farber Cancer Institute in Boston, Massachusetts. He earned an A.B. from Cornell University and an M.D. and Ph.D. from the University of Michigan. He then completed his residency in internal medicine at Brigham and Women's Hospital and a fellowship in medical oncology at the Dana-Farber Cancer Institute, followed by a postdoctoral fellowship at Boston Children's Hospital where he focused on transcriptional regulation of hematopoiesis, or how the body generates new blood cells. For the past 20 years, he has been conducting innovative research on gastrointestinal development, differentiation, and tumorigenesis. Dr. Shivdasani is a principal member of the Harvard Stem Cell Institute, where he also serves as co-director of the Institute's Cancer Program. He also is a principal investigator in the NIDDK-supported Intestinal Stem Cell Consortium, a highly productive network of researchers who are conducting research to better understand the biology of intestinal stem cells during development, homeostasis, regeneration, and disease. At the January 2018 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Shivdasani presented an overview of scientists' research into the mechanisms of intestinal renewal. The following are highlights from his presentation.

Regenerative medicine is the process of creating living, functional tissues to repair or replace tissue or organ function that was lost because of age, disease, damage, or congenital defects. This field holds the promise of fixing damaged body parts by stimulating previously irreparable organs to heal themselves. Regenerative medicine also empowers scientists to grow tissues and organs in the laboratory and safely

implant them when the body cannot heal itself. Thus, importantly, regenerative medicine has the potential to solve the problem of organ shortage—that is, the relatively small number of organs that are currently available for a large number of people who need a life-saving organ transplantation. But before scientists can optimally generate organs for use in people, they first must understand how organs grow, develop, maintain a healthy state, and repair themselves when damaged.

THE INTESTINE AS A MODEL FOR REGENERATIVE MEDICINE

Dr. Shivdasani began his presentation by remarking that the intestine is an ideal model for regenerative medicine research, partly because the intestine is the most rapidly regenerating organ in the body. In fact, the intestinal lining completely renews itself once every five days. Research into how this feat is accomplished has provided valuable insight into how the intestine repairs itself. And, as Dr. Shivdasani hinted, it has forced scientists to rethink their assumptions about organ regeneration in general.

Dr. Shivdasani described the intestine as being carpeted with millions of fingerlike projections called villi. These millimeter-long protrusions add to the surface area of the intestine, effectively increasing its absorptive power. Coating the villi are the most common cells in the intestinal lining—the enterocytes—whose primary role is to absorb water and nutrients. Interspersed among the enterocytes is a diverse mix of other cell types that secrete mucus, produce hormones, and regulate immune responses.

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In the 1960s, scientists began to suspect that all the different cell types in the intestine descend from a small population of cells—stem cells—that are nestled at or near the bottom of “crypts,” which are pit-like structures that are contiguous with villi. With the hope of finding the key to intestinal regeneration, scientists began searching for these stem cells.

THE SEARCH FOR INTESTINAL STEM CELLS

Dr. Shivdasani explained what scientists started looking for in their search for intestinal stem cells: cells that could reproduce to form more stem cells, or differentiate—turn into cells with specialized intestinal functions. They made three assumptions based on what was currently known about the stem cells in the bone marrow that give rise to all red and white blood cells in the body. The first assumption was that all stem cells would divide slowly. This would be important for preserving the stem cell population because cells that divide slowly are less susceptible to genetic damage, such as from radiation or chemicals. The second assumption was that all stem cells would divide into two cells asymmetrically—that is, they would make one copy of themselves along with another cell that would take on a more specialized role. This starts some cells on the path to specialization while maintaining the valuable stem cell population. The third assumption was that all populations of stem cells would be rare, which would make them difficult to find.

Researchers began looking for cells in the intestinal crypts that fit these descriptions, but they were hampered by technological limitations. Eventually they narrowed their search to a population of slender cells wedged in the bottom of the crypts. Labelling these so-called “crypt-based columnar cells” (CBCs) in mice, so that scientists could track them and their progeny, revealed that CBCs were the source of several different intestinal cell types, implicating them as stem cells. But Dr. Shivdasani noted that CBCs were found to be constantly

dividing and exclusively producing exact copies of themselves (*i.e.*, dividing symmetrically), which did not fit the traditional assumptions of what stem cells should do. They were also not a rare population—it is not uncommon to find a dozen of these cells within each of the millions of crypts.

Advances in technology eventually made it possible to define cells in the crypts by scanning them for molecular markers, which are proteins that can be used to identify cells. The CBC cells were found to produce a protein called LGR5, so these cells are also referred to as “LGR5-positive cells.” The use of molecular markers also allowed scientists to find another population of possible stem cells that were located several cells up from the bottom of the crypts, at the so-called “+4 position.” These “+4 cells” were rarer than LGR5-positive cells and, unlike LGR5-positive cells, they were slow to replicate—characteristics that fit the assumptions of what stem cells should be. This led to a model of intestinal regeneration that uses two populations of stem cells—the LGR5-positive population that lies near the base of the crypts, and the +4 cells, which were rarer, more dormant, and act as a backup in case the LGR5-positive cells become damaged.

This model provided a novel and intricate view of organ regeneration. However, the biology of intestinal crypts—and the stem cells they harbor—was about to get even more complicated.

REDEFINING STEM CELLS

Dr. Shivdasani explained another important discovery that introduced a twist to the intestinal stem cell model. Experiments in an animal model showed that both the LGR5-positive and +4 cell populations could be eliminated, and the intestine could still recover. In fact, the lost stem cells would eventually be replenished. This led to the discovery that other, more differentiated cells—the cells that had arisen from the stem cells—could return to a stem cell state, and this appeared to be regulated by signals produced in different areas of the crypt.

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Dr. Shivdasani went into further detail, explaining how cells in the crypt transition back and forth between a stem cell state and a more specialized state. The dividing LGR5-positive cells at the bottom of the crypt exert force on adjacent cells, providing the “motor” for an escalator-like mechanism that pushes all the cells above towards the top of the crypt and into the villi. As the cells are pushed upwards, they lose contact with signals at the bottom of the crypt and are exposed to new signals that originate closer to the top. This change in environment coaxes the cells to take on more specialized characteristics, eventually becoming enterocytes or secretory cells. However, if the cells at the bottom of the crypt are damaged or lost, cells from higher up can relocate to the bottom and become re-exposed to factors that revert them to a stem cell state. Thus, the transition between intestinal stem cells and specialized cells is a two-way street.

Scientists are still sorting through all the signals that drive the transitions to and from the stem cell state, but the Wnt family of proteins appears to have a major role. Wnt protein levels are highest near the bottom of the crypt, where they stimulate the cell division that pushes all other cells upwards. In fact, interfering with Wnt signaling disrupts the entire architecture of the intestinal lining and reduces the fingerlike villi to a flat sheet of cells. But Wnt proteins do not act alone—other proteins called R-spondins are important for amplifying the effects of Wnt signaling, and additional factors (such as Notch and bone morphogenic protein) trigger signals in the cells to steer them towards specialization.

Dr. Shivdasani noted that this model of intestinal cell production offers an important lesson about regenerative medicine. The assumptions that were made about intestinal stem cells were based on what was known about hematopoietic (blood) stem cells, but the intestinal lining takes a different route to regenerate itself. For example, intestinal stem cells divide symmetrically, which, from blood cell research, would be counterintuitive. However, the intestinal crypt itself is asymmetric, so the population of dividing stem cells at the base of the

crypt is preserved while its progeny become more specialized as they move upwards. Thus, almost all the attributes that had been thought to be necessary characteristics of stem cells are simply the success story of the hematopoietic system, which happens to use a rare population of stem cells that replicate infrequently and divide asymmetrically. But another organ may use a different process to regenerate itself. And studies of the intestine have convinced scientists to revisit their assumptions of stem cell properties, allowing for a broader definition.

From a gut regenerative medicine standpoint, the important idea is that almost any intestinal cell that has started down the road of becoming a specialized cell retains the potential to move back to a stem cell state if necessary. This is a “game changer,” Dr. Shivdasani explained, because it may no longer be crucial to specifically target and isolate the intestinal stem cells as a source for regenerative tissue. Instead, nature has provided a way to work with several populations of cells that are readily convertible.

PUTTING KNOWLEDGE TO USE: MAKING MINI-INTESTINES AND DEVELOPING THERAPIES

Scientists are applying what they have learned to demonstrate the potential for using intestinal stem cells to generate laboratory models of disease, and for building tissue for the therapeutic purpose of replacing damaged intestinal lining. For example, scientists in NIDDK’s Intestinal Stem Cell Consortium have successfully grown intestinal organoids—miniature versions of intestines—by culturing intestinal stem cells or crypts in collagen-based scaffolds along with a cocktail of growth factors. Another avenue is to start with induced pluripotent cells, adult cells that have been “reprogrammed” to assume a primitive state with the ability to give rise to cell types as diverse as nerve, blood, and pancreas cells. Consortium members recently developed methods to generate intestinal organoids from such pluripotent cells and also to generate tissue layers underneath the crypt, such as the system of nerve cells in the gut.

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One eventual goal of building mini-intestines in the lab would be to replace damaged intestinal tissues in people with inflammatory bowel disease and reduce the odds of transplant rejection by using the patients' own cells as starting material. There are still several obstacles to overcome before scientists are ready to test transplants in humans, however. In mouse models, the efficiency of successful implantation is low, and the process of generating organoids requires culturing the cells in an expensive form of collagen scaffolding, although other substrates are being explored. Also, to be effective, the implant would need to cover vast areas in the gut and be connected to the vascular system.

Nevertheless, there are plenty of examples of how using a regenerative medicine approach could treat gastrointestinal diseases and disorders. One would be Crohn's disease, which involves inflammation and ulceration of the intestinal tract and is often treated by removing portions of the gut. Regenerating the intestinal tissue for individuals with this condition would help to avoid short bowel syndrome, a disorder that causes chronic diarrhea. Another disease that could potentially be treated with regenerative

medicine is necrotizing enterocolitis, a devastating and poorly understood condition of some premature infants wherein a portion of the intestine is destroyed by infection and inflammation. Restoration of the damaged tissue could be a reasonable treatment component for this potentially fatal disease.

Dr. Shivdasani finished by discussing how the discovery and characterization of intestinal stem cells could play an important role in designing future therapies for diseases beyond the gut. It is worth considering, he noted, that just as bone marrow transplantation and hematopoietic stem cells have been enormously valuable models for understanding the nature of certain stem cells, the intestine offers a parallel model. It is possible that the lessons learned from the intestine could be applied to repair other organs. The urgency of developing such regenerative medicine-based therapies is underscored by the problems currently faced by many people waiting for the availability of replacement organs. But because of scientists' innovative research advancing the field of intestinal stem cell biology, the future of regenerative medicine in the gut—and beyond—looks promising.

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Raeann and Shirley: Giving Back to Other Families Affected by Celiac Disease



Raeann (left) and Shirley (right)

In the Winter of 2003, Shirley noticed that her younger daughter, Raeann, then just shy of 2 years old, would tire easily when chasing her older daughter around the house. Not long after, when Raeann started throwing up frequently at meals, the family knew something was wrong. They would eventually learn that Raeann had celiac disease. As Shirley later read through the information available about celiac disease and its treatment on the internet, she sensed the challenge that lay before them. It was just the beginning of her family's journey learning about life with celiac disease, empowering themselves and others along the way with the knowledge needed not only to survive with the disease, but to thrive.

WHAT IS CELIAC DISEASE?

Celiac disease damages the digestive tract and results from an autoimmune reaction triggered by consuming gluten, a protein found mainly in foods containing wheat, barley, and rye. Gluten can also be found in other foods, as well as some supplements and other products. This autoimmune reaction—where the body damages its own cells—occurs in the small intestine, limiting nutrient absorption and potentially resulting in gas and bloating, diarrhea or constipation, and abdominal pain, as well as nausea and vomiting. However, a wide range of symptoms may occur outside the gastrointestinal tract as well. In children, celiac disease can have severe consequences, such as delayed growth and development, while in adults it may manifest as anemia, bone loss, rashes, fatigue, or other complications. Celiac disease affects an estimated 1 in 141 Americans.¹ The disease is more common in Caucasians, females, and those with a family history of celiac disease or with other specific diseases, including other autoimmune conditions such as type 1 diabetes. Diagnosis of celiac disease can be difficult and is often delayed; it is based on a series of physical exams, blood and genetic tests, and, lastly, an intestinal biopsy if other tests suggest the disease is present. Celiac disease usually resolves quickly with strict adherence to a gluten-free diet and avoidance of any other products containing gluten—but it can take longer for healing of any intestinal damage that may have already occurred, and the necessary dietary and other changes require constant, lifelong vigilance.

¹ Rubio-Tapia A, et al. The prevalence of celiac disease in the United States. *Am J Gastroenterol*. 107: 1538–1544, 2012.

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TUMMY TROUBLES

Initially, it took some sleuthing and persistence to get to the bottom of Raeann's vomiting and lethargy. Though her vomiting was frequent, it was not at every meal. Their pediatrician ruled out a viral infection but was not able to pinpoint the cause. Shirley kept a journal noting when the vomiting occurred and what foods Raeann had eaten. "It was to the point that I took out strawberries and I took out chocolate and different things out of her diet, but nothing really seemed to fit," says Shirley. After some scary incidents where Raeann gagged while eating in her high chair, they returned to the pediatrician's office, who referred them to a gastroenterologist. There, they had some tests done that finally gave them an answer. "They did blood work, and they called and said 'I think she has celiac disease,'" remembers Shirley. "My first question was 'what is that?'" recalls Shirley. The doctor then explained what celiac disease is and how it is

"My first question was 'what is that?,' says Shirley, recalling when the doctor diagnosed her daughter Raeann's celiac disease. "He said 'you go on a gluten-free diet' ... I thought 'what is gluten?' I had no idea."

treated. "He said 'you go on a gluten-free diet,'" says Shirley. "I thought 'what is gluten?' I had no idea." No one else in their family had ever been diagnosed with celiac disease or any other autoimmune disease. The doctor informed Shirley that they would need to take a biopsy from Raeann's intestine to confirm the diagnosis. Reluctant to put their young child under general anesthesia for the biopsy procedure, Shirley sought a second opinion from the head of the Celiac Disease Program at Children's National Health System, a children's hospital in Washington, D.C. They called day after day, hoping for an appointment to open up, and when one did, they drove there through the snow. After seeing Raeann, the doctor strongly supported the diagnosis. "He kind of pointed out these things we hadn't noticed

much, like ... a distended belly," says Shirley. "Then he said 'she has really skinny arms, her hair's kind of thin, you're telling me that she's kind of tired a lot, and looking at the blood work I'm 98 percent sure she has celiac, but you want to be 100 percent sure before you change her diet, so you need to do the endoscopy.'" They went ahead with the procedure that took a biopsy of Raeann's intestine, showing a flattening of the villi—tiny hair-like projections on the inside of the intestine that absorb nutrients—a sign that the intestinal lining was being eroded by her overactive immune system. The doctor confirmed the diagnosis of celiac disease and advised that Raeann immediately start eating a gluten-free diet.

A RADICAL DIETARY CHANGE

Thankfully, Raeann's celiac disease was caught early, before long-term gluten exposure could cause more lasting damage. After six weeks of eating 100 percent gluten-free, when they brought Raeann back to the gastroenterologist's office, she was well on her way to recovery. "She was like a changed child," remembers Shirley. "She was starting to run and jump and laugh, and, over the next year, she talked." In retrospect, the family realized that not only had Raeann experienced a delay in her language and other development, but her growth had also been slower. Once on the gluten-free diet, she caught up and gained 10 pounds over the next year. "It was really kind of a miracle—no medication, no surgery, just a very radical diet change," says Shirley.

But, as easy as the prescription of a gluten-free diet sounded, it required Raeann and her family to approach food in a whole new way. They went online and studied the lists of foods that Raeann could and could not eat. "We got rid of every bit of gluten. We went through all of our pantries and looked at everything," says Shirley. Shirley put together a notebook with information on gluten-free foods and recipes. As there were few stores where gluten-free items, such as baked goods like breads and cookies, were available, they ordered many items online. "I would order a

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dozen loaves of bread from a company, and they would deliver it packed in dry ice on my doorstep and then I'd put it in the freezer," recalls Shirley. Over time, they identified gluten-free baked goods and mixes that Raeann liked. Shirley and her husband helped Raeann come to terms with the diagnosis by reading her a children's book about a little girl diagnosed with celiac disease. "It was this whole thing about 'she's not different, she's just special,'" recalls Shirley. "It was nice to have a story she could relate to and feel like 'oh yeah, that's just like me.'"

"It was really kind of a miracle—no medication, no surgery, just a very radical diet change," remembers Shirley of the period after daughter Raeann went on a gluten-free diet for her celiac disease.

LEARNING TO THRIVE WITH CELIAC

Despite learning as much as they could about celiac disease, the family experienced its share of challenges and setbacks along the way. But they took these in stride as learning experiences that helped them achieve the minor victories necessary for Raeann to thrive throughout childhood and now, as a healthy and active teenager. On the rare occasions when Raeann accidentally consumed even small amounts of gluten, she would become violently ill 2 to 3 hours later. Raeann's family ingrained in her that she had to ask what was in any food she consumed and empowered her with the knowledge of what to avoid. Over the years, the family evolved strategies for managing Raeann's celiac disease. They slowly re-introduced gluten into the house for other family members to consume, along with a set of strict rules that the family and any visitors must follow to keep Raeann safe: no double-dipping in foods like peanut butter, a separate toaster for gluten-free bread, repeatedly wiping down the counters, and a place reserved for Raeann at the family table. "Everyone in our family

is very good with it, and I have my own cabinet with all gluten-free stuff," says Raeann. The family also juggles her brother's tree nut allergy. Every Halloween, Raeann and her brother would trade candy based on their different food restrictions.

As Raeann grew, she served as her own advocate, making sure as much as possible not to consume any gluten even when her family was not around to protect her. When Raeann was in first grade, Shirley would include a note reassuring her daughter that her packed lunch was gluten-free. "There was one day," says Raeann, "where she wrote a note that said 'your sandwich is gluten-free,' and then I looked at the sandwich and it wasn't the same bread that I usually had." She asked her teacher, who thought it was OK to eat, but called Shirley to check. When Shirley arrived at the school, she realized that she had accidentally switched Raeann's sandwich with her sister's, which did contain gluten. Although Shirley and Raeann's teacher felt terrible about the mix-up, they were glad that Raeann had spoken up and taken care of herself. Another time, when she had a friend sleep over, Raeann became ill after dinner. Her friend had to leave, and the family were left wondering whether the cause was cross-contamination from her friend's pizza to Raeann's gluten-free one or another source. "When something like that happens, we always wonder, 'what could it have been?' ... there's no way to really know," says Shirley. Due to the severity of her symptoms when she accidentally consumes gluten, Raeann must avoid foods that well-meaning friends and neighbors try to make gluten-free for her, in case their preparation or handling introduced any gluten contamination.

Eating out presents its own unique challenges for someone with celiac disease. Raeann is always careful and plans ahead when eating in a restaurant, sometimes bringing her own food or eating at home before or afterwards, but even foods that are advertised as gluten-free can be problematic. Recently, while eating out with friends for her junior year homecoming dance, she pre-ordered a gluten-free meal. "When the waitress served me

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my meal, she said this was the allergy meal, and I said ‘it’s gluten-free, right?’ and she seemed kind of confused,” recalls Raeann. “Everyone was eating their meals, so I said ‘oh, I’ll just eat it.’” She went home sick that night, missing out on time with friends and tarnishing her memories of the dance.

When Raeann was growing up, Shirley would always make gluten-free birthday cakes for everyone in the family, so Raeann could enjoy them. Raeann would always bring her own gluten-free cupcake that her mom had made to any birthday party she attended. But in recent years, a growing awareness of celiac disease and the wider availability of new foods and dining options for those following a gluten-free diet has made life easier in some ways. For Raeann’s sixteenth birthday, her friends and family went to one of her favorite restaurants featuring a gluten-free menu, culminating with a delicious, gluten-free cake. “It was a big cookie cake,” Raeann recalls fondly. “All my friends ate it too and it was the best cake I’d had.”

A CIRCLE OF GIVING

In the early years after Raeann’s diagnosis, Shirley and Raeann went to events hosted by the local chapter of a support group for families with children with celiac disease, where they found a welcoming community. There, Raeann participated in activities such as making gluten-free holiday cookies while Shirley learned from other parents of children with the disease. Eager to learn all they could about their daughter’s condition, Shirley and her husband also attended a celiac disease group event at a local library, featuring a presentation by a researcher in the field.

Starting when Raeann was in middle school, the family began sharing with others what they had learned in their years managing her celiac disease. At that time, two other parents contacted Shirley about meeting up and sharing experiences. “They were really interested in talking to me because I had had so many years [dealing with a child’s celiac disease], and their children had been more recently diagnosed,” says Shirley. They learned from her

experience, but Shirley also learned from them, including finding out about an app for her phone with information on sources of gluten-free food in the area or when traveling. “That sharing is so important,” remarks Shirley.

In describing why she and mother Shirley reach out to share their experiences managing her celiac disease, Raeann says, “People get so scared about it ... so it’s good to talk to people.”

When Raeann and her sister went through their bat mitzvah ceremonies—Jewish coming of age rituals—they chose as their community project to raise money for celiac disease research through a 5K race called “Making Tracks for Celiacs” in Baltimore, Maryland. In addition to collecting donations for the cause, they also participated in the walk/run with Shirley. Afterwards, they enjoyed the rows of gluten-free vendors and raffles. “There were people doing raffles for all different things, and the money goes to celiac research,” says Raeann. They also enjoyed the camaraderie of being around other families managing celiac disease.

In the past year, the family has reached out to area elementary and middle schools, on their neighborhood Facebook page, and through email groups, such as one for parents at the NIH, where Shirley works in a communications office, to share what they have learned. “We said ‘I have celiac, if you or anyone you know has celiac, we’d be happy to help,’” says Raeann. “It had been several years, and we feel like we have our arms around the problem,” adds Shirley. Both Raeann and Shirley responded to people who contacted them, sharing information on their experiences and resources, such as how Raeann approaches life with celiac disease and her favorite area restaurants. “We want to give back a little bit because people helped us and it was useful,” says Shirley. “People get so scared about it ... so it’s good to talk to people,” says Raeann. Shirley also stays current on the latest advances in celiac disease

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research by subscribing to an email newsletter from the NIDDK. (See further information below related to celiac disease available through the NIDDK.)

"It's one of the interesting things about myself," says Raeann about her celiac disease, adding that "it comes up right away when I make friends ... it's kind of like part of me."

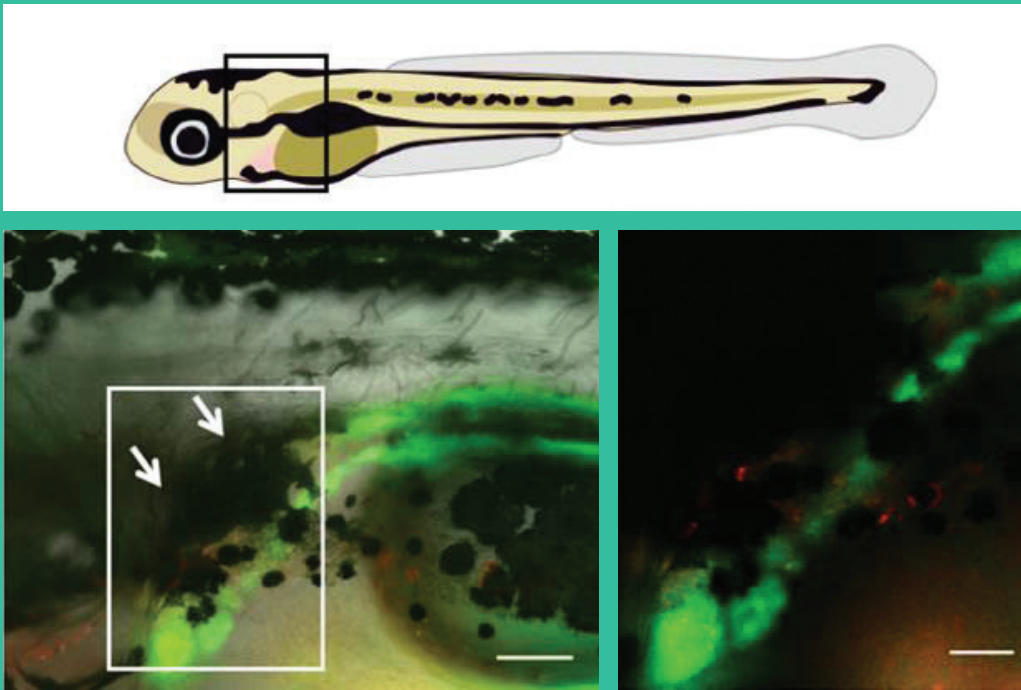
In June 2018, Raeann and Shirley attended the Washington, D.C. Gluten-Free Expo and Education Day, where Raeann participated as a speaker on a teen panel. The session was part of a day-long series of meetings on challenges confronting those on a gluten-free diet, with this particular session exclusively by and for teens. "I thought it was just going to be us talking," recalls Raeann. Instead, "they were all sharing their experiences and asking questions, so it was actually a lot of fun," she says. Raeann and Shirley also volunteered with the vendors, handing out gluten-free samples. There, they marveled at the wealth of gluten-free foods available and watched some cooking demonstrations, a stark

contrast from the scarcity of gluten-free options and more limited awareness of celiac disease when Raeann was first diagnosed.

These days, Raeann stays in excellent health as she juggles her academic work, including honor societies and tutoring responsibilities, sports such as lacrosse and cross-country, and planning for college. Like the message of the children's book her parents used to read her, Raeann views her formative experiences growing up with celiac disease in a positive light, writing about them in school essays and openly sharing her story with people she meets. "It's one of the interesting things about myself," she says, adding that "it comes up right away when I make friends ... it's kind of like part of me." While she and her family all wish she didn't have celiac disease, their advice to others affected by the disease is that it is manageable. "It may seem hard to change your whole diet, but it's not as bad as you think," says Raeann. "This is something that affects my food and my eating, but that's not a big barrier ... I can still do so many things," she notes. "Anything really," adds Shirley.

NIDDK CELIAC DISEASE RESEARCH AND EDUCATION

The NIDDK engages in multiple activities to advance research on celiac disease, including supporting studies conducted by scientists at institutions across the country to gain insight into the underpinnings of the disease and enable the development of improved diagnostics and treatments. Researchers have identified some of the molecular, dietary, and immune mechanisms underlying celiac disease development; revealed the prevalence of celiac disease, including a growing number of undiagnosed cases; and identified subclinical symptoms and tested new diagnostics in children with the disease. For example, The Environmental Determinants of Diabetes in the Young (TEDDY) is a long-term study supported by the NIDDK to understand environmental factors causing type 1 diabetes and celiac disease in children with high genetic risk living in the United States and European countries. In another celiac disease research effort, the Institute provided support for the National Health and Nutrition Examination Survey (NHANES), a program led by the Centers for Disease Control and Prevention (CDC), to gather data that are being used for ongoing analyses on the disease risk factors and dietary intake over time nationwide. The NIDDK also provides online fact sheets for the public and health care professionals with information on celiac disease symptoms, diagnosis, and a gluten-free diet, as well as updates on research. Information is available through the NIDDK website at www.niddk.nih.gov



Akin to the bone marrow in mammals, the zebrafish kidney marrow is responsible for the production of new blood cells. Research described in this chapter provides evidence that a cluster of cells called melanocytes form a shield to protect blood stem cells in the zebrafish kidney from damaging UV light.

Top illustration: The immature zebrafish.

Bottom left panel: White arrows point to melanocytes (black spots) that form a cluster that obscures the blood stem cells (red spots) from visualization.

Bottom right panel: Close-up (higher magnification) of the boxed area shown in left panel.

Image courtesy of Leonard Zon, M.D., Department of Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Harvard University. Kapp FG, Perlin JR, Hagedorn EJ,...Zon LI. Protection from UV light is an evolutionarily conserved feature of the haematopoietic niche. Nature 558: 445-448, doi: 10.1038/s41586-018-0213-0, 2018. Reprinted with permission from Springer Nature, copyright 2018.

Kidney, Urologic, and Hematologic Diseases

Diseases of the kidneys, urologic system, and blood are among the most critical health problems in the United States. They affect millions of Americans, and their impact is felt across the lifespan. To improve our understanding of the causes of these diseases, and to identify potential new treatments for them, the NIDDK supports basic and clinical research studies of the kidney and urinary tract and of the blood and blood-forming organs. The overall goal of the NIDDK's research programs is to increase our understanding of kidney, urologic, and hematologic (blood) diseases in order to enhance approaches to prevent and treat these serious conditions.

Normal, healthy kidneys filter about 200 quarts of blood each day, generating about 2 quarts of excess fluid, salts, and waste products that are excreted as urine. Loss of function of these organs, either for a short period of time or as a consequence of a gradual, long-term decline in kidney function, is a life-threatening condition.

It has been estimated that more than 30 million American adults have impaired kidney function—also called chronic kidney disease (CKD).¹ CKD has two main causes: high blood pressure and diabetes. The increases in obesity and type 2 diabetes in the United States in recent years—especially among children and adolescents—have grave implications for the Nation's health, as young people with these conditions are likely to face serious health complications at an earlier age than people who historically have developed these conditions later in life.

One feature common to kidney diseases arising from varying causes is the deposition of fibrotic scar tissue in the kidney. Research supported by the NIDDK has enhanced our understanding of the origin of this scar tissue, how it can impair kidney function, and how it might be prevented or treated. CKD, especially if undetected, can progress to irreversible kidney failure, a condition known as end-stage renal disease (ESRD). People with ESRD require dialysis or a kidney transplant to live. In 2015, over 701,000 patients received treatment for ESRD: over 493,000 received either hemodialysis or peritoneal dialysis, and

over 207,000 were living with a kidney transplant.² Racial and ethnic minority populations in the United States, particularly African Americans, Hispanic and Latino Americans, and American Indians and Alaska Natives, bear a disproportionate burden of CKD and ESRD. Compared to non-Hispanic Whites, ESRD prevalence in 2015 was about 3 times greater in African Americans, 1.3 times greater in Hispanics, 1.2 times greater in American Indians and Alaska Natives, and 1.0 times greater in Asians.² In recent years, scientists supported by the NIDDK have uncovered important genetic clues that may play a role in some of the health disparities related to kidney disease susceptibility and progression in minority populations.

The Institute supports a significant body of research aimed at understanding the biology underlying CKD and developing treatment strategies. The NIDDK's chronic renal diseases program supports basic and clinical research on kidney development and disease, including the causes of kidney disease,

¹ Centers for Disease Control and Prevention. National Chronic Kidney Disease Fact Sheet, 2017. Atlanta, Georgia: U.S. Department of Health and Human Services, 2017.

² U.S. Renal Data System, USRDS 2017 Annual Data Report: Epidemiology of Kidney Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland, 2017.

the underlying mechanisms leading to progression of kidney disease to ESRD, and the identification and testing of possible strategies to prevent development or halt progression of kidney disease. The NIDDK also supports studies of inherited diseases, such as polycystic kidney disease, congenital kidney disorders, and focal segmental glomerulosclerosis; and immune-related kidney diseases, such as IgA nephropathy and hemolytic uremic syndrome. The CKD Biomarkers Consortium (CKD BioCon) promotes the discovery and validation of novel biomarkers for CKD initiation, progression, and development of complications. A more complete understanding of biomarkers could allow physicians to detect kidney disease earlier and perhaps identify people at greater risk of progression, allowing them to tailor treatments to a specific individual. The Kidney Precision Medicine Project aims to obtain and evaluate human kidney biopsies from participants with acute kidney injury (AKI) or CKD for the purpose of creating a kidney tissue atlas, defining disease subgroups, and identifying critical cells, pathways, and targets for novel therapies.

The NIDDK's National Kidney Disease Education Program (NKDEP) is designed to raise awareness about the problem of kidney disease and steps that should be taken to treat CKD and prevent kidney failure. NKDEP represents a major educational outreach effort to patients, physicians, and the public. NKDEP also promotes the inclusion of estimates of kidney function as a part of routine blood testing and seeks to standardize measurements of protein in the urine, often a sign of underlying kidney disease.

Urologic diseases affect people of all ages, result in significant health care expenditures, and may lead to substantial disability and impaired quality of life. The NIDDK's urology research program supports basic and clinical research on the normal and abnormal development, structure, function, and injury repair of the genitourinary tract. Areas of interest include the causes of and treatments for urologic diseases and disorders such as benign prostatic hyperplasia, urinary incontinence, urinary tract infections, and urinary stone disease. To spur research in urinary stone disease, the Urinary Stone Disease Research Network (USDRN) is: a) conducting a randomized clinical trial to investigate the impact of increased fluid intake and increased urine output on the rate of recurrence of urinary stones in adults and children; b) conducting clinical research to understand and mitigate ureteral stent-related pain and symptoms; and c) providing

data and collecting biological samples from the studies to create a resource for future researchers.

Other disorders of the genitourinary tract, such as interstitial cystitis/bladder pain syndrome (IC/BPS)—also known as IC/painful bladder syndrome (PBS)—in women and men and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) in men, are also important research topics of the NIDDK's urology program.

IC/BPS is a debilitating, chronic, and painful urologic disorder. Based on a recent large, national interview survey, it is estimated that among U.S. women 18 years old or older, 3.3 million (2.7 percent) have pelvic pain and other symptoms, such as urinary urgency or frequency, that are associated with IC/BPS.³ Using a community-based epidemiologic survey, researchers have estimated that among U.S. men ages 30 to 79 years old, 1.6 million (1.3 percent) have persistent urologic symptoms, such as pain with bladder filling and/or pain relieved by bladder emptying, that are associated with BPS.⁴

NIDDK-supported basic and clinical research on IC/BPS and on CP/CPPS is focused on elucidating the causes of these conditions, identifying important subsets of patients to aid diagnostic stratification, and improving treatment and interventions. One example of an ongoing study is the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network, which supports research designed to uncover the underlying causes of IC/BPS and CP/CPPS and to characterize the disease profiles in patients.

Based upon national public health surveys conducted over several years, it is estimated that 1 in 10 U.S. adults (18 years of age and older) suffer from daily urinary incontinence; most of those affected are women.⁵ Many suffer in silence due to embarrassment and lack of knowledge about treatment options available. NIDDK-supported studies over the past several years have helped to advance knowledge about the efficacy of surgical treatment of urinary incontinence, as well as to

³ Berry SH, et al. *J Urol* 186: 540-544, 2011.

⁴ Link CL, et al. *J Urol* 180: 599-606, 2008.

⁵ *Urologic Diseases in America*. NIDDK, NIH Publication Number 12-7865, 2012.

provide new insights into non-surgical alternatives. As researchers continue to investigate treatment options, an equally important challenge is to identify and understand the important subgroups of patients with lower urinary tract symptoms (LUTS) through improved measurement of patient experiences of LUTS in men and women. To address this challenge, the NIDDK supports the multi-site Symptoms of Lower Urinary Tract Dysfunction Research Network (LURN). The NIDDK is also leading new efforts to explore whether it may be possible to prevent symptom onset and/or progression, thereby improving health. The NIDDK, in conjunction with the National Institute on Aging and the NIH Office of Research on Women's Health and Office of Behavioral and Social Sciences Research, established the Prevention of Lower Urinary Tract Symptoms (PLUS) Research Consortium to develop the evidence base for normal or healthy bladder function and to identify behavioral and other risk factors for conditions associated with lower urinary tract symptoms in women.

The NIDDK's hematology research program uses a broad approach to enhance understanding of the normal and abnormal function of blood cells and the blood-forming system. Research efforts include studies of a number of blood diseases, including sickle cell disease, the thalassemias, aplastic anemia, iron deficiency anemia, hemolytic anemias, thrombocytopenia, and the anemia of inflammation and of chronic diseases. To promote high-impact basic or pre-clinical research, the Institute supports the Stimulating Hematology Investigation: New Endeavors (SHINE) program and includes the following current research topic areas: regulation of blood (hematopoietic) stem cells, factors that play a role in the development of different types of blood cells, and red blood cell maturation. The Institute's SHINE II program seeks to further catalyze research in basic or pre-clinical, proof of principle research projects that are tightly focused and directed at validating novel concepts and approaches that promise to open up new pathways for discovery in benign hematology research. The NIDDK is also keenly interested in the basic biology of adult hematopoietic stem cells, which are used clinically in bone marrow transplants and may have broader application in gene therapy research.

KIDNEY FORMATION AND FUNCTION IN HEALTH AND DISEASE

Kidney Protein Could Expand the Window for Developmental Nephron Production: A study in mice showed that a partial reduction of the protein hamartin in the developing kidney leads to larger numbers of nephrons. Nephrons—the basic functional unit of the kidney—consist of various cells and structures that work together to filter waste products and excess fluid from the blood. The final number of nephrons in the kidney is widely variable, but research has shown that higher numbers of nephrons correlate with improved kidney function, and that nephron loss occurs with aging. While this variability is not completely understood, several factors, such as premature birth, lead to low nephron numbers. In mammals, the production of nephrons in the kidney begins during prenatal development and ends before birth (e.g., in humans) or shortly thereafter (e.g., in mice), at which point the body cannot generate additional nephrons. Therefore, understanding the mechanisms that govern nephron generation during early development has the potential to inform strategies to increase nephron numbers in those at risk, thereby reducing the likelihood of kidney disease later in life.

Previous research demonstrated that a pool of kidney stem cells, called nephron progenitor cells (NPCs), can turn into nephrons when signaled to do so at just the right time during kidney development. A team of scientists discovered that a protein called Mtor seemed to be one of these important signals, and thus used genetic mouse models to investigate the role of Mtor—as well as its functional inhibitor, hamartin—in shaping the number of nephrons in the kidney. Mice engineered to completely lack either Mtor or hamartin in their NPCs did not survive beyond 2 days after birth because they were unable to develop functional kidneys. However, mice engineered to lack just one of the two gene copies encoding Mtor (effectively reducing Mtor levels by half) in NPCs survived but had significantly lower numbers of nephrons and smaller kidneys than their control counterparts. Conversely, genetic deletion of one copy of the gene encoding hamartin led to a greater number of nephrons than in control mice. Further analysis of the mice with a partial reduction in hamartin revealed that the developmental “window” for generating nephrons in the kidney extended by about 1 extra day, resulting

in the significant increase in nephron number that was observed. Surprisingly, by combining these modified genetic backgrounds in mice, the scientists determined that the higher nephron number observed in mice with reduced hamartin was independent of the Mtor pathway. These findings define hamartin as part of an important pathway in mice that determines nephron number by regulating the window of time in which nephrons can form. Additionally, hamartin is coded by the gene *Tsc1*, which is involved in the development of a rare, multi-system genetic disease called tuberous sclerosis complex that causes benign tumors to grow in the kidney and other organs. Thus, future research building on these findings may shed light on cellular pathways in tuberous sclerosis complex, and, if the connection between numbers of nephrons and hamartin is conserved in humans, the hamartin signaling pathway could represent an important therapeutic target for people with or at risk for kidney disease.

Volovelsky O, Nguyen T, Jarmas AE,...Kopan R. Hamartin regulates cessation of mouse nephrogenesis independently of Mtor. *Proc Natl Acad Sci USA* 115: 5998-6003, doi: 10.1073/pnas.1712955115, 2018.

Identifying Patients with Chronic Kidney Disease Who Are at Increased Risk of Death: A recent study has established a correlation between the level of fibroblast growth factor-23 (FGF-23) in the blood of a person with chronic kidney disease (CKD), measured over time, and the risk of death. This research could alert physicians to the need for improved care for these individuals. Previous studies have found that the hormone FGF-23 may play a key role in kidney function and in the initiation and progression of CKD. Premature death from all causes, and from cardiovascular disease in particular, is higher in people with CKD than in healthy adults. Increased FGF-23 levels in the blood have been shown to be associated with increased mortality in CKD. However, it has been unclear how these levels change over time in a person with CKD, and whether repeated testing of FGF-23 levels over long periods of time can better predict clinical outcomes.

In 2001, the NIDDK established the Chronic Renal Insufficiency Cohort (CRIC) study to identify the risk factors for loss of kidney function and the link between kidney and heart diseases, and to apply this acquired knowledge to improving health. In the current study, CRIC investigators examined

circulating FGF-23 levels to see whether they increase over time and whether a rise in FGF-23 levels (i.e., a rising trajectory) is associated with an elevated risk of death among men and women with CKD. FGF-23 levels were measured in 1,135 CRIC participants at enrollment and then again 1, 2, 3, and 4 years later. Compared to levels when the participants first enrolled, FGF-23 levels rose slightly in later years for the group as a whole. However, using a special statistical analysis technique, the investigators identified three FGF-23 trajectory subgroups: stable, slowly rising, and rapidly rising. Unlike the subgroup whose FGF-23 levels remained stable over time, the 37 percent of participants in the slowly rising FGF-23 subgroup were at 4.5-fold higher risk of death, and the 7 percent of participants in the rapidly rising FGF-23 subgroup were at 15.2-fold higher risk of death. Thus, although stable in the majority of CRIC participants, rising FGF-23 levels in some people with CKD place them at exceptionally high risk of death. This finding could help kidney specialists identify patients with CKD at particularly high risk, based on rising FGF-23 levels, and thus provide more personalized care.

Isakova T, Cai X, Lee J,...Wolf M on behalf of Chronic Renal Insufficiency Cohort (CRIC) Study Investigators. Longitudinal FGF23 trajectories and mortality in patients with CKD. *J Am Soc Nephrol* 29: 579-590, doi: 10.1681/ASN.2017070772, 2018.

Health Benefits of Intensive Blood Pressure Control Outweigh a Slight Risk of Developing Kidney Disease: A new study found that in people who do not have chronic kidney disease (CKD), an intensive blood pressure control regimen increases risk of declining kidney function; this risk is generally outweighed by a reduced risk for cardiovascular events and death. Elevated blood pressure is relatively common in the U.S. population and is a risk factor for heart disease, stroke, and kidney disease. The Systolic Blood Pressure Intervention Trial (SPRINT) was designed to test whether using medications to reduce systolic blood pressure to a lower goal than currently recommended will reduce cardiovascular disease risk in people with high blood pressure but not diabetes. ("Systolic" refers to the higher of the two numbers in a blood pressure reading; it measures the pressure in the arteries when the heart beats. "Diastolic" refers to the lower of the two numbers and measures the blood pressure when the heart rests between beats).

SPRINT researchers previously reported that, among the subset of study participants who did not have CKD at the start of the trial, those who received an intensive blood pressure control regimen for more than 3 years were at a slightly higher risk of developing CKD than those who received a standard blood pressure control regimen. Researchers have now followed up on this initial observation by performing a more detailed analysis of SPRINT data to understand the broader health benefits and risks of intense blood pressure control. The scientists analyzed data from more than 6,600 study participants without CKD, about one-third of whom were women, to determine the rates of CKD development, cardiovascular events, and death after about 3 years. The scientists defined new-onset CKD as a reduction in kidney function of at least 30 percent, to a level of function considered less than normal. In the intensive group, 3.7 percent of participants developed CKD as compared to 1.0 percent in the standard group. The researchers also found that 4.9 percent of the intensive group experienced cardiovascular events or death, compared to 7.1 percent in the standard group. Further computational analysis of these data revealed that when compared with standard care, intensive blood pressure control led to a 2.6 percent increase in risk of developing CKD, but the risk of death or of cardiovascular events decreased by 2.2 percent. Through their calculations, the scientists predicted that statistically, for each death or cardiovascular event prevented by the intensive therapy regimen, there would be 1.2 cases of new-onset CKD.

In the intensive group, the reasons for and long-term consequences of the observed reduction in kidney function remain unclear. The researchers point out that because kidney filtration rates are dependent on blood pressure, reduced kidney function to some degree would be expected. Because cardiovascular events and, of course, death are far more serious outcomes, the health benefits of intensive blood pressure therapy outweigh the risk of developing CKD. However, the scientists note that additional follow-up time will help in understanding the longer-term risks and benefits of the two blood pressure control regimens described in the study. They also caution that in some cases, intensive therapy might not be the best option for blood pressure control because the risk of CKD development could be a higher priority than other health considerations. These findings, which add to those from other SPRINT studies, could help provide

valuable insights that inform decisions made by patients and health care providers.

Beddhu S, Rocco MV, Toto R,...Whelton PK; for the SPRINT Research Group. Effects of intensive systolic blood pressure control on kidney and cardiovascular outcomes in persons without kidney disease: a secondary analysis of a randomized trial. Ann Intern Med 167: 375-383, doi: 10.7326/M16-2966, 2017.

KIDNEY STONE TREATMENT

Treatment for Common Urinary Ailment No Longer Carved in Stone: Newly published results from a large clinical trial indicate that a drug frequently used in the emergency room (ER) to treat people with urinary stone disease has no benefit if the stones are below a certain size. Urinary stones, also called kidney stones, are pebble-like masses that can form in the kidneys if there is an excess of certain minerals in the urine. Stones vary in size from a grain of sand to (rarely) a golf ball. Those that get lodged in the ureters—the tubes that carry urine from the kidneys to the bladder—cause extremely painful symptoms. Although many symptom-causing stones subsequently pass out of the body on their own, appropriate medical evaluation is important because, if they do not, they can lead to infection and loss of kidney function. Men are much more prone to stones than women, although recent research suggests that rates may be rising in women. People with symptomatic urinary stones are often first seen in emergency departments. Under current guidelines, if a special imaging technique reveals a stone up to a certain size trapped in a person's ureter, a recommended first line of treatment is to prescribe a type of drug thought to promote stone expulsion, called an alpha adrenergic receptor blocker, or alpha blocker. One such drug is tamsulosin. The drug is taken daily by mouth for about a month, along with medication to alleviate pain until the stone passes.

However, because recent studies have called into question whether alpha blockers actually promote passage of stones that are below a certain size (including stones commonly considered large), NIDDK initiated the Study of Tamsulosin for Urolithiasis in the Emergency Department (STONE) clinical trial. The goal of the trial was to determine whether symptomatic patients given tamsulosin actually passed a symptomatic stone at

a significantly higher rate over the course of 28 days than patients given placebo (no medication) pills. Conducted at six different emergency departments, the STONE trial enrolled 512 participants 18 years of age or older with a symptom-causing stone of less than 9 millimeters (i.e., up to the size of a large green pea); the average size was close to 4 millimeters, and over half of the symptom-causing stones fell within the 3 to 4 millimeter range. About three-quarters of participants were men. Participants were randomly assigned to either tamsulosin or an identical looking placebo pill to be taken once a day, and then contacted by phone multiple times during the treatment phase to find out whether the stone had passed—i.e., whether he or she had seen or even collected it after urination. The STONE researchers found that, among the 497 persons for whom they had data, 49.6 percent of those in the tamsulosin group and 47.3 percent in the placebo group reported stone passage at 28 days—this small difference was not statistically significant (i.e., it likely happened by chance). Further, while the researchers also did not find significant differences between the groups in factors such as pain medication usage, time away from work, or return ER visits due to a stone, they did find that men on tamsulosin were more likely than men on placebo to report problems with ejaculation (a side effect of tamsulosin that has been noted in the past). These findings, in combination with similar findings from two other trials conducted in the United Kingdom and Australia, indicate no benefit of tamsulosin for promoting passage of stones of the sizes tested and suggest that guidelines for ER treatment of urinary stone disease may need to be revised.

Meltzer AC, Burrows PK, Wolfson AB,...Brown J. Effect of tamsulosin on passage of symptomatic ureteral stones: A randomized clinical trial. *JAMA Intern Med* 178: 1051-1057, doi: 10.1001/jamainternmed.2018.2259, 2018.

URINARY TRACT INFECTIONS AND THE MICROBIOME

Microbial “Irony”—Bacteria Provide Keys to Battling Urinary Tract Infections: Researchers have identified two different molecules that can limit growth of microbes that cause urinary tract infections (UTIs), adding to approaches being pursued to develop new strategies for clinical treatment of UTIs. UTIs are very

common and primarily affect women, who often go on to suffer long-lasting or repeated infections. The majority of UTIs are caused by bacteria. Although still treatable with available antibiotics, a steady and alarming increase in antibiotic-resistant bacteria has led researchers to seek out other ways to prevent or treat UTIs. Exploiting the knowledge that bacteria need host-supplied iron in order to survive—and that human hosts limit iron access during infection—is one very promising avenue of research.

UTIs are most commonly caused by uropathogenic *Escherichia coli* (*E. coli*), or UPEC. Whereas *E. coli* normally live in the human intestinal tract and benefit health, some strains possess factors that help them, with varying degrees of success, to colonize the urinary tract and cause disease. For example, UPEC that possess a genetic element called the *Yersinia* high pathogenicity island (HPI) can make a special iron-scavenging molecule, called a siderophore, that helps them obtain iron even as the host tries to fight infection by limiting its availability. Scientists have now found evidence that the HPI also enables UPEC to produce a second molecule, called escherichelin, that inhibits a siderophore produced by a totally different species of bacteria that can also cause UTIs. This finding—together with some initial findings in humans—not only provides insight into how some UPEC may eliminate microbial competitors, but also suggests that *E. coli* strains that can colonize the urinary tract and produce escherichelin but not cause disease (asymptomatic strains) might be useful as “probiotics” to prevent opportunistic UTIs caused by other bacteria.

In a second study, a different team of researchers sought out molecules that could inhibit UPEC growth itself. One of the first antibiotics ever identified, penicillin, is a natural product of a certain mold, and kills susceptible bacteria by inhibiting a key step in how they create their unique cellular “wall.” Similarly, the research team looked to an extensive library of naturally produced molecules, rather than synthesized chemicals, to screen for any that could limit UPEC growth under low iron conditions similar to those found during UTI. They hoped to find molecules that didn’t bind up (chelate) iron and further reduce its availability to have this effect, but instead could target UPEC cellular processes active during “normal” infection. From their screening process, they isolated a novel molecule produced by a bacterial species called *Streptomyces nicotiae* (*S. nicotiae*), which they

termed nicoyamycin A, or NicA. Testing revealed that purified NicA and two similar molecules subsequently isolated from *S. nicotiae* are potent inhibitors of UPEC growth in low iron conditions. However, despite efforts during the initial screens to exclude iron-chelating molecules, purified NicA (as well as the other two molecules) was subsequently found to be a potent iron chelator. Still, it is known that some antimicrobial molecules, such as the antibiotic tetracycline, have iron-chelating properties, even though that is not their primary mode of action. Thus, further studies of NicA and related molecules from *S. nicotiae* could reveal if this is the case, and, if so, the relative contributions of different molecular properties to their inhibition of UPEC growth. Encouragingly, a second set of molecules similar to NicA that were also tested inhibited UPEC growth but did not potently chelate iron, suggesting a greater likelihood that NicA and the other molecules act through another mechanism. In the meantime, the identification of multiple bacterial-produced molecules, such as escherichelin and NicA, that hamstring UTI-causing organisms under conditions of low iron is an encouraging step forward in finding new therapeutic strategies to combat UTIs.

Mike LA, Tripathi A, Blankenship CM,...Moblely HLT. Discovery of nicoyamycin A, an inhibitor of uropathogenic *Escherichia coli* growth in low iron environments. *Chem Commun (Camb)* 53: 12778-12781, doi: 10.1039/c7cc07732g, 2017.

Ohlemacher SI, Giblin DE, d'Avignon DA, Stapleton AE, Trautner BW, and Henderson JP. Enterobacteria secrete an inhibitor of *Pseudomonas* virulence during clinical bacteriuria. *J Clin Invest* 127: 4018-4030, doi: 10.1172/JCI92464, 2017.

INTERSTITIAL CYSTITIS DISEASE MEDIATORS

A Molecule in the Urine May Hold the Key to Improved Bladder Health for People with Interstitial Cystitis: New research has revealed that reduced levels of menthol in the urine are associated with interstitial cystitis, a finding with implications for improving both diagnosis and treatment. Interstitial cystitis (IC), also called IC/bladder pain syndrome (BPS), is a urologic chronic pelvic pain syndrome with symptoms that include urinary urgency and frequency, and pelvic pain. The causes and risk

factors for IC are not well understood, and there is no fully effective treatment. A better understanding of IC could lead to improvements in diagnosis and the development of new therapeutic approaches. Because people with IC often report foul odors in their urine, the researchers, in a recent study, examined a class of odorant molecules called volatile organic chemical compounds (VOCs) that may be altered with disease progression.

The scientists sought to identify differences in VOCs by comparing urine samples from women with IC to those from women with normal bladder health. Using sophisticated techniques, the researchers separated the various urinary VOCs from one another, determined their identities, and compared the results. These analyses yielded twelve distinct VOCs that were different between urine samples from the two groups. One of these compounds, menthol, was reduced in urine from women with IC. Menthol is widely used for a range of medicinal purposes, in part due to its anti-inflammation properties. Because inflammation of the bladder is a hallmark of IC, the researchers tested whether menthol plays a role in disease progression. They exposed mouse macrophages—immune cells that initiate inflammation—to menthol under conditions that instruct the macrophages to begin the inflammatory response. These cells did not turn on the genes or secrete the proteins necessary for inflammation, in contrast to the robust inflammation program initiated in cells that were not treated with menthol. Further analysis identified the specific cellular pathways normally triggering inflammation that were disrupted by menthol. These findings suggest that urinary menthol could serve as a useful diagnostic indicator of IC. Additional research will be needed to determine whether menthol, or the cellular pathways targeted by the VOC, could be utilized as a treatment to quell bladder inflammation in people with IC.

Shahid M, Lee MY, Yeon A,...Kim J. Menthol, a unique urinary volatile compound, is associated with chronic inflammation in interstitial cystitis. *Sci Rep* 8: 10859, doi: 10.1038/s41598-018-29085-3, 2018.

RED BLOOD CELL MATURATION AND RETENTION

New Tool Reveals Insights into Maturation of Red Blood Cells: Using detailed genetic data along with a recently developed analytical tool called “population

balance analysis” (PBA), investigators have developed models capable of predicting the mature blood cell type of early stage (progenitor) blood cells. Scientists have grappled with how to design studies that will reveal what controls or drives progenitor cells to undergo a process that ultimately leads to becoming more specialized mature cells (i.e., red blood cells). With previous significant technological advances, scientists are now able to use an approach called single-cell expression profiling to begin to understand what cell and tissue types will arise from an individual progenitor cell. In single-cell expression profiling, the “transcriptome” is characterized to provide a measure of a single cell’s gene activity. Active genes produce transcripts, which may serve as instructions for making proteins. A transcriptome is a collection of all the transcripts present in a given cell and gives an internal snapshot of what the cell is doing or how it is changing.

Using the transcriptome data of 4,763 individual cells, researchers showed that progenitor cells from female mice mature along distinct branches leading to one of seven blood cell fates. To understand pathways that progenitor cells might follow as they mature, the investigators had previously developed the analytical tool, PBA, to predict cell fate likelihood using snapshots of single-cell transcriptomes. PBA identified the commitment likelihood for each blood progenitor cell to each of the seven blood cell fates, and these predictions were confirmed by tracking the cell fate process. Unlike the classical models of blood cell maturation, the details provided by PBA revealed that the process is a continuous progression (rather than discrete stages of cell development). For example, the researchers were able to map the continuity of the red blood cell maturation process, called erythropoiesis, from the earliest progenitor cell to the final maturation stage. During these experiments, the researchers uncovered new insights in red blood cell development, including the first identification of IL-17A, a growth factor, as a strong stimulator of red blood cell maturation. The new knowledge obtained from single-cell data combined with PBA has allowed an in-depth examination of early stage blood cell maturation. This approach may provide similar insights into other cell maturation processes, and provides tools to further the fields of stem cell biology and regenerative medicine.

Tusi BK, Wolock SL, Weinreb C,...Socolovsky M. Population snapshots predict early haematopoietic and erythroid hierarchies. *Nature* 555: 54-60, doi: 10.1038/nature25741, 2018.

Zebrafish Employ Cellular Shield To Protect Blood Stem Cells from Damaging Ultraviolet Light: While studying the blood stem cells that reside in the kidney of the zebrafish embryo, researchers observed that cells called melanocytes were positioned above the stem cells in an umbrella pattern, and subsequently showed this umbrella served as a shield protecting stem cells from damaging ultraviolet (UV) light. When stem cells divide, they can form more stem cells or other cells that perform specialized functions. The blood stem cell niche is crucial for regulating the process of new blood cell formation, and its location varies among species—for example, it is found in the bone marrow of adult mammals and in the kidney of the majority of fish species. Little is known about the factors (e.g., environmental) that have influenced the placement of stem cell niches in mammals and fish.

A recent report described an opportune observation that a cluster of melanocytes formed an umbrella pattern directly above the kidney stem cell niche in zebrafish. Zebrafish are an important animal model system that scientists use to better understand human physiology and disease. One distinct advantage that zebrafish embryos have is that they are “transparent”—allowing scientists to watch the fertilized eggs grow into fully formed baby fish under a microscope. Previous research has shown that the melanocyte is a cell in human skin that produces and contains the pigment melanin; melanin protects skin cells from DNA damage caused by UV light.

This led the research team to hypothesize that melanocytes may serve as a shield to protect the blood stem cells in the zebrafish kidney from damaging UV light. They tested this hypothesis by studying a mutant form of zebrafish embryo that lacks melanocytes. After UV-light exposure, stem cells from mutant zebrafish embryos contained significantly more DNA damage compared to stem cells from normal zebrafish embryos with melanocytes. Furthermore, mutant zebrafish embryos showed a significant decrease in stem cell numbers compared to those in normal embryos. These findings suggest that the melanocytes were protecting the stem cell niche from damaging UV light. The researchers then sought to determine whether it was the “orientation” of the melanocyte umbrella that played a protective role. When anesthetized, zebrafish embryos flip upside-down—this change in orientation no longer positions the melanocyte umbrella between the UV light and

kidney stem cell niche. Under these conditions, stem cell numbers in normal zebrafish were reduced to the same level as those in mutant embryos after UV treatment. These findings confirm that the orientation of the melanocyte umbrella is critical to protect stem cells from UV damage and suggests that melanocytes form an optical shield.

The research team also showed that other types of fish contain a melanocyte shield and postulated that land-based animals such as mammals employ a “bone” shield to protect the blood stem cell niche in the bone marrow from damaging UV light. These new findings suggest that protection against damage from UV light plays an important role in influencing the placement of animals’ stem cell niche.

Kapp FG, Perlin JR, Hagedorn EJ,...Zon LI. Protection from UV light is an evolutionarily conserved feature of the haematopoietic niche. *Nature* 558: 445-448, doi: 10.1038/s41586-018-0213-0, 2018.

TREATING BLOOD DISORDERS

Identification of Potential Therapeutic Target for Sickle Cell Disease and Other Hemoglobinopathies:

Researchers recently identified a protein governing production of a type of hemoglobin, called fetal hemoglobin (HbF), in adult human red blood cells, a finding that may have important implications for treating red blood cell diseases. People with sickle cell disease suffer from chronic anemia and episodes of bone, joint, and muscle pain, as well as other complications because their red blood cells form rigid, “sickle” shapes, leading to impaired blood flow in small blood vessels and reduced oxygen delivery to tissues. The disease results from genetic mutations that affect the form of hemoglobin often called “adult” hemoglobin, even though its production begins soon after birth. Such diseases, affecting hemoglobin structure and/or production, are referred to as hemoglobinopathies. Individuals with another hemoglobinopathy, called β -thalassemia, also suffer from chronic anemia. In this case, impaired adult hemoglobin production results in reduced numbers and viability of red blood cells. Although HbF is mostly undetectable in adults and children (after about 6 months of age) in the general population, increased levels safely persist to varying degrees in some people. Researchers previously observed that

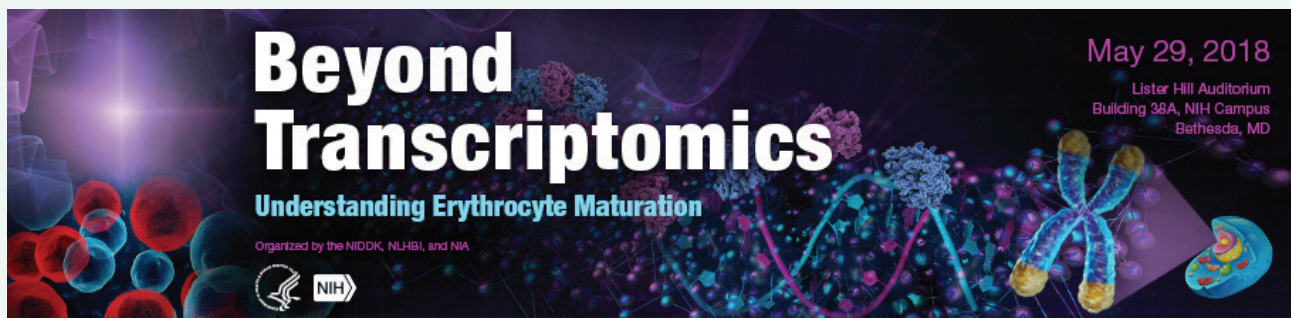
people with sickle cell disease who retain higher HbF levels have less severe disease. Thus, one potential treatment approach is to reactivate HbF production to sufficient levels in adult red blood cells such that it compensates for the defects in hemoglobinopathies.

One way to achieve such reactivation of HbF would be to use a drug to target proteins that regulate its production. Thus, for the current study, the researchers aimed to identify regulators of HbF production that would potentially be “druggable.” To do this, they designed a screen for proteins called kinases in adult red blood cells, to see if any of the numerous kinases in human cells might affect HbF production. They focused on this type of protein because many protein kinases are druggable with small molecules that inhibit their activity. For their screen, the scientists used a genome editing tool called the CRISPR/Cas9 system to target genes encoding protein kinases. The CRISPR/Cas9 system allows for a gene’s DNA to be edited with unprecedented precision, and the resulting changes can be screened for the desired outcome—in this case, a change in a sought-after regulator would result in a change in HbF levels. This approach led to the identification of a protein kinase called heme-regulated inhibitor (HRI) as a repressor of HbF production. Specifically, HRI depletion in normal adult red blood cells resulted in significantly increased HbF levels. Furthermore, in subsequent experiments the scientists found that HRI depletion in red blood cells from patients with sickle cell disease led to increases in HbF levels, which suggests that this strategy may be beneficial to people with sickle cell disease.

The discovery of HRI as a regulator of HbF opens the possibility for future research to identify a small molecule drug that would selectively inhibit it; such a drug may have profound clinical benefit to people with sickle cell disease or β -thalassemia by increasing HbF to sufficient levels to overcome the pathology associated with these red blood cell diseases.

Grevet JD, Lan X, Hamagami N,...Blobel GA. Domain-focused CRISPR screen identifies HRI as a fetal hemoglobin regulator in human erythroid cells. *Science* 361: 285-290, doi: 10.1126/science.aaa0932, 2018.

Scientific Workshop: Exploring Approaches To Bolster Understanding of Red Blood Cells



To identify gaps in our knowledge of red blood cell (also known as the erythrocyte) maturation, the NIDDK, in collaboration with the National Heart, Lung, and Blood Institute and the National Institute on Aging, held a workshop titled “Beyond Transcriptomics: Understanding Erythrocyte Maturation” in May 2018.

In a healthy person, the production and destruction of erythrocytes is maintained at a certain level. With respect to production, approximately two million newly formed erythrocytes leave the bone marrow and enter the blood stream every second in the adult human. Unlike most cells of the body, the mature erythrocyte no longer contains a nucleus—the cellular compartment that serves as the cell’s command center by sending directions to the cell to grow and mature. Pathways critical to understanding how the erythrocyte matures and how the cell maintains its metabolism after the nucleus is jettisoned are not completely understood. Knowledge gained from new research into pathways and metabolism may shed light on disorders that affect erythrocyte levels such as certain types of anemia (decreased erythrocyte level) or polycythemia (increased erythrocyte level).

The main purpose of the workshop was to stimulate research interest beyond the recent progress that has been made using an approach called “transcriptomics”—the study of messengers, or “transcripts,” between genes and proteins that eventually carry out the instructions encoded in the genome. To explore a range of other research avenues, workshop attendees, who were drawn from across the United States, Canada, and Europe, had expertise in several different scientific disciplines including cellular biology, molecular biology, molecular genetics, physiology, bioinformatics, developmental biology, pathology, and hematology. By the conclusion of the meeting, the participants identified several research questions that, if answered, could move the field forward, including the: characterization of the structures of the erythrocyte cell membrane that ensure integrity and survival in the blood; identification of external and internal factors that modify erythrocyte structure and function in normal and diseased states; determination of the mechanisms in the bone marrow that allow for and regulate the differences found in normal erythrocytes; and identification of the signals for destruction and production of proteins in developing early stage erythrocytes or fully mature erythrocytes.

Workshop Charts a Path Forward to Imaging the Kidney and Its Function

On July 12–13, 2018, the NIDDK held a workshop on the NIH campus in Bethesda, Maryland, to discuss current approaches to renal (kidney) imaging and to improve the characterization of kidney function, structure, and damage.

Kidney disease remains an enormous public health burden, but discovery of novel treatment and prevention strategies has been challenging. Research to develop new therapies for kidney disease would greatly benefit from an improved ability to visualize the structure of the kidney in finer detail and to evaluate its functional status. In this workshop, NIDDK brought together experts from different fields to explore opportunities to advance the development of new technologies and approaches to kidney imaging. A range of topics was covered in the presentations, including current cutting-edge science in renal imaging, examples of clinical areas in which imaging approaches have been successfully applied, and technological hurdles that impede translation of laboratory discoveries to clinical settings. The workshop featured a poster session that focused on additional

technologies and recent studies, and provided an opportunity for junior investigators and trainees in this area to present their research.

The workshop also convened several breakout group sessions to explore topics in greater depth. Through these sessions and subsequent discussion, ideas emerged to tackle several important challenges in renal imaging development and implementation, such as identifying research areas essential for scientific advancement; defining critical measurements of kidney structure and function; improving the drug development process; and validating clinical imaging methodologies. The workshop revealed that a robust scientific community is currently working toward each of these goals. However, workshop participants agreed that the development of multidisciplinary teams that include nephrologists (specialists in kidney disease), radiologists, engineers, and the biomedical industry will likely accelerate progress. Scientific opportunities discussed during the meeting are expected to inform new approaches to renal imaging, thereby paving the road for the next generation of kidney disease therapeutic strategies.

Workshop Forges Collaborative Approach to Understanding Chronic Kidney Diseases in Agricultural Communities

On June 25–26, 2018, the NIDDK co-sponsored—together with the National Institute of Environmental Health Sciences (NIEHS) at NIH—a workshop in Bethesda, Maryland focusing on research to understand the causes of and potential treatments for chronic kidney diseases in agricultural communities. This workshop brought together clinicians, basic scientists, epidemiologists, and public health officials to discuss current gaps in knowledge and to develop a coordinated scientific research agenda on this topic. In addition to NIDDK and NIEHS staff, other organizers included representatives from the National Institute for Occupational Safety and Health at the Centers for Disease Control and Prevention (CDC), the Fogarty International Center at the NIH, and the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán in Mexico. The workshop was part of ongoing efforts to advance research in this area, including NIEHS's formation of a working group on this topic in December 2017 and related work through their collaborating center with the World Health Organization.

In recent years, chronic kidney diseases of unknown or unexpected origins have been on the rise, particularly in agricultural communities around the world, including in the United States. These diseases go by many names depending on where they occur, including chronic kidney disease of unknown or non-traditional etiology, chronic interstitial nephritis in agricultural communities, and Mesoamerican nephropathy. Possible contributing factors identified to date include heat stress, dehydration, drugs or herbal supplements, environmental chemicals, and a combination of exposure with genetic susceptibility. Many questions remain about them, such as: 1) whether these are one common disease or a syndrome of related conditions that are unique to a given region, and 2) which factors are most important to their development and progression.

Workshop participants reflected the international and interdisciplinary expertise needed to tackle this problem through building consensus around the scientific path forward. The speakers presented on available information, challenges, and research opportunities related to these chronic kidney diseases in agricultural communities occurring in such regions as the Americas (mainly the United States and Central America), Sri Lanka, the Balkans, and other parts of the globe. Session themes focused on defining these diseases, using geography to provide new insights, investigating causes, studying the agricultural environment, gaining trust in the community, and studying the entire population for genetic and epidemiologic clues. Breakout working groups were tasked with addressing challenges in areas such as defining cases, identifying common data elements, understanding ethical barriers to studying agricultural workers, and creating a research agenda to advance understanding of this mysterious and far-reaching disease.

The meeting concluded with a summary of the wide range of inter-related topics touched on by the speakers that will inform future research on chronic kidney diseases in agricultural communities, including key questions in defining the disease, its causes, and its susceptibilities; developing necessary research tools; designing animal and clinical studies; and identifying opportunities for prevention. Meeting organizers plan to develop a summary for publication in the scientific literature describing the workshop proceedings. Scientific opportunities discussed during the meeting will inform future efforts to advance research addressing the many unanswered questions surrounding chronic kidney diseases affecting agricultural communities.

STORY OF DISCOVERY

Getting a Notch Up on Cord Blood Cell Transplantation

Blood stem cell transplants can be life-saving for people with a number of conditions. However, it can be challenging to find blood stem cells in needed quantities from a donor whose cells are similar enough to a patient's cells to be a sufficient “match” for transplantation. Cells from bone marrow and from circulating blood have been used in treatments for many years, and blood from the umbilical cord, collected after a baby is born, has been another source of blood stem cells used more recently. Cord blood from donors is available in public cord blood “banks,” and the match between donor and recipient may not need to be quite as close as is needed for transplants of other types of blood cells. However, cord blood from each donor exists in only limited quantities. Researchers supported by NIDDK have discovered key factors that promote blood stem cell growth and maturation, and, with these insights, have developed ways to expand the numbers of cells from cord blood for use in transplants. This research may increase the availability of such treatments to benefit many more people.

CORD BLOOD CELL TRANSPLANTATION

Cord blood is found in blood vessels of the placenta and the umbilical cord—tissue that is normally discarded. It is collected after a baby is born and after the umbilical cord is cut between the mother and the baby. Approved by the U.S. Food and Drug Administration only for use in blood (hematopoietic) stem cell transplantation procedures, cord blood has been transplanted into thousands of patients who have serious medical conditions such as bone marrow failure syndromes, blood disorders, immunodeficiencies, certain metabolic disorders, and cancers. Cord blood stem cells (which are

different from embryonic stem cells) facilitate healing and repair of damaged cells and tissue—that is, they are early stage blood cells that, like other blood stem cells, provide a source for the regrowth of all the types of specialized blood cells. Cord blood transplantation, therefore, is an example of regenerative medicine.

A critical advantage of cord blood is that cord blood stem cells can be easily transplanted; the transplanted cells do not have to be an exact match with the recipient's tissues. However, the main disadvantage is that a single umbilical cord is insufficient; a single umbilical cord contains a limited number of stem cells that do not mature into a sufficient number of specialized types of blood cells needed for adults. Cord blood cell transplants (from a single umbilical cord) could thus place an adult patient at increased risk of life-threatening infections due to the inadequate number of infection-fighting types of blood cells. Such patients may need two or more units of cord blood. Yet, they may still be at risk because, compared with conventional bone marrow transplantation, it may take longer for transplanted cord blood stem cells to engraft—that is, to start working properly in a patient's body—and give rise to other types of blood cells. For this reason, cord blood cell treatment is used more often in children, who have a small body size and thus require fewer cells. Basic research on blood cells has sparked ideas for expanding the numbers of cells in a unit of cord blood.

NOTCH: THE STORY BEGINS

In 1994, while investigating the cellular and molecular mechanisms underlying the production

STORY OF DISCOVERY

and function of blood cells, NIDDK-supported researchers discovered for the first time that in human early stage blood cells, a gene encoding a protein called Notch is “turned on.” NIDDK-supported researchers then set out to determine whether Notch influences the function of blood stem cells. Notch, which sits in the cell membrane, transmits signals originating from the outside of the cell to direct the turning on or turning off of genes. Using laboratory mice, they demonstrated, in 2002, that activated Notch had two distinct activities—1) the inhibition of stem cell maturation into different blood cell types and 2) the stimulation of stem cell self-renewal (*i.e.*, an increase in stem cell number). In 2003, with support from NIDDK and other sources, researchers reported that they could induce early stage mouse blood cells to proliferate by exposing the cells to an engineered protein that binds to Notch; this protein was a modified version of a protein called Delta1. Interestingly, the researchers did not see this effect if they simply mixed the modified Delta1 protein into the liquid in which the cells were growing; through their experiments, they realized they had to immobilize this protein on the dish in which the cells were growing for it to have the desired effect of increasing the numbers of cells.

NOTCH-DRIVEN ENGRAFTMENT OF CORD BLOOD CELLS IN HUMAN PILOT STUDY

Taking advantage of their knowledge of the Notch signaling pathway and blood stem cells and the modified Delta1 protein, investigators supported by NIDDK and others were able to significantly increase, by greater than 100-fold, the number of early stage blood cells from a unit of cord blood. The researchers then conducted, and reported in 2010, a pilot clinical study of 10 participants with leukemia to begin to assess the safety of infusing patients with cord blood stem cells that had been expanded to greater numbers using the modified Delta1 protein/Notch procedure, and to begin evaluating the engraftment properties of the expanded stem cells. In the course of their

treatment for leukemia, the participants were given radiation therapy and chemotherapy to destroy the blood cancer cells; these treatments also destroyed the stem cells in their bone marrow. Cord blood cell transplantation was subsequently used to repopulate the bone marrow. Each participant received two units of cord blood—one unit of non-expanded blood and one containing expanded numbers of blood cells, or two units of non-expanded blood. In this small group of participants, no safety issues were encountered. In addition, the time for engraftment, measured in terms of white blood cell recovery, was significantly shorter for participants who received Delta1/Notch-expanded cells than for those who received only non-expanded cord blood.

MANIPULATING BLOOD STEM CELLS INTO MATURE BLOOD CELLS

Blood stem cells mature along two tracks: 1) the myeloid lineage, which produces white blood cells (*e.g.*, macrophages and neutrophils), red blood cells, and other cells, and 2) the lymphoid lineage, which produces T cells, B cells, and other cells. The immune system consists of two major pillars: the innate (general defense) and the adaptive (specialized defense). Both systems work closely together and take on different tasks. Researchers have sought to identify a minimum cocktail of factors necessary to enable the reconstitution of both myeloid and lymphoid lineages from blood stem cells. Previous NIH-supported research found that experimentally increasing production of a factor called HoxB4 in mouse early stage blood stem cells could reconstitute the myeloid lineage but only minimally reconstituted the lymphoid lineage. In further experiments to try to reconstitute both lineages, in 2016, investigators supported by NIDDK and others discovered that Delta1/Notch activation of mouse early stage blood stem cells, in combination with increased HoxB4 in these cells, enabled the cells to fully reconstitute the myeloid and lymphoid lineages when transplanted into mice lacking their own blood cells.

STORY OF DISCOVERY

CLINICAL TRIAL ASSESSMENTS OF NOTCH-ACTIVATED CORD BLOOD CELL TRANSPLANTATION

The NIDDK-supported research to increase numbers of blood stem cells led one of the investigators to start a clinical cell therapy company. The company is investigating (in two Phase 2 clinical trials) whether Delta1/Notch-expanded cord blood cells could reduce the time to white blood cell recovery and reduce the rate of infections in individuals with blood cell cancers.

NIDDK-SUPPORTED RESEARCH

The translation of scientific knowledge and technology into improvements in the practice of medicine is central to the missions of the NIH and the NIDDK. As this story illustrates, the initial investment in basic science research has led to the development of a laboratory-based methodology to expand the stem cell population in cord blood for potential beneficial transplantation treatment of people with a myriad of blood disorders and diseases.

SCIENTIFIC PRESENTATION

Dr. Iain Drummond—Kidney Regenerative Medicine and the (Re)Building a Kidney Consortium

Dr. Iain Drummond is an Associate Professor at Massachusetts General Hospital and Harvard Medical School, and the Director of the Kidney Group of the Harvard Stem Cell Institute. An expert in kidney developmental biology and kidney disease, Dr. Drummond is a member of the NIDDK-supported (Re)Building a Kidney Consortium.

At the September 2018 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Drummond presented an overview of regenerative medicine challenges and approaches as applied to the kidney and kidney disease, with a special focus on the work of the (Re)Building a Kidney (RBK) Consortium, including work from his own laboratory.

Regenerative medicine is a multidisciplinary field focused on developing ways of replacing, engineering, or regenerating human cells, tissues, or organs to restore, establish, or enhance normal biological function that has been lost to disease or trauma. Its progress relies on concerted and collaborative efforts in the life and physical sciences and engineering. Currently, the scientific knowledge and approaches to regeneration vary greatly by organ and tissue. A common thread is that directing regeneration in any tissue is a complicated process that will likely require multiple strategies.

Many of the diseases and conditions within the NIDDK research portfolio, from diabetes to liver and digestive disorders to problems of the bladder and kidney, are amenable to regenerative medicine approaches. Regarding kidney disease in particular, the last few years have seen fundamental advances

that have helped contribute to different strategies toward rebuilding a kidney or regenerating kidney tissue. Human kidneys are bean-shaped organs about the size of a fist that perform many critical tasks, including cleansing metabolic waste products from the blood and maintaining proper salt and mineral balance and fluid volume in the body; each of the two kidneys normally found in a person is comprised of approximately 1 million basic functional units, called nephrons, that carry out the majority of these tasks. Loss of kidney function can thus lead to build up of toxins in the blood and other problems, and total kidney failure is deadly without dialysis or a kidney transplant. In his presentation, Dr. Drummond described recent advances, pressing questions, and the development and testing of various strategies by which researchers may be able to accomplish restoration of kidney function via regenerative medicine approaches.

KIDNEY REGENERATIVE MEDICINE AND HUMAN DISEASE: MULTIPLE CHALLENGES, TWO OVERARCHING GOALS

Dr. Drummond reminded the group of the prevalence of chronic kidney disease, which has a number of causes, including diabetes, hypertension, and inherited diseases such as polycystic kidney disease. There has been little advance in the last 50 years in treating chronic kidney disease or end-stage renal disease (ESRD, or kidney failure) since dialysis treatment became available—a treatment, he noted, that is really a “holding pattern” as it does not solve the underlying problem. Kidney transplantation

SCIENTIFIC PRESENTATION

to replace the function of at least one damaged or diseased organ can be life-saving for people with ESRD, but limited organ availability means that people die every day waiting for a kidney. Regenerative medicine could thus be an answer if it provides functional kidney tissue or entire organs—but how to go about it?

Dr. Drummond broke down the regenerative medicine challenge of building a kidney, or making new kidney tissue, into steps, using the analogy: An iPhone is composed of many component parts—phone, camera, touch screen, microphone, *etc.*—that together enable the device to act as a single complex unit capable of carrying out numerous tasks. At the same time, each contributing component can be developed independently of the others and as such provide milestones of success for development of the final, complex device. Similarly, while much more complicated than a smartphone, the kidney has many component parts with specific functions that contribute to overall organ function, but which can potentially be isolated and worked on individually.

Looking at the kidney in this way, according to Dr. Drummond, the challenges of generating and using a kidney/kidney tissue *de novo* become somewhat more tractable as specific questions:

- How do we make kidney cells?
- How do we know we have made the right kidney cells?
- How do we organize them into useful structures?
- How can we show they are useful for functional replacement?
- How do we integrate them into patient tissue to augment or replace renal function?

At the same time, he noted, it is equally important to consider how it might be possible to keep people from progressing to ESRD through studies to better understand deterioration and damage in key parts of the kidney. This leads to a sixth question:

- How do we promote regeneration of damaged kidney tissue?

Approaches for addressing the burden of kidney disease and kidney failure—for organ/tissue replacement and prevention of disease progression—are being pursued in the (Re)Building a Kidney (RBK) Consortium. RBK was started in 2015 to optimize approaches for isolation and expansion of different kidney cell types, and their integration into complex structures that replicate human kidney function.

Dr. Drummond broke down the RBK mission in terms of the two overarching goals: (1) engineer and engraft new, functional kidney tissue and (2) identify repair mechanisms in the injured kidney that can be exploited therapeutically.

Multiple research groups in the United States, Australia, and New Zealand are part of RBK, tackling the different challenges. Using a published schematic diagram that captures the six challenges and how they fit together as a reference map, Dr. Drummond proceeded to lead the audience on a journey highlighting scientific advances from the RBK Consortium and other research groups that are occurring along the way to the ultimate goals of functional kidney tissue and reparative processes.

MAKING (ENOUGH) KIDNEY CELLS WITH THE POTENTIAL TO WORK TOGETHER

Dr. Drummond began with the fundamental question: how do we make cells? It turns out that the initial objective is to begin with human-derived pluripotent stem cells—*i.e.*, cells that retain the potential to be coaxed into becoming many different types of cells found in the human body, find a way to drive them specifically into a nephron progenitor cell state (nephrogenic stem cells), and optimize the expansion of those cells to produce the billions of cells that will be needed to create replacement tissues for patients.

Dr. Drummond summarized a number of advances in the field that have yielded a group of signals and growth factors that, when sequentially applied to pluripotent cells, produce cells that resemble early nephrogenic cells found during kidney development.

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He noted that successful generation of these cells in the laboratory is usually measured using the level of expression of certain marker genes (e.g., *OSR1*, *Six2*, and *PAX2*). Further, he noted that the field is at a stage now where people have optimized these procedures, citing in particular the work of RBK member Dr. Melissa Little and researchers at the Harvard Stem Cell Institute (Drs. Ryuji Morizane and Joseph Bonventre), in which almost 90 percent of pluripotent cells driven through their protocol emerge with markers denoting them as potential kidney progenitor cells.

RBK has adopted these successful and highly reproducible strategies to create new nephron progenitor cells—and to expand their numbers. Dr. Drummond described how RBK scientist Dr. Leif Oxburgh has generated growth conditions that support the development and expansion of mouse kidney cells and adapted that to nephrogenic cells derived from human-derived pluripotent stem cells—meaning that researchers can now reliably create mouse and human kidney stem cells and expand them.

Moreover, these cells apparently have the potential to work, as suggested by experiments in which Dr. Oxburgh has taken some of these nephrogenic cells, allowed them to differentiate, and implanted them in mice under the outer covering (capsule) of the kidney, a procedure referred to as engraftment. The result was the growth of a ball of cells that contained many elements of a fully functional kidney (segmented tubules, podocytes, and vascular components), a very encouraging finding.

However, according to Dr. Drummond, the current challenge is whether researchers can actually integrate this type of engrafted tissue into the body such that it has basic kidney functions—e.g., blood filtration (cleansing) and drainage into a host kidney collecting system. So, stepping back, kidney regenerative medicine scientists are working on generating “organoids”—miniature, multicellular, three-dimensional structures that resemble and/or mimic (but usually do not yet fully replicate) corresponding organs, such as was seen in the mouse

kidney capsule experiment—in the laboratory, outside of a living organism, for subsequent engraftment and testing. This means first taking the experimentally created nephrogenic progenitor cells and trying to drive them into organs and tissue structures, and there are multiple approaches to doing that.

For example, Dr. Little and her research group have basically concentrated nephrogenic progenitor cells into small pellets, finding that this approach enhances their differentiation (maturing into more specialized cells) into organoids in which different nephron segments and kidney cell types can be detected visually through special cellular staining techniques. Dr. Drummond pointed out that this experiment and others underscore how, intriguingly, most of the approaches to creating kidney organoids do not involve use of growth factors at the final differentiation stage, suggesting that there is a lot of self-differentiation and self-organization of tissue on the part of these cells, once they get started—and also a great deal of mystery about how these nephrons are forming. Hence, there is also a lot to learn about whether it might be possible to develop directed and organized approaches to drive the cells into specific/desired structures and shapes. This is another highly complex research problem being taken on by RBK scientists (led by Drs. Oliver Wessely and Jan Jensen), who are using specialized computational approaches to help reduce the number of variables and experiments that need to be done in order to answer some of the questions about what factors are driving differentiation into what types of cells in the organoids. The caveat remains, however, that the kidney organoids are still limited—in their structure, level of maturity, and functional capacity versus what exists in a normal, human adult kidney.

“HAVE WE MADE THE RIGHT CELLS?”

Dr. Drummond turned to another of the fundamental challenges for kidney regenerative medicine: what are all of the cells in the kidney? That is, do scientists even know what they all are and their key functional features?

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For example, he noted that the kidney stroma—the mixture of supportive cells and molecular matrix surrounding more discrete structures—was originally thought to be homogeneous, but studies from RBK member Dr. Thomas Carroll's group are showing that it has at least six different zones defined by different gene activity patterns in the kidney. This is important because kidneys without stroma do not make nephrons; thus, Dr. Drummond observed that in the organoids there are no doubt stromal cells that are understudied, and we need to know how they support growth of the kidneys. Similarly, work from RBK member Dr. Ondine Cleaver's research group is showing that blood vessels in the kidney are highly heterogeneous; a greater understanding of these vessels is critical to future strategies for conveying blood to the filtration unit of newly generated/regenerated kidney tissue.

Another consideration, Dr. Drummond observed, is that most of the work on kidney development has been performed in animal models—specifically, mice and zebrafish—meaning that it is critical to figure out how the data derived from those studies applies to human biology. For example, the human and mouse kidney differ in shape and size, and that is reflected in differences in gene expression (which genes are turned on or off), as revealed in part by research performed by RBK member Dr. Andrew McMahon and his group; they continue research toward understanding the differences in factors governing gene expression and also differences in cell types among species.

Dr. Drummond also remarked on how, in addition to a static description of cell types and the genes they express, developmental studies inherently need to integrate a time factor and account for cellular changes, similarities, and interactions that occur over the course of and during transitions in development of an organ. This perspective can also be applied to models of the progression of injury in the kidney, and to understanding where cells have productive responses and where cells do not.

STRUCTURAL CONSIDERATIONS AND PLUMBING

For externally generated kidney cells, tissues, and organoids to be tested and ultimately useful, Dr. Drummond noted they will need to be placed in structures that can be used by the body, so the essential question is: how to build these and make them do what they need to do? Dr. Drummond described a number of approaches being pursued in RBK. For example, RBK member Dr. Jennifer Lewis and her group are focused on “printing” materials—*i.e.*, cells and supports—and showing what can be done with intentional organizations of tissue. Her group's approach allows the researcher to start with simple structures and build up iteratively. Dr. Drummond noted the hope that this research will, in the future, enable use of organoids or simple kidney tissues to build a complex three-dimensional structure; currently, Dr. Lewis and her group are working on printing tubular structures and testing them for function.

Dr. Drummond then described some of his own group's research on zebrafish, which are of interest because, unlike humans, they can regenerate entirely new kidney tissue as adults if injured. The relevance of this model to structural considerations for human kidney tissue replacement is that all nephrons ultimately must fuse with the collecting system that carries away fluid containing filtered waste, *etc.* So, similarly, new nephrons that grow out in the course of regeneration in the zebrafish must establish proper connections (plumbing joints) to enable flow and filtering of blood and excretion of wastes.

Dr. Drummond's group has found that a signaling factor highly conserved among animals, called Wnt, is essential to the interconnection of regenerating nephrons in zebrafish. This finding means that kidney regenerative medicine researchers have a new guidepost for understanding how to build a complex structure if they can provide signals that promote tubule interconnection at key parts of an assembly.

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Another facet of his research is building on the finding that zebrafish that regenerate their kidneys activate growth factors that humans and other mammals use during early development (embryogenesis). With those observations in mind, Dr. Drummond's group is pursuing the idea of "turning a mouse into a fish." The concept is to use a genetic approach designed to introduce and produce growth factors in a kidney-specific fashion in mice—creating discrete sites of growth factor or morphogen expression—and then introduce laboratory-grown nephrogenic progenitor cells and ask whether it is possible to promote development and connection of organoids, and also whether it is possible to promote blood vessel growth in the new tissues. This is a prime example of combining technologies to achieve a regenerative medicine end: derivation of human pluripotent stem cells and subsequent nephrogenic progenitor cells, promoting differentiation to kidney cells types, and providing the right environment for them spatially for assembly into essential interconnected structures.

TOWARD FUNCTIONAL REPLACEMENT

While pursuing the generation of kidney cells and organoids, dissecting and facilitating proper organization, and developing methods and structures, key issues that needs to be considered simultaneously are how to determine and demonstrate that they will actually work as kidney replacements. Approaches under consideration include attempting to restore function to mutant mouse kidneys with implanted organoid tissue, and generating transgenic organoids that secrete molecules that can be measured in the urine to show there is functional interconnection.

Currently, some other research is being pursued in RBK to get a better handle on the function of various types of engineered cells and tissues. For example, Dr. Drummond noted that Dr. Lewis and her group are testing their three-dimensional "printed" kidney structures for functions such as active transport of larger molecules between physically distinct but adjacent tubular structures, mimicking what would

be observed in a normal nephron, and thus far is seeing positive results. Another RBK member, Dr. Lisa Satlin, is studying the Lewis group's printed tubules to assess how fluid flow rate affects the flux of essential salt ions, potassium and sodium, in the engineered structures—to see how well these generated tubules mimic the native kidney. Dr. Drummond also noted the work of a recent addition to the RBK consortium, Dr. Thomas Kleyman, who is examining organoid cells and is finding that they possess physiologically relevant and active potassium transport channels.

PROMOTING REGENERATION OF DAMAGED KIDNEY TISSUE TO PREVENT PROGRESSION

Alluding to the other overarching goal of RBK, Dr. Drummond remarked that as the consortium's research leads to discoveries of new growth and differentiation factors, some of these might be therapeutic targets for regeneration or regrowth of existing kidney structures when they are damaged.

Thus, in addition to their work on the relationships between gene expression and kidney differences in the human and mouse, he noted, as one example, that Dr. McMahon's group is using gene expression profiling to discover how to distinguish states of cells that can repair certain damaged kidney structures versus those that result in scarring (fibrosis) or cell death. Already, the group has identified genes, such as *SOX9*, that seem to be activated in injury and recapitulate certain developmental events in repair. These approaches, along with work from RBK member Dr. Benjamin Humphreys' lab to develop an "atlas" of cells active during repair, are enabling researchers to examine how well current mouse models replicate human data sets.

FINAL REMARKS

In the time remaining, Dr. Drummond acknowledged the work of all the individual research groups involved in RBK and provided

SCIENTIFIC PRESENTATION

some perspectives on what has made the RBK Consortium work well thus far both scientifically and logistically. These include a highly effective coordinating center that maintains an informative website for the consortium; effective communication across the RBK, including discussion of failures that sometimes leads to solutions; development of new collaborations within the Consortium over time; addition of new groups who bring new technologies and other expertise to the Consortium; and public data-sharing that benefits the entire NIDDK investigator community. In addition, the RBK has a partnership program that actively recruits new labs with grant funding, which, Dr. Drummond noted, recently brought in expertise to advance RBK's research; collaborating projects are brought in as well.

Looking forward, Dr. Drummond noted activities and efforts that could ultimately emerge from RBK's research even though they are not part of its near-term aims, such as drug screens that could yield small molecule therapeutics to help heal damaged tissues, and studies of disease using the organoids. He noted the importance of communication with other organizations who have shared goals in reducing kidney disease. Finally, he expressed his appreciation for the NIDDK program staff and leadership most closely involved in the initiation and continued work of the RBK Consortium to pave the way to regenerative medicine approaches for treating people with kidney disease and damage.

PATIENT PROFILE

Charles: Participation in a Pragmatic Clinical Trial To Bring Hope to People Coping with Multiple Chronic Diseases



Charles

People who have multiple chronic diseases often lead difficult lives, struggling with effects of these conditions on their health and well-being. “Well, everything with me has slowed down ... I’m not so active anymore,” says Charles, who has type 2 diabetes, hypertension (high blood pressure), and chronic kidney disease (CKD). “I used to be active all day.” In his mid-thirties, Charles was initially told by his doctor that his blood glucose (sugar) levels were elevated. Although he has struggled with his weight, he was

somewhat surprised to learn of this diagnosis because he had always led an active life, playing sports such as football. “I had never been the person who went to the doctor because I was always athletic ... always feeling good,” he explains, “all my life, I always felt good.” As his condition worsened to type 2 diabetes, he began taking insulin to manage his blood glucose levels. In the years that followed, he was also diagnosed with CKD and hypertension. For Charles, coping with these three chronic diseases would prove to be a major focal point in his life. By choosing to participate in the NIH-supported Improving Chronic Disease Management with Pieces (ICD-Pieces) clinical trial, Charles is helping researchers find new pathways to better health for people with these life-altering diseases.

THE CONTINUAL CHALLENGES OF MANAGING MULTIPLE CHRONIC DISEASES

Charles, now in his late-fifties, has adjusted many aspects of his life to manage these diseases. His health was a factor in his decision to work as a driver for a day care facility, noting that he was limited in what he could physically do. “Standing—I can’t do that ... [lifting] heavy things—I can’t do that either,” he notes, “I’m just able to get by by driving ... so I do that.” However, as is often the case, these three chronic diseases may have been the causes of other health issues that have come up to further complicate Charles’s life. He also has other

PATIENT PROFILE

conditions, including bone disease and eye-related health issues. A few years ago, Charles faced another serious health scare—he suffered a stroke, which left an enduring mark, still affecting the right side of his body. “My body is breaking down a little bit,” Charles reflects. In order to manage these diseases, he takes different medications that require specific timing during the day. After his stroke, Charles’s long-term memory is not quite what it used to be, so he has come up with a plan to make sure he takes all of his medicines in a timely manner. “I make things simple for myself,” he explains, “everything is laid out for me in a simple location, in a simple bag ... I try to keep it plain and simple for me because I will, I will forget.” Adherence to the proper regimen for each of his medications is just one of the many daily challenges faced by Charles and others with type 2 diabetes, hypertension, and CKD, as well as the spectrum of health complications that these diseases can cause.

“Well, everything with me has slowed down ... I’m not so active anymore,” says Charles, who has type 2 diabetes, hypertension (high blood pressure), and chronic kidney disease (CKD). “I used to be active all day.”

THE ICD-PIECES TRIAL—A PRAGMATIC APPROACH TO CLINICAL RESEARCH

Charles’s struggles illustrate how multiple chronic conditions can exact a serious toll on people’s health and their daily lives. Although research studies over the years have identified potential treatments for these diseases, the application of scientific advances to usual clinical settings has proven difficult. Earlier studies have focused on each disease individually, but not in combination—a complex, clinically important health scenario for many people in the United States. In 2014 the NIH Common Fund and NIDDK began supporting the Improving Chronic Disease Management with Pieces (ICD-Pieces) study—a clinical trial designed to improve health

outcomes in people with coexisting type 2 diabetes, hypertension, and CKD. Four health-care systems, three based in Texas and one in Connecticut, are participating in the study. The study implemented a new technological tool, called “PIECES,” that uses electronic health records (EHRs) in the four participating health care systems. The PIECES platform, developed by Pieces Technologies, utilizes data from EHRs in real time to help researchers and clinicians identify patients managing the triad of chronic diseases, improve data collection, discover complications of the three diseases in patients at an early stage, and coordinate care for study participants. Patients enrolled in the ICD-Pieces trial are assigned to one of two groups. In one group, the participants’ health care does not change, but their outcomes are closely monitored—this will serve as the “control” group. In the second group, the patients’ primary care physicians collaborate with subspecialists, for example, kidney disease specialists (nephrologists); these collaborations are supported by “practice facilitators” who work with physicians to help implement best practices and tailor health care plans based on available resources at each site. Frequency of hospitalization for any reason, over the course of 1 year, is the main, or primary, outcome measured in the study. However, researchers are also evaluating a number of secondary outcomes, including disease-specific hospitalizations, emergency room visits, cardiovascular events (e.g., stroke or heart attack), and death.

“I make things simple for myself,” he explains, “everything is laid out for me in a simple location, in a simple bag ... I try to keep it plain and simple for me because I will, I will forget.” Adherence to the proper regimen for each of his medications is just one of the many daily challenges faced by Charles and others with type 2 diabetes, hypertension, and CKD, as well as the spectrum of health complications that these diseases can cause.

PATIENT PROFILE

ICD-Pieces falls under the “pragmatic clinical trial” category of research studies, which uses real-world, large-scale, often multiple-center health care settings to evaluate different approaches to disease management, leading to findings that are likely to have broader translatability to clinical care. However, in order to conduct a pragmatic clinical trial, several practical challenges unique to these settings must be overcome, such as implementation of appropriate and standardized rules and regulations across the different health care systems. By contrast, more traditional “randomized controlled trials” often occur at a smaller scale and in settings where most variables can be controlled, but findings may not necessarily be as applicable beyond the clinical settings used in the trial. ICD-Pieces plans to enroll more than ten thousand study participants who are already patients across the four participating health care systems. This engagement of diverse health care systems and a broad range of study participants will help expand translation of the research findings.

Charles remains positive about his circumstances, noting that he didn't choose to have these conditions. “And I can only adapt to it,” he says, “and keep going with my life, and don't give up.”

Charles, a patient within one of the Texas-based health care systems participating in ICD-Pieces, was identified as a potential candidate for the study. He agreed to enroll and in February of 2018 began his 12-month participation in the trial, in which he continued to receive care from his primary care physician, but also began seeing new subspecialists for his chronic conditions. Other health care

professionals, including a nutritionist and physical therapist, provided additional support and guidance as well.

Findings from ICD-Pieces and other clinical trials will continue building the foundation for better health in people living with complex multiple chronic diseases. “I keep moving,” Charles reflects, “I keep moving every day, and not let this disease ... get me down.”

HOPE THROUGH RESEARCH

Charles struggles daily with the burden of managing the many health conditions he has—the primary effects of which can change over time. “[My back pain] has made it difficult for me to function like a normal person would, all day,” he explains; “now my back is really my biggest problem.” However, Charles remains positive about his circumstances, noting that he didn't choose to have these conditions. “And I can only adapt to it,” he says, “and keep going with my life, and don't give up.” With the invaluable participation of Charles and thousands of others, ICD-Pieces is paving the way to improved treatments for people with type 2 diabetes, hypertension, and CKD, identifying therapeutic approaches that have the highest likelihood of improving health in the real world, not just in an experimental setting. Findings from ICD-Pieces and other clinical trials will continue building the foundation for better health in people living with complex multiple chronic diseases. “I keep moving,” Charles reflects, “I keep moving every day, and not let this disease ... get me down.”

Extramural Funding Trends and Support of Core Values

The NIDDK's core values emphasize maintaining a vigorous investigator-initiated R01 research portfolio, supporting pivotal clinical studies and trials, preserving a stable pool of talented new investigators, and continuing to foster exceptional research training and mentoring opportunities, consistent with the vision of NIDDK Director, Dr. Griffin P. Rodgers (see Director's Message).

At the NIDDK's May 2012 Advisory Council meeting, the NIDDK Deputy Director Dr. Gregory Germino highlighted these values and reviewed the NIDDK's resource focus on areas supporting them.

Following that presentation, the NIDDK generated additional data on application and funding trends to help our research community understand application and funding dynamics over recent years and demonstrate the NIDDK's commitment to research and programs associated with the NIDDK's core values. The Institute posted these data on the NIDDK website and has since updated them annually. The data shown here were recently updated to include Fiscal Year (FY) 2018.

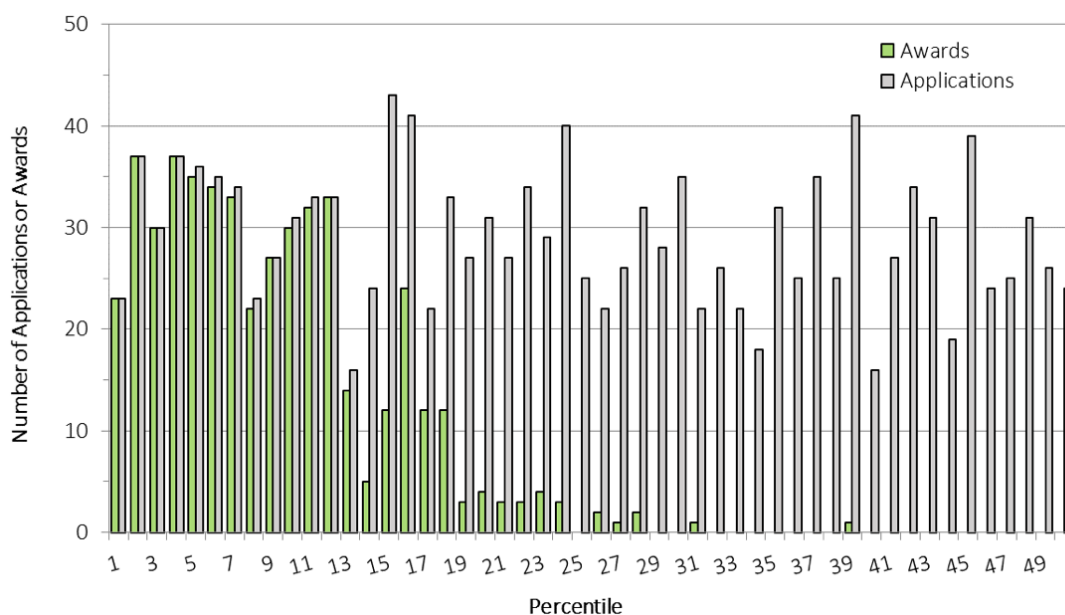
NIDDK Funding Outcomes for FY 2018 and Historical Application and Funding Trends

With the exception of Figure 8 (which includes initiative data), the data in all charts exclude initiatives (i.e., Requests for Applications, or RFAs), grants funded through the *Special Statutory Funding Program for Type 1 Diabetes Research*, and funds appropriated through the *American Recovery and Reinvestment Act (ARRA)*.

Most charts and tables in this report show data for the past 10 FYs. Figure 1 only focuses on FY 2018. In Figures 3 and 5-7, the time horizon is expanded to include FYs starting in 1997, the year before the start of the doubling of the NIH budget from FYs 1998 through 2003. This expansion provides some perspective on application and funding trends occurring through the doubling period and then in the post-doubling era. Figures 11 and 12 are focused on Early Stage Investigators (ESIs) and build upon an initial set of charts that include data starting in FY 2010.

FIGURE 1

Number of NIDDK Competing R01 Applications Scoring Within the Top 50th Percentile and Number of NIDDK Percentiled R01 Applications Funded in FY 2018

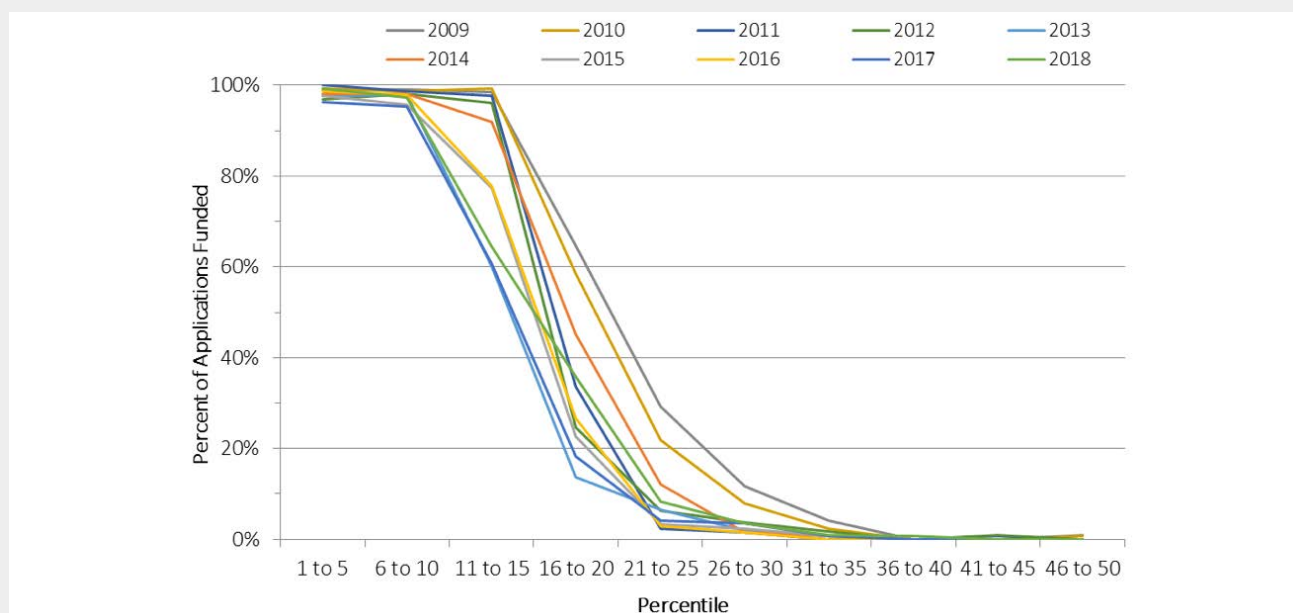


Note: “Applications” shown in the chart above include all R01 applications that scored 50th percentile or better. Unscored applications, scored applications with no percentiles, and applications scoring above the 50th percentile are not shown. (Fifty-nine percent [n=1,413] of the applications received were unscored, scored but did not receive a percentile, or scored above the 50th percentile.) No unscored applications were funded in FY 2018.

The NIDDK nominal payline in FY 2018 for most R01 applications was the 13th percentile for established investigators and the 18th percentile for Early Stage Investigators (ESIs). The payline and additional programmatic scrutiny for R01 applications requesting more than \$500,000 in direct costs are substantially more stringent. These data show that the NIDDK adheres closely to its payline but does exercise programmatic discretion to include a limited number of programatically important applications beyond the payline.

FIGURE 2

NIDDK Competing R01 Application Funding Curves for FYs 2009-2018



To generate the data for Figure 2, R01 applications were placed into “percentile bins” as follows: bins 1 to 5 include all applications with percentile scores from 0.1 to 5.0, bins 6 to 10 include applications with percentile scores from 5.1 to 10.0, etc. Only R01 applications that scored 50th percentile or better were included in the analysis. The data demonstrate steep deflections in the percentage of applications funded at or above the nominal payline for each year. The R01 paylines for the years included in Figure 2 are shown in Table 1.

Note: In FY 2012, the NIDDK began focusing on Early Stage Investigators (ESIs; see definition on the NIH “New and Early Stage Investigator Policies” webpage at http://grants.nih.gov/grants/new_investigators/index.htm), a subset of New Investigators. For more information on the benefits that the NIDDK conveys to ESIs, see the NIDDK New and Early Stage Investigators page at <https://www.niddk.nih.gov/research-funding/process/apply/new-early-stage-investigators> (See also Figures 11 and 12.)

Fiscal Year	General Payline	>\$500K Payline	New Investigator Payline	Early Stage Investigator (ESI) Payline	ESI First Competitive Renewal Payline
2008	17	11	19	–	–
2009	17	11	19	–	–
2010	17	11	19	–	–
2011	15	10	17	–	–
2012	13	9	13	18	–
2013	11	7	11	16	–
2014	13	8	13	18	–
2015	13	8	13	18	15
2016	13	8	13	18	15
2017	12	7	12	17	15
2018	13	8	13	18	16

FIGURE 3

Number of Competing NIDDK R01 Applications (Including Resubmissions) Received for Funding Consideration in FYs 1997-2018

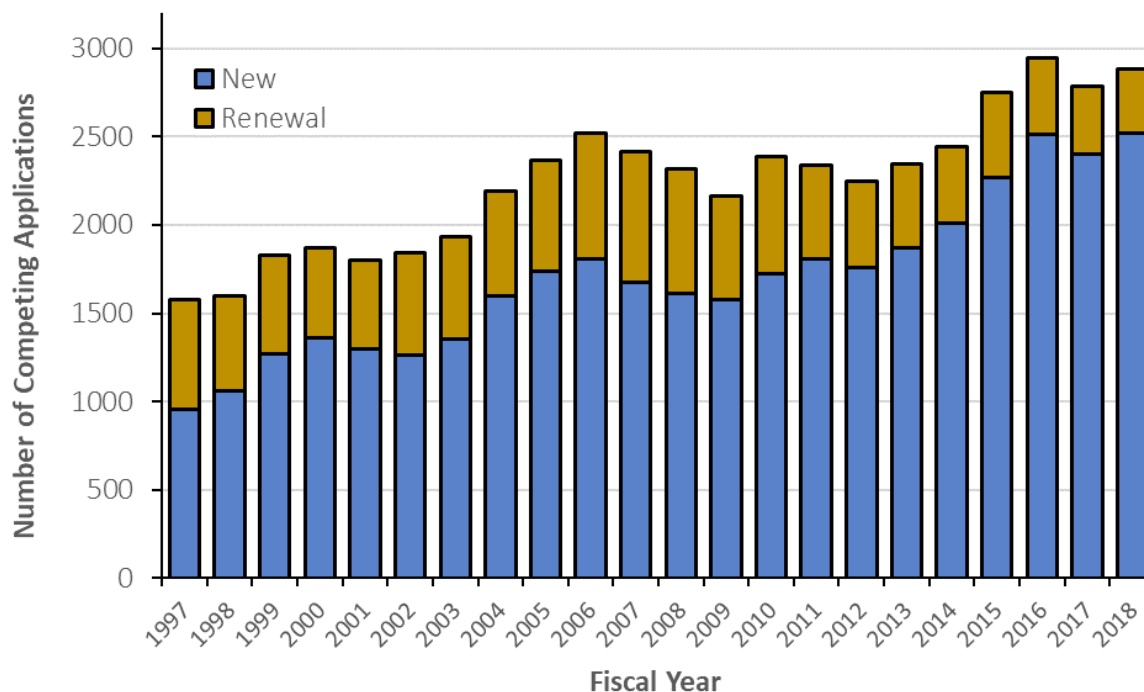


Figure 3 shows a substantial increase in the number of competing R01 applications received by the NIDDK between FYs 1997 and 2018. After an initial increase that followed the doubling of the NIH budget in FYs 1998-2003, the number of competing R01 applications received by the NIDDK leveled out for almost a decade before increasing by over 30 percent in FYs 2013-2016. The number of submitted applications has again flattened over the past three years. The observed increases between FYs 1997 and 2006 were primarily due to increases in the number of new (Type 1) applications. This same trend of increasing numbers of new (Type 1) applications was also seen between FYs 2013 and 2016 (see also Figure 4).

FIGURE 4

Number of Competing NIDDK R01 Applications Received for Funding Consideration in FYs 2009-2018: New Versus Renewal Application Numbers

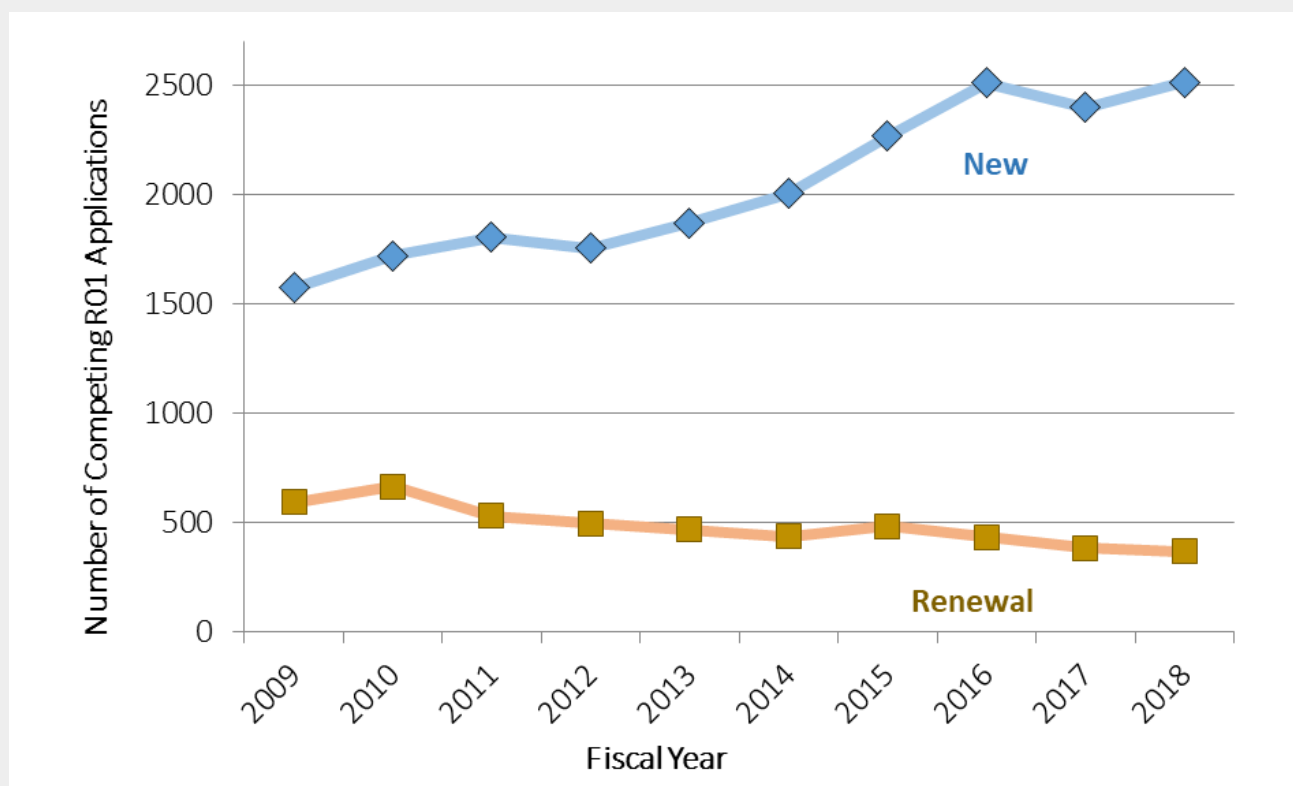
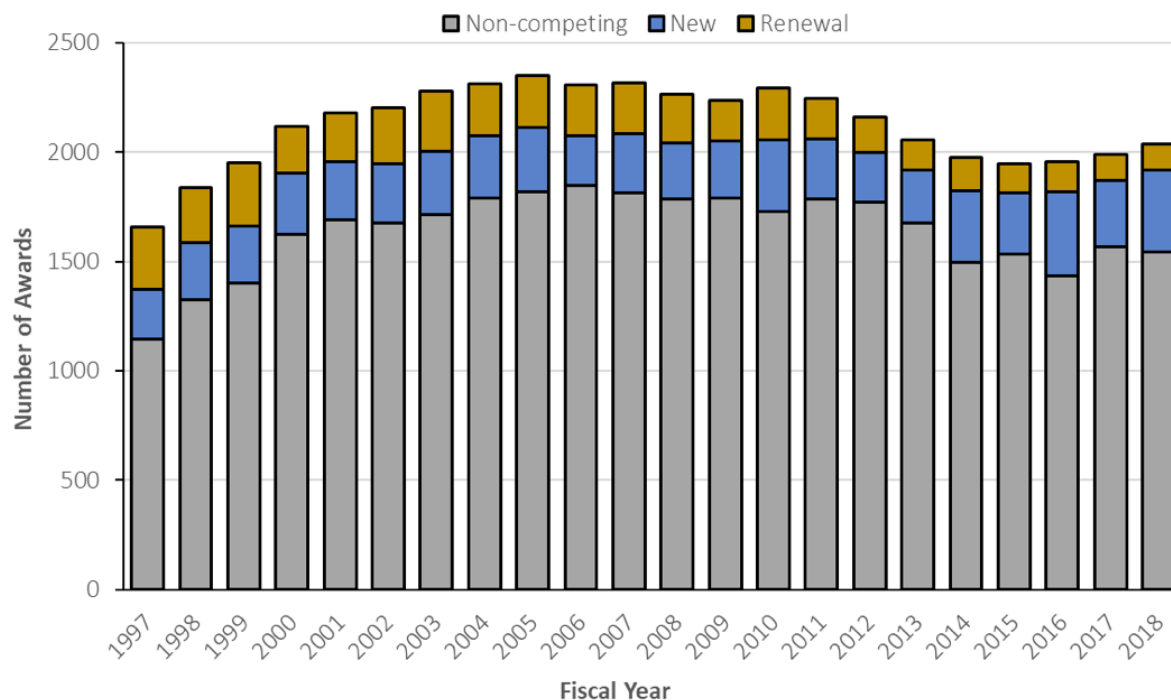


Figure 4 shows the last ten years of competing and non-competing NIDDK R01 application numbers from Figure 3, to allow more detailed visualization. In the last decade (FY 2009 to FY 2018) the number of competing R01 applications submitted to the NIDDK has risen by over 30 percent (an increase of 717 more R01 applications received in FY 2018 than FY 2009). This was due to an increase in the number of new applications, which rose by 60 percent, whereas the number of competing renewal applications dropped by 38 percent (from 592 in FY 2009 to 367 in FY 2018). The number of new applications leveled out after FY 2016, but the downward trend in renewal applications continued. The recent surge in new applications may, in part, be explained by the change in NIH policy that discontinued A2 submissions, but the reason for the decrease in number of renewal applications is less clear.

FIGURE 5

Number of NIDDK R01 and R37 Grants (Competing and Non-Competing)
Funded In FYs 1997-2018



During the doubling of the NIH budget (FYs 1998-2003), the total number of R01/R37 grants funded by the NIDDK increased significantly. After leveling off following the doubling, the number of R01/R37 grants funded by the NIDDK from FY 2010 to FY 2015 declined. From FY 2016 to FY 2018, there has been a slight but steady increase in the number of R01/R37 awards supported by the NIDDK. Prior to FY 2009, approximately half of the competing grants funded by the NIDDK were new (Type 1) awards in most FYs. However, in the last 10 years, the majority of competing awards are new: in FY 2018, 76 percent of competing NIDDK R01/R37 awards were new.

FIGURE 6

Overall NIDDK Expenditures (Includes Direct and Facilities and Administrative Costs) on R01 and R37 Awards (Competing And Non-Competing) in FYs 1997-2018

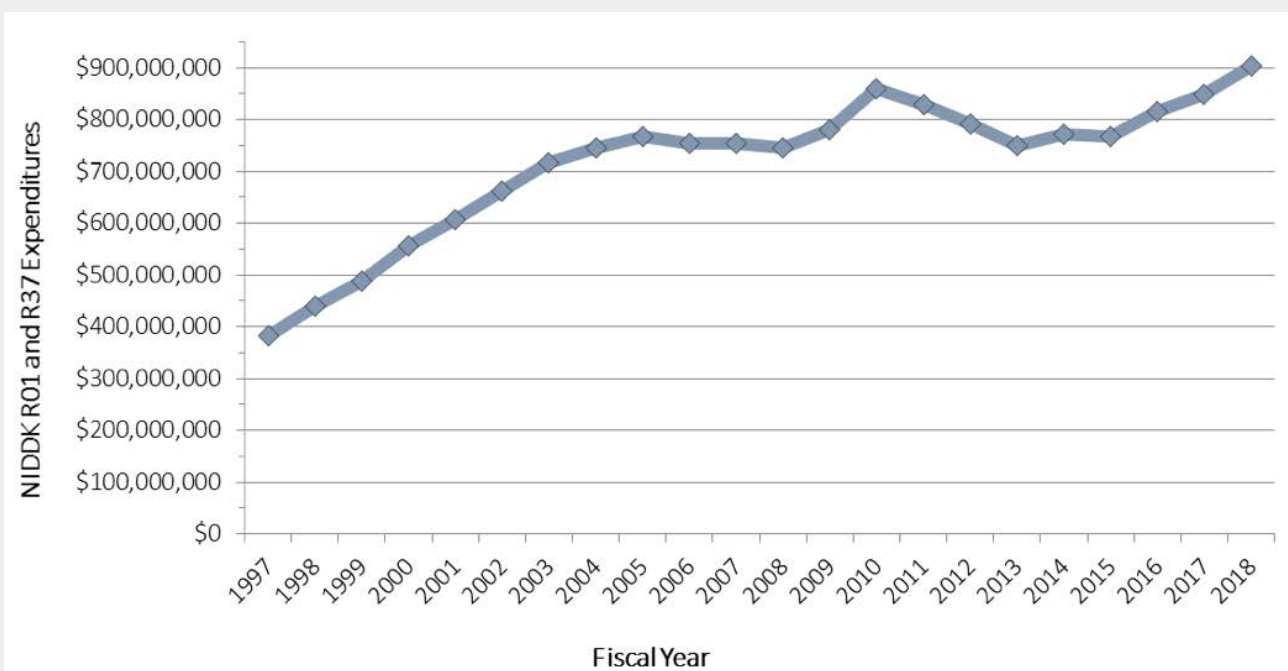


Figure 6 shows that NIDDK total expenditures on R01 and R37 grants have more than doubled since FY 1997 (a 136 percent increase from \$383.5M to \$903.3M). This is because the NIDDK is funding more of these awards (Figure 5), and because the median cost of an R01 has increased substantially (Figure 7) since FY 1997.

FIGURE 7

Median Total Costs (Includes Direct and Facilities and Administrative Costs) of NIDDK R01 and R37 Grants (Competing and Non-Competing) in FYs 1997-2018

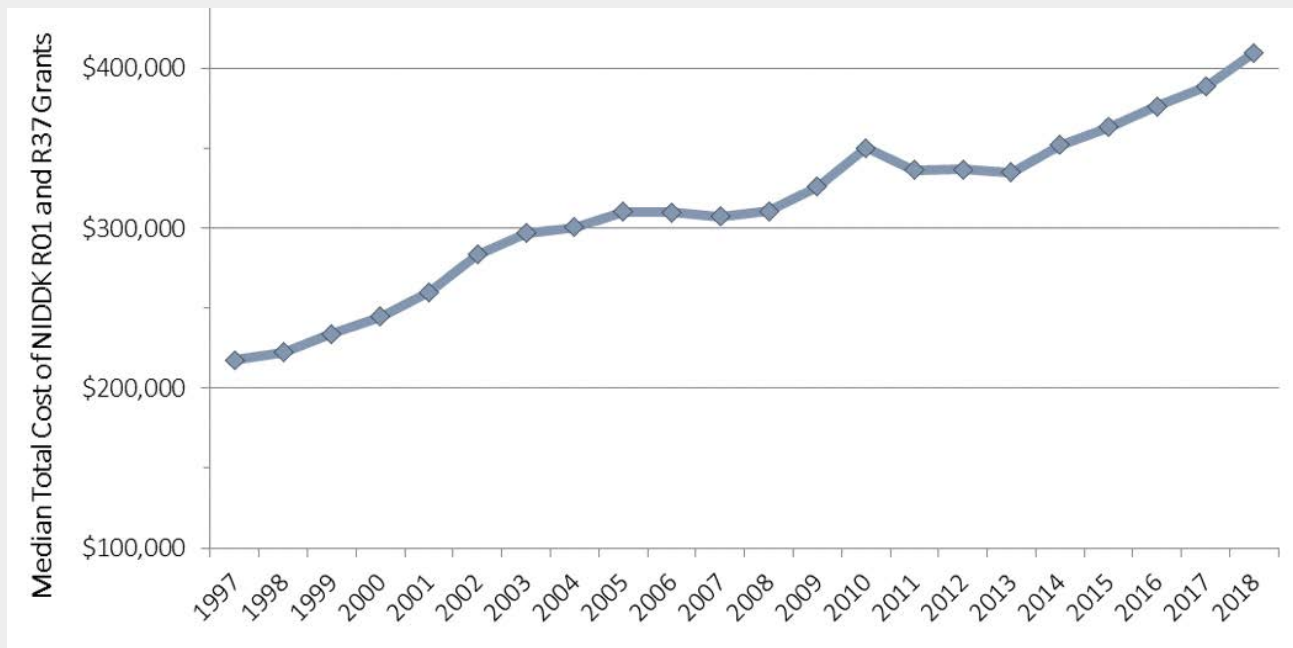


Figure 7 illustrates that the median cost of R01 and R37 awards has increased approximately 88 percent since FY 1997 from about \$217,638 to \$409,993 in FY 2018.

FIGURE 8

**NIDDK Extramural Research Funding by Category
(Competing and Non-Competing) in FYs 2009-2018**

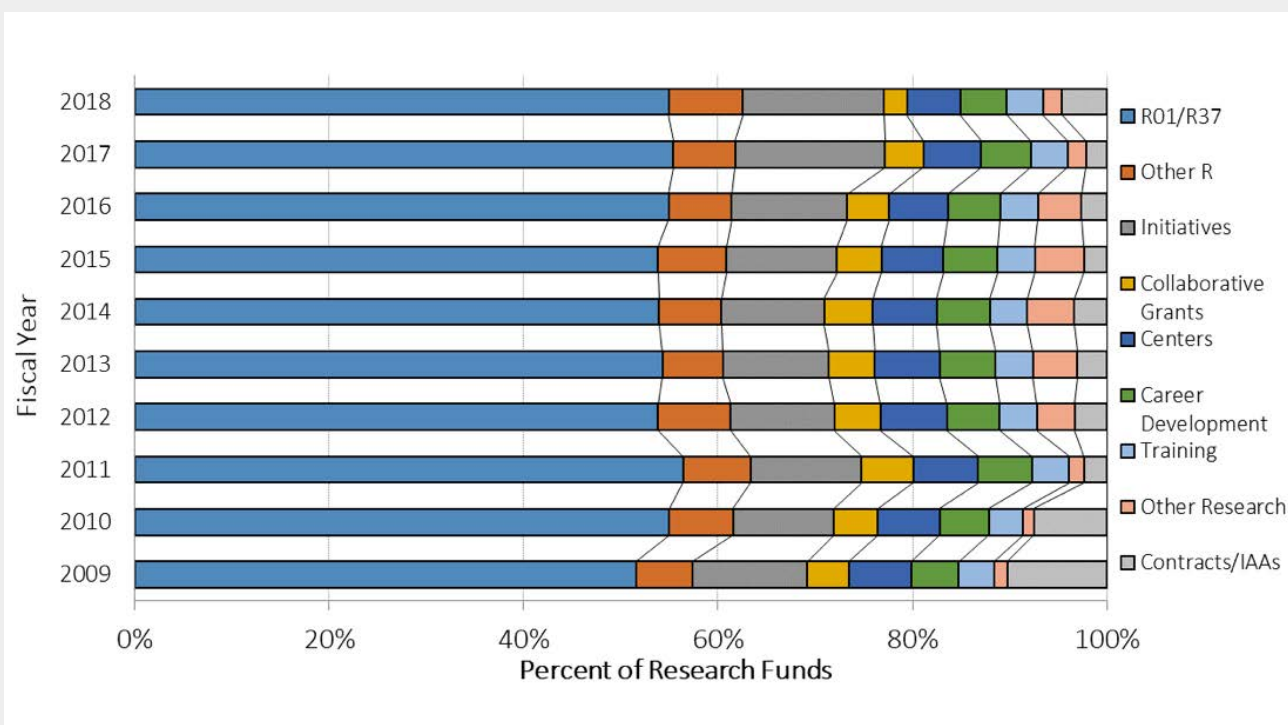


Figure 8 shows that relative funding levels of most NIDDK extramural research categories have remained fairly stable since FY 2009.

NIDDK Portfolio Categories:

- **R01/R37** – Investigator-initiated (excludes R01s responding to NIDDK RFAs)
- **Other R** – Includes other R activities (*i.e.*, R03, R13, R15, R18, R21, R34, SBIR/STTR, *etc.*) but excludes R24s and applications submitted to NIDDK RFAs
- **Initiatives** – Awards made in response to NIDDK RFAs; includes most NIDDK large clinical trials and consortia
- **Collaborative Grants** – P01s and R24s that are not “mini-Centers”
- **Centers** – Includes all non-P01 P awards and R24 “mini-Centers”
- **Career Development** – Includes all Ks (including K99/R00)
- **Training** – Includes all F and T activities
- **Other Research** – Everything not captured in the other categories, including D43, SB1, U24, U54, UH3, and U2C awards
- **Contracts and Interagency Agreements (IAAs)** – Includes some large clinical studies

FIGURE 9

Maintaining a Stable Pool Of NIDDK Investigators—Number of Investigators Supported by at Least One R01 or R37

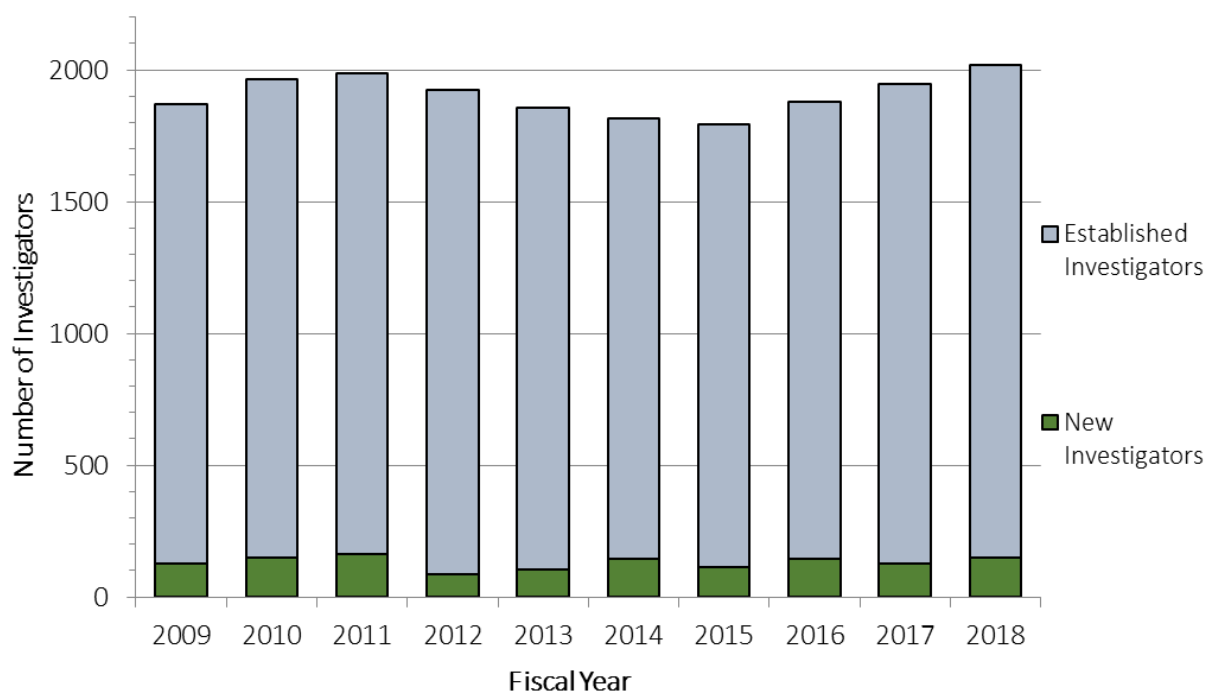


Figure 9 shows that the number of principal investigators (PIs) supported by at least one R01 or R37 remained relatively stable between FYs 2009 and 2018, with slight increases from FYs 2010-2012 and FYs 2016-2018. The reduction in the number of PIs supported by the NIDDK from FY 2012 to FY 2015 may be the result of more stringent paylines, as well as other factors, during that period.

FIGURE 10

Maintaining a Stable Pool Of NIDDK Investigators—Number of Investigators Supported by at Least One R01 or R37 and Growth of Multiple PI (MPI) Awards

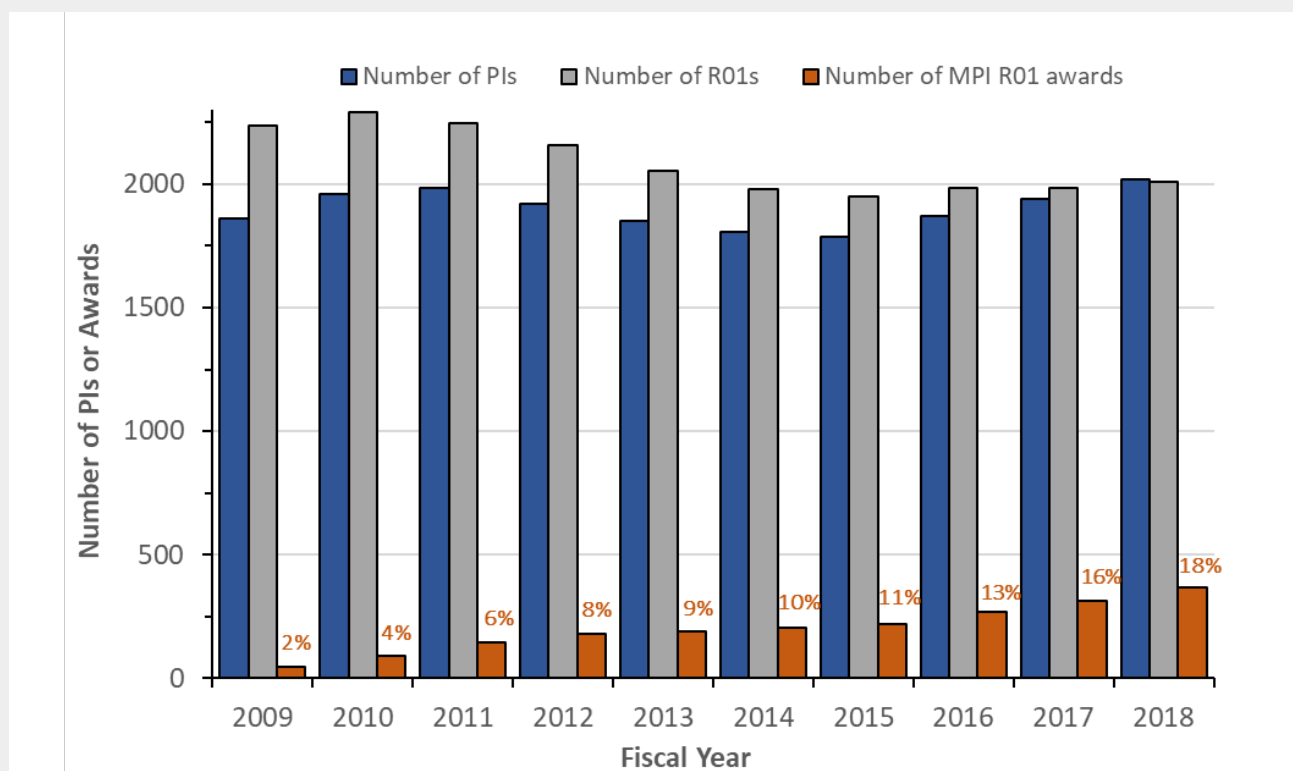


Figure 10 shows that Multiple Principle Investigator (MPI) awards make up a growing fraction of all R01 (competing and non-competing) awards. Two percent of R01s were MPI in FY 2009, rising to 10 percent in FY 2014, and reaching 18 percent in FY 2018. The number of MPI awards is growing more rapidly than either the total number of R01 awards or the number of R01 PIs. Between FY 2015 and 2018, the total number of grants rose only by 59 whereas the number of PIs and MPI awards increased by 232 and 147, respectively. The disproportionate increase in the number of PIs is likely explained by an increase in the number of MPI awards, the steady infusion of ESIs since inception of that program, and select use of Special Emphasis awards.

FIGURE 11

Preserving a Stable Pool of New Investigators—Number of NIDDK Early Stage Investigator (ESI) R01 Applications and Number of Unique ESIs Applying and Awarded

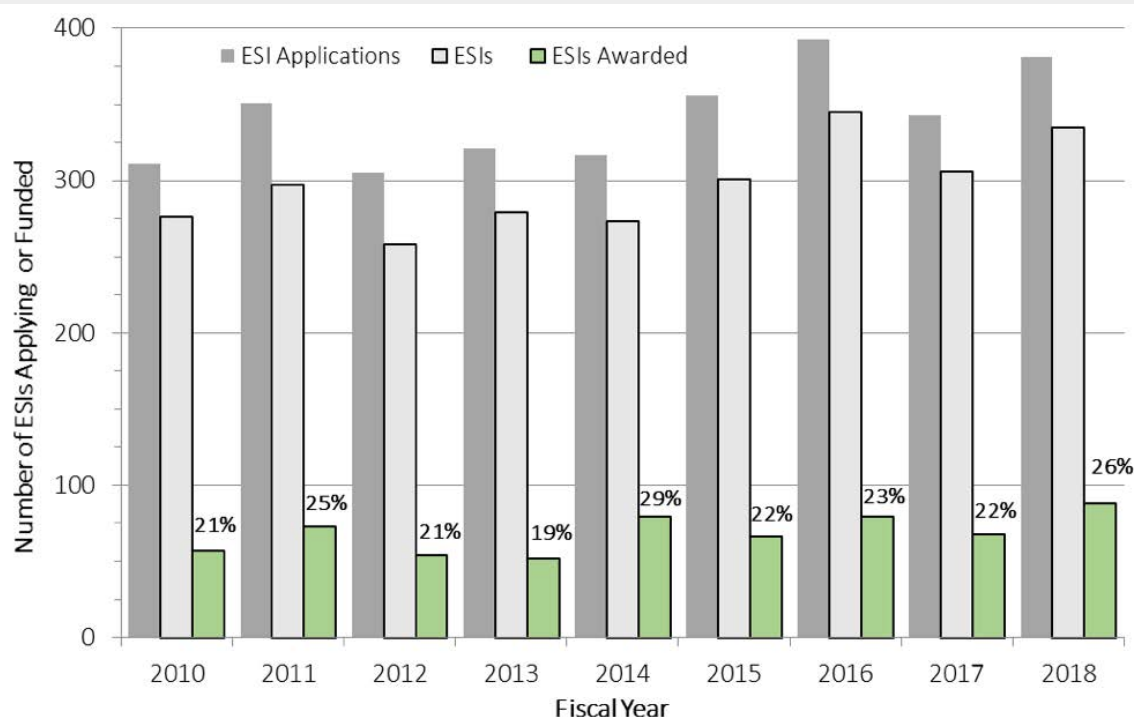


Figure 11 shows that numbers of NIDDK Early Stage Investigator (ESI) R01 applications have increased in the last nine years, and numbers of ESIs applying to and being funded by the NIDDK have also trended up. Numbers of ESI applications are higher than the numbers of unique ESIs applying, as some ESIs submit multiple R01 applications in a given FY. Although there is moderate fluctuation from year to year in the numbers of ESI applications and awards, the differential payline is contributing to a healthy success rate for these applications. Success rate is shown in Figure 11 above the bar indicating the number of ESIs awarded and is calculated as the percentage of unique ESIs that applied to the NIDDK that were funded.

FIGURE 12

Preserving a Stable Pool of New Investigators—Percent of NIDDK New Competing R01 Applications Submitted and Awards Received by Early Stage Investigators (ESIs)

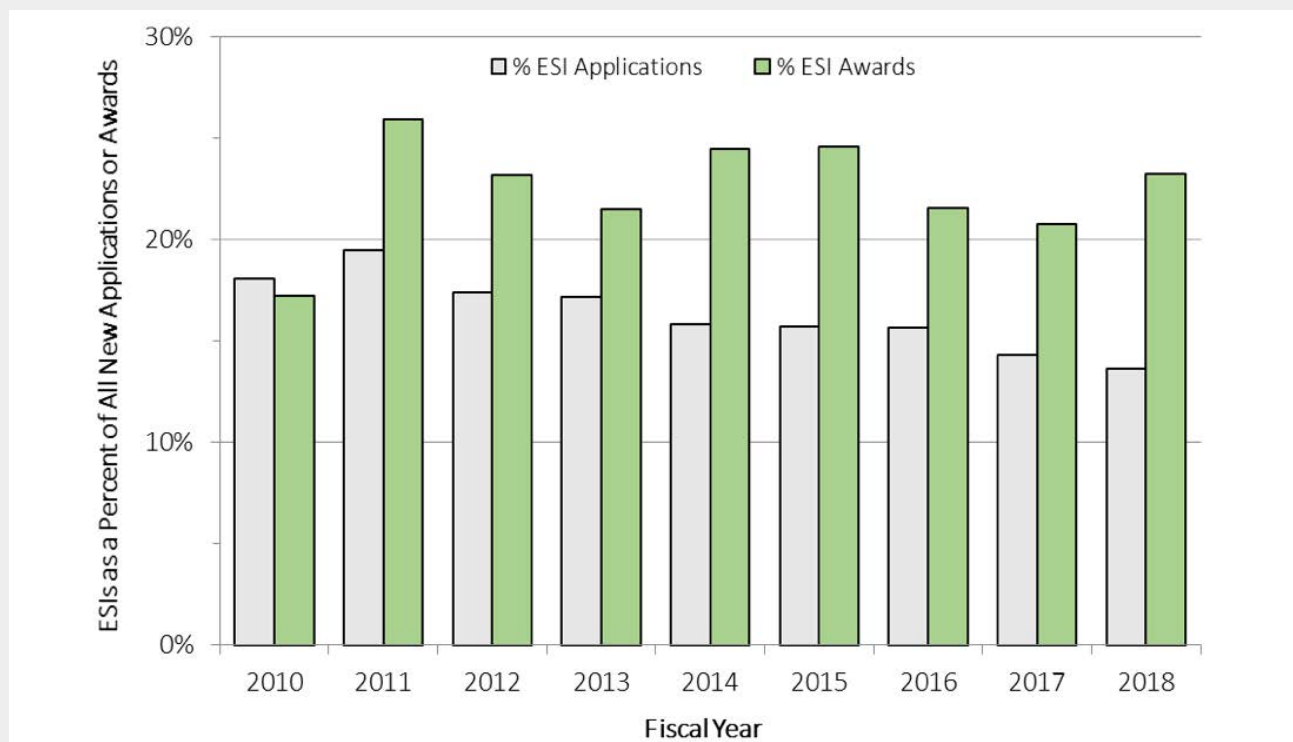
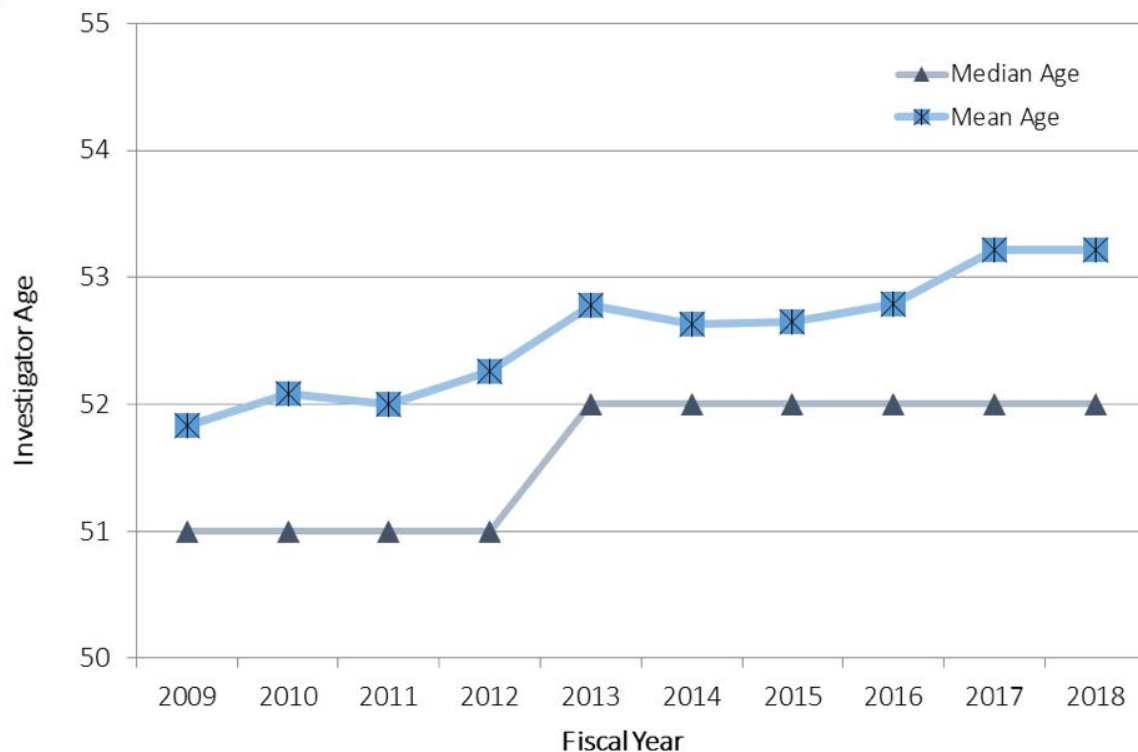


Figure 12 shows that the NIDDK's differential payline for ESIs from FYs 2012-2018 (see table accompanying Figure 2 and the NIDDK New and Early Stage Investigators page at <https://www.niddk.nih.gov/research-funding/process/apply/new-early-stage-investigators>) has been effective in enhancing ESI representation among new (competing) R01 awards. Numbers of new R01 applications increased 46 percent from FY 2010 to FY 2018 (see Figure 3), whereas numbers of ESI R01 applications increased only slightly, resulting in a gradual reduction of ESI applications as a percentage of all new R01 applications, from 18 percent down to 14 percent. The ESI differential payline has increased the number of awards to ESIs, so that about 23 percent of all new NIDDK R01 awards go to ESIs.

FIGURE 13

Median and Mean Ages of NIDDK R01 and R37 Investigators



Over the past 10 years, the median ages of investigators holding R01 or R37 awards (competing and noncompeting) increased by 1 year, and mean age of these investigators has increased by 1.4 years. Mean age increased gradually from FYs 2009 through 2013, then held relatively constant from FY 2013 through 2016. In FYs 2017 and 2018, mean age increased to approximately 53.2 years, continuing the overall upward trend over time. Median age increased from 51 to 52 in FY 2013 and has remained constant since then.

FIGURE 14

Support Pivotal Clinical Studies and Trials—NIDDK Human Subjects Research Funding as a Proportion of All Extramural Research Funding

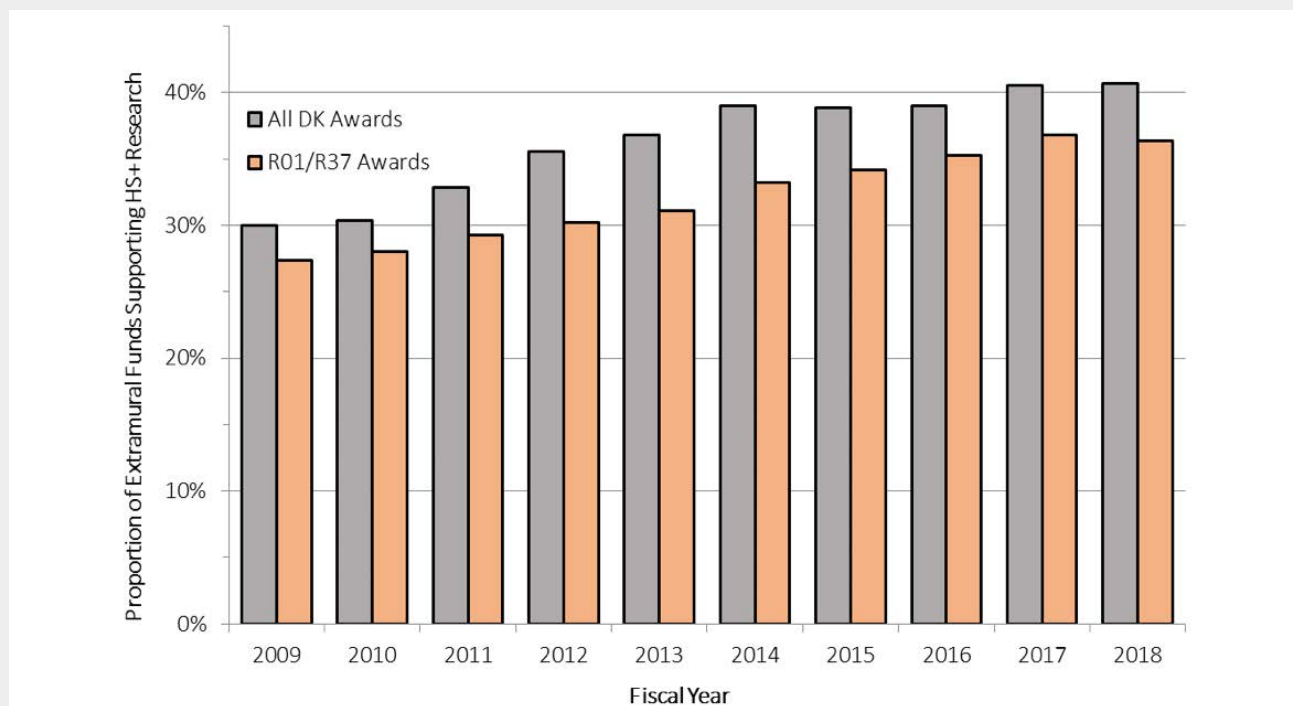


Figure 14 demonstrates that the NIDDK continues to commit a substantial proportion of its research funding to the support of clinical research involving human subjects. The proportion of NIDDK funds supporting Human Subjects research has increased in the last 10 years, from 30 percent of all NIDDK-funded research in FYs 2009-2010 to about 40 percent in FYs 2014-2018. This same steady increase was seen in the fraction of R01 and R37 funding for human subjects research, rising from 27 percent in FY 2009 and leveling out at about 36 percent in FY 2016 through 2018. For the purpose of this analysis, we used the definition described in Kotchen *et al.*, (*JAMA* 291: 836-843, doi:10.1001/jama.291.7.836, 2004) and included all studies coded as using human subjects (HS+). We are monitoring this trend to be sure that we maintain an appropriate balance between clinical and basic research.

FIGURE 15A TO 15E

Figures 15A To 15E: The NIDDK Is Committed to Training the Next Generation of Scientists

Figures 15 A to E demonstrate that the NIDDK's commitment to training and developing the careers of the next generation of scientists remains strong.

Figure 15A: NIDDK Fellowship (F), Career Development (K), and Training (T) Awards as a Percent of Total Extramural Research Funding

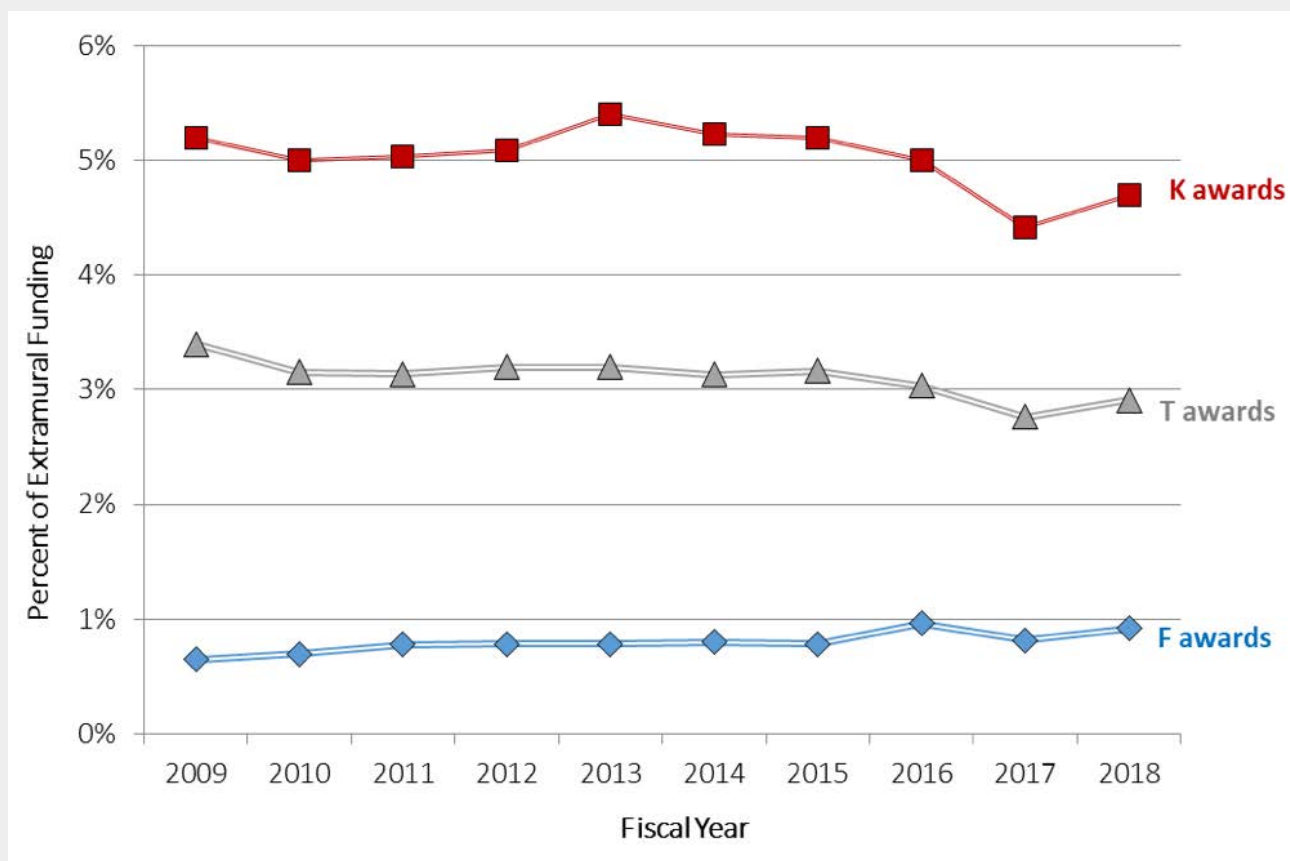


Figure 15A shows that overall support of training and career development programs has remained fairly stable. Funding for K awards remained stable at about \$72 million a year in FYs 2010–2017, with an increase to about \$74.9 million in FY 2018. K awards comprise about 5 percent of the NIDDK overall extramural research budget, T awards about 3 percent, and F awards just under 1 percent.

FIGURE 15B

Numbers of NIDDK Fellowship (F), Career Development (K), and Training (T) Awards by Fiscal Year

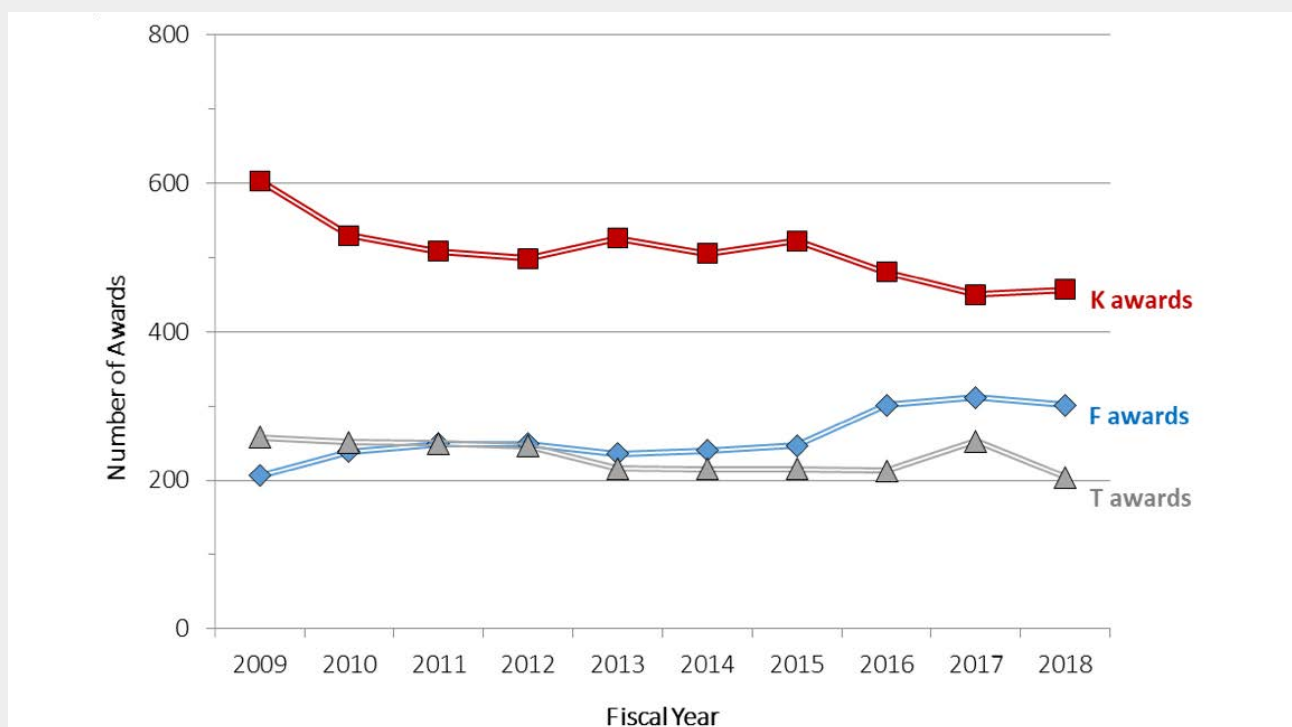


Figure 15B shows that the number of NIDDK F awards has increased slightly since FY 2009 and held steady at just over 300 F awards in FYs 2016-2018. The number of K awards has decreased in the last decade, dropping from about 600 in FY 2009 to about 450 in FYs 2017 and 2018. The number of T awards has held relatively constant over the last 10 years. Trends in specific K mechanism awards that contributed to this effect are shown in detail in Figure 15C.

FIGURE 15C

Numbers of NIDDK Career Development (K) Awards by Activity and Fiscal Year

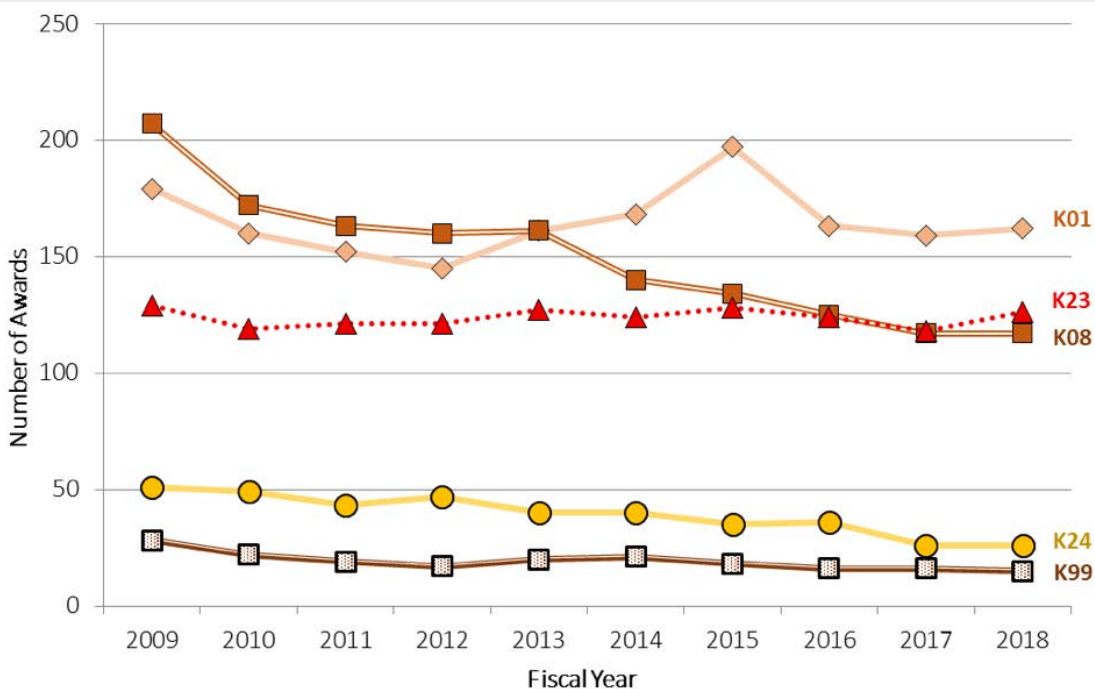


Figure 15C shows that the overall decrease in numbers of K awards in the last decade (Figure 14B) is due primarily to a decrease in numbers of NIDDK K08 (Mentored Clinical Scientist Development Awards) and K24 (Midcareer Investigator Awards in Patient-Oriented Research). The numbers of other K mechanism awards have shown no such overall trend. FY 2017 was the last year that the NIDDK accepted K24 applications and no new NIDDK K24 awards are expected after FY 2018.

FIGURE 15D

Numbers of NIDDK Career Development (K) Applications by Activity and Fiscal Year

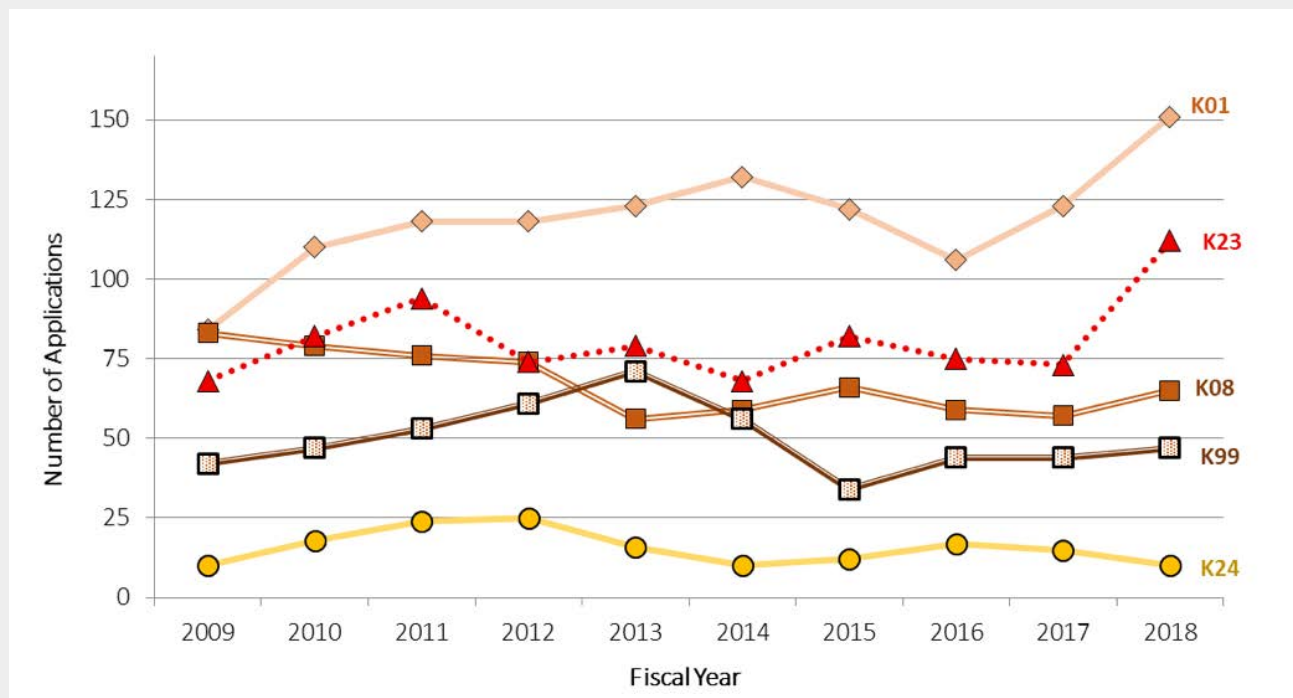


Figure 15D shows that K application numbers have fluctuated over time, with substantial increases in K01 and K23 applications in FY 2018. The number of K08 applications, which had been steadily declining since FY 2009, has stabilized over the last 5 years. Other K application types show some year-to-year fluctuations or short-term trends but relatively comparable numbers of applications overall between FY 2009 and FY 2018. 2017 was the last year that the NIDDK accepted K24 applications and no new NIDDK K24 awards are expected after FY 2018.

FIGURE 15E

Numbers of NIDDK Training (T32) Award Slots by Fiscal Year

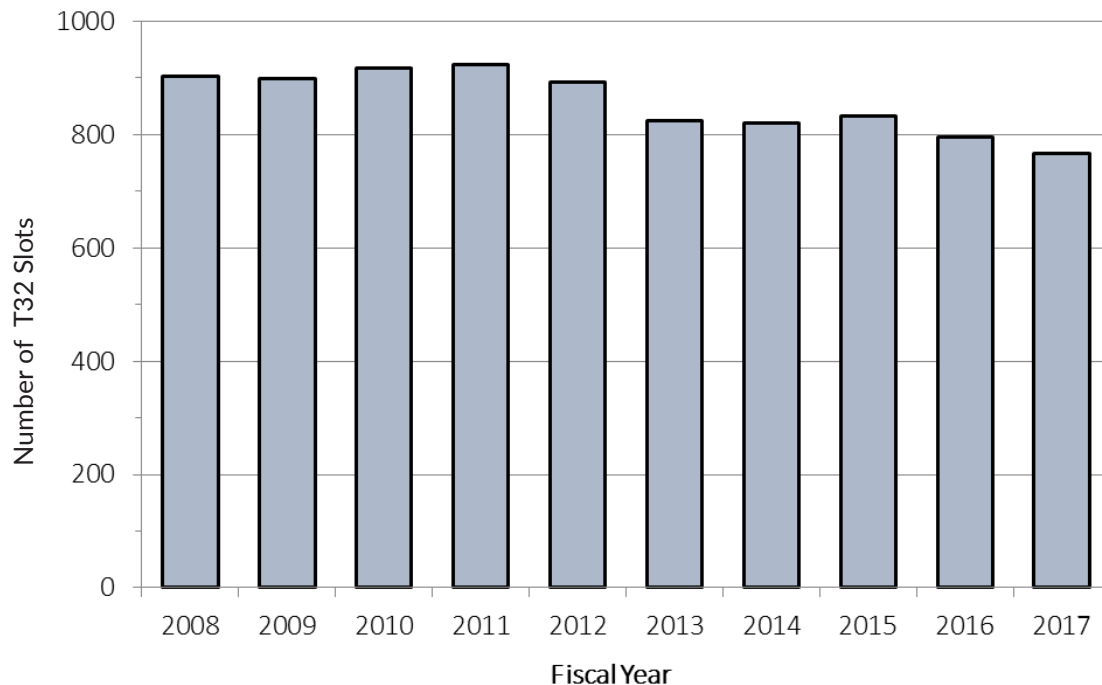


Figure 15E illustrates that the numbers of NIDDK T awards and associated training slots/positions have decreased slightly in the last decade. Between FYs 2008–2012, the NIDDK supported about 907 T32 training slots, which dropped to about 824 slots in FYs 2013–2015. The number of slots decreased in both FYs 2016 and 2017. The NIDDK will continue to monitor carefully its training and career development programs to identify factors behind trends and to ensure appropriate balance.

Note: T32 awards made in FY 2018 continue into FY 2019. The total number of T32 slots are reported at the end of the award period. Therefore, the FY 2018 information on T32 slots will not be available until later in FY 2019; thus, unlike the other charts in this section, FY 2018 data are not included here.

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NIDDK Funding Trends and Support of Core Values

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