NIDDK

Recent Advances & Emerging Opportunities

January 2015

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

ON THE COVER

The NIDDK research mission encompasses a broad array of diseases and conditions affecting people of all ages. This “word cloud,” built from terms representing NIDDK mission areas and efforts, illustrates how scientific inquiry and disciplines, the people who conduct and participate in research, and the dissemination of discoveries are all interwoven in the NIDDK research enterprise—highlights of which are presented in this annual publication.

Note: The relative size of words in this cloud is a design choice and is not meant to imply differences in importance and/or funding levels.

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ACKNOWLEDGEMENTS

Message from the Director

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, such as cystic fibrosis; liver disease and other digestive diseases and conditions, such as inflammatory bowel disease and irritable bowel syndrome; nutritional disorders and obesity; kidney diseases, such as polycystic kidney disease; urologic diseases and conditions, such as interstitial cystitis/painful bladder syndrome and prostatitis; and hematologic diseases, such as sickle cell disease.

The 15th edition of this report illustrates recent NIDDK-supported scientific advances, such as:

- Insight into how a risk gene for type 1 diabetes might exert its effect, with potential therapeutic implications
- Preliminary evidence that bariatric surgery may be more effective than current non-surgical treatments for type 2 diabetes, after a year of treatment, in individuals with mild to moderate levels of obesity
- Finding that rare mutations reduced people's risk of type 2 diabetes by halving the amount of a certain protein in their cells, suggesting therapeutics targeting the protein may be safe and effective for diabetes prevention or treatment
- Highly encouraging results from studies testing how well artificial pancreas technologies help people with type 1 diabetes improve their blood glucose (sugar) control in "real-world" settings outside the clinic
- Fundamental insights into the identity, activities, and impact of microbes present in the gut, and how changes in this "microbiome" early in life may have important clinical implications for treating or preventing malnutrition, obesity, and other diseases
- Identification of host and microbial factors associated with inflammatory bowel disease that could pave the way to new treatment approaches
- A potential new model for predicting liver toxicity of drugs before they are tested in humans
- Discovery of molecular mechanisms that turn calorie-storing white fat into calorie-burning "beige" fat, which may open the door to new ways of treating obesity and improving metabolic health
- New data on the impact of *APOL1* gene variants on risk of kidney disease progression and cardiovascular disease in African Americans
- A new understanding of why a cystic fibrosis treatment strategy is not working as expected, providing important insights in the quest to improve therapy for people with the most common mutation causing this disease
- Demonstration that long-term use of a drug combination dramatically reduces the risk of recurrent urinary tract infection in children with vesicoureteral reflux
- Success in reversing sickle cell disease with a new, less aggressive blood stem-cell transplant regimen that could be an option for treating older or sicker adults with the disease

This report also includes personal stories of those who have given time and effort to participate in NIDDK-sponsored clinical research. A woman whose liver had been damaged by the hepatitis C virus describes the road she has traveled with family and scientists on the way to living virus-free. A man who has lived with type 1 diabetes for over 50 years joyfully shares how his life has been transformed by an islet transplant. A Marine with polycystic kidney disease describes

his determination to make a healthy future for himself and others, including participating in a clinical trial even while deployed overseas. A teenager and her mother discuss how getting a diagnosis and the right treatment for a rare metabolic disorder has changed her life for the better.

The NIDDK is continuing efforts to ensure that knowledge gained from its research advances is disseminated to health care providers, patients, and the general public. Such efforts include the Institute's education programs: the National Diabetes Education Program and the National Kidney Disease Education Program. Recent activities of both these programs are highlighted in this publication. Additionally, the Weight-control Information Network, the National Diabetes Information Clearinghouse, the National Digestive Diseases Information Clearinghouse, and the National Kidney and Urologic Diseases Information Clearinghouse develop and distribute science-based information on diseases and disorders within the NIDDK mission. Several hundred brochures, fact sheets, and publications are available to patients, health care providers, and the public both in printed format and on the NIDDK website. I invite you to visit us at *www.niddk.nih.gov*

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

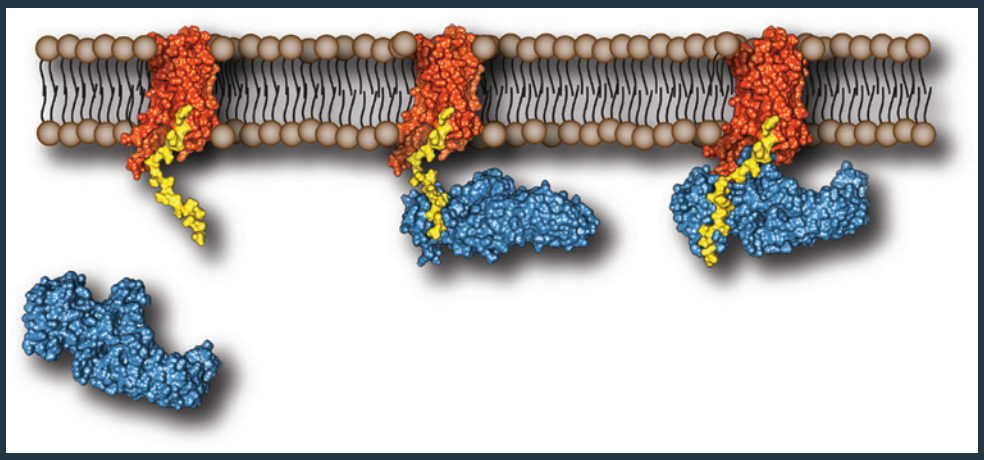
More information on how the NIDDK's activities support these core values can be found in the "NIDDK Funding Trends and Support of Core Values" section at the end of this report and on our website at *www.niddk.nih.gov*

If you have visited the NIDDK online in the past year, you may have noticed some changes. On December 16, 2013, the NIDDK launched its dynamic new website. Created through the dedicated labor and teamwork of staff across the NIDDK, this redesigned website enables us to continue to help our scientific community, patients, and the public. The NIDDK's health information has always been well received by more than 25 million online users annually. Our major goal with the revised website is to provide the same level of clear and current information for researchers. Our research community can now more easily access information about current funding and research training opportunities, NIDDK personnel, and research resources. The site also highlights important NIDDK-supported basic, clinical, and translational advances in the wide range of acute and chronic diseases and conditions in our mission, and includes a quarterly "Director's Update," which provides updates on NIDDK-supported research, health information and education programs, and other news of interest at the NIDDK and the National Institutes of Health (NIH).

This report reflects only a fraction of the immense body of NIDDK-funded research 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

Scan the QR code with your mobile device to link to the NIDDK website at *www.niddk.nih.gov*



G-protein-coupled receptors (GPCRs), found in the cell membrane, transmit biological signals to play critical and diverse roles in maintaining health, and are important targets for about 40 percent of all pharmaceutical drugs on the market today. This illustration depicts three phases of a new model of GPCR regulation by the protein β arrestin. To discover how these proteins interact, researchers slightly modified a GPCR, called β_2 adrenergic receptor, to facilitate their experiments, and used the powerful tools of electron microscopy and mass spectrometry, along with other techniques. (Left) The GPCR core (orange) sits in the cell membrane (row of brown circles and lines) with its "tail" (yellow) extending to the interior of a cell. (Middle) The regulatory protein β arrestin (blue) grasps the GPCR tail. (Right) The regulatory protein then changes orientation to interact with both the GPCR core and tail, blocking the receptor's ability to transmit cellular signals. As described in this chapter, the detailed structural knowledge gained from this fundamental study may help scientists develop additional medications targeting GPCRs to improve health.

Image courtesy of Dr. Georgios Skiniotis, University of Michigan Medical School, Dr. Brian Kobilka, Stanford University School of Medicine, and Dr. Robert J. Lefkowitz, Duke University Medical Center. Adapted by permission from Macmillan Publishers Ltd: Nature, AK Shukla et al. *Visualization of arrestin recruitment by a G-protein-coupled receptor. 512:218–222, copyright 2014.*

Cross-Cutting Science

Medical advances are not always achieved in great, intuitive leaps. More often, new prevention strategies, treatments, and cures result from a long, gradual accumulation of new 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. 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.

Described in this chapter are several recent studies that illustrate the Institute's commitment to basic and applied research relevant across a broad spectrum of scientific disciplines. Also featured are several programs that demonstrate the breadth and collaborative nature of the NIDDK's research efforts, including innovative programs to engage K-12 and undergraduate students from rural or underrepresented groups in STEM fields—science, technology, engineering, and mathematics. Other features describe the creation of the NIDDK Information Network (dkNET), which serves to allow users to simultaneously search multiple databases to locate a biomedical resource, and the development of new NIH policies seeking balance of both males and females in animal and cell studies.

PROTEIN STRUCTURE AND THERAPEUTICS

Structure of Blood Clotting Receptor Identified: Two recent studies have described the discovery of the three-dimensional structure of a protein receptor called P2Y12 that plays a role in blood clotting. Normally, a blood clot forms to seal small cuts or breaks on blood vessel walls and stop bleeding. After the bleeding has stopped and healing has occurred, the body breaks down and removes the clot. Excessive blood clotting can occur if the body's clotting process is altered or wrongly triggered. In addition, blood clots can form in, or travel to, the blood vessels in the brain, heart, kidney, lungs, and limbs—causing such dire medical consequences as heart attack or stroke. The P2Y12 receptor is found on the surface of blood cells called platelets. When the P2Y12 receptor is activated by a molecule called ADP, platelets cluster and a clot forms. The receptor is one of the most prominent clinical drug targets for the inhibition of platelet clustering. Although U.S. Food and Drug Administration (FDA)-approved drugs to limit clot formation are available, their use can cause unwanted side effects.

New research eloquently shows how the P2Y12 receptor's structure undergoes a pronounced shape change when bound to the activator molecule ADP versus a potent inhibitor called AZD1283. Understanding how the P2Y12 receptor responds to drug molecules is the first step toward developing the next generation of clot inhibitors. With the knowledge of a high-resolution structure of the receptor, research chemists can now use computer modeling to identify potentially more efficacious drugs with fewer side effects.

Zhang K, Zhang J, Gao ZG,...Zhao Q. Structure of the human P2Y12 receptor in complex with an antithrombotic drug. Nature 509: 115-118, 2014.

Zhang J, Zhang K, Gao ZG,...Zhao Q. Agonist-bound structure of the human P2Y12 receptor. Nature 509: 119-122, 2014.

Molecular Model Sheds Light on How Antibiotics Are Synthesized: The first high-resolution snapshots of a protein "assembly line" that synthesizes a medically important class of natural products have shed light on how antibiotics and other drugs are produced naturally. This assembly line, a group of polyketide synthase (PKS) enzymes, is found in many organisms, including those that are routinely used for producing antibiotics and several other drugs. PKS enzymes are the molecular machinery responsible for synthesizing polyketides, a class of long, chain-like molecules that includes drugs such as the antibiotic erythromycin and the anti-cholesterol drug lovastatin. PKS enzymes act as modular assembly lines, with different PKS modules modifying a polyketide chain in a particular sequence. Despite the fact that PKS enzymes are the key enzymes responsible for the production of many drugs, the molecular details of how they operate were unknown.

To fill this information gap, researchers used sensitive electron microscopy to zoom in on one PKS enzyme called PikAIII and map its molecular structure both at rest and at work. These "snapshots" of PikAIII as it assembled a polyketide provided crucial clues about how PikAIII's different modules are shaped and how they interact during polyketide synthesis. The researchers found that PikAIII is assembled as an arch around a central reaction chamber. Surprisingly, their data showed for the first time that a mobile "carrier module" inside the reaction chamber shuttles the assembling polyketide between module "workstations" to allow the growing molecular chain to be modified in a precise, orderly fashion. The sequence of these movements is determined by the dynamic movements of the different PikAIII modules as they interact with the assembling polyketide and each other. After PikAIII has completed its work, it then passes the elongated and processed polyketide to the next enzyme in the assembly pathway. These new data clearly demonstrated how PikAIII's structure makes it uniquely suitable for its particular function, including suggesting how PikAIII may "shield" modules from unnecessary contact with growing polyketides to reduce errors in this complex process.

This new information about PikAIII's structure gives exciting new insight into the inner workings of PKS enzymes. Armed with this new understanding of how PKS molecules work, it may now be possible to design PKS enzymes to produce a wide range of clinically relevant drugs more efficiently. Finally, this detailed knowledge about how these drugs are synthesized naturally might also guide the development of new antibiotics and other drug candidates.

Dutta S, Whicher JR, Hansen DA,...Skiniotis G. Structure of a modular polyketide synthase. Nature 510: 512-517, 2014.

Whicher JR, Dutta S, Hansen DA,...Skiniotis G. Structural rearrangements of a polyketide synthase module during its catalytic cycle. Nature 510: 560-564, 2014.

Structural Studies Reveal Details of Early Stages of HIV Infection: NIDDK intramural scientists have described new details of the structural changes that occur at the moment that the virus that causes AIDS penetrates the outer membrane of a target cell.

The first step in viral infection is the attachment of the virus to the surface of a target cell. After latching on to the cell, the virus fuses its outer membrane with that of its target and inserts its genetic material into the cell. In HIV infection, the attachment of the virus is mediated by the protein Env, which consists of three subunits each of the proteins gp41 and gp120. Recent studies have shown that, upon binding to the target cell, Env alters the arrangement of gp41 and gp120, extending gp41 like a spear and pulling the virus and cell closer together to facilitate fusion.

Previous research has given scientists "snapshots" of what gp41 looks like before and after fusion, but the intermediate steps have been poorly understood. Researchers have now gathered new data that give a clearer picture of how gp41 "moves" during fusion. These studies suggest that, upon cell binding, the

three copies of gp41 in an Env molecule spring apart, embedding in the host cell and viral membranes. As gp41 folds back to its resting state, it pulls the membranes with it, bringing viral and cell membranes into close proximity so that membrane fusion can occur and infection can proceed.

This information may shed new light on how existing anti-HIV medications inhibit virus entry and may open the way for novel strategies to disrupt the fusion process that could have important therapeutic implications for the prevention of HIV infection.

Roche J, Louis JM, Grishaev A, Ying J, and Bax A. Dissociation of the trimeric gp41 ectodomain at the lipid-water interface suggests an active role in HIV-1 Env-mediated membrane fusion. Proc Natl Acad Sci USA 111: 3425-3430, 2014.

A Molecular Picture of Key Proteins That Send and Stop Biological Signals: Seeking new insight into the structure of proteins called G-protein-coupled receptors (GPCRs), which transmit important signals in cells throughout the body, researchers discovered how the signals may be stopped by a regulatory protein, β-arrestin. GPCRs play critical and diverse roles in maintaining health and are the targets for about 40 percent of all drugs that are in the market today. For example, a GPCR called the β_2-adrenergic receptor plays important roles in multiple tissues and is the target of drugs for treating respiratory diseases and other conditions. Like other GPCRs, the β_2-adrenergic receptor sits in the membrane at the outer edge of a cell. This provides an ideal vantage point from which the GPCR can bind specific hormones or factors from the nervous system and then send signals into the cell to direct a response, assisted by its namesake G-protein companion. Past research has shown that when cells need to halt or change the signals, they recruit a regulatory protein, β-arrestin. However, it has been difficult to decipher exactly how β-arrestin impedes the signaling of GPCRs such as the β_2-adrenergic receptor. Recently, scientists devised a way to capture and study this elusive protein duo. They slightly modified the β_2-adrenergic receptor to facilitate their experiments, and used the powerful tools of electron microscopy and mass spectrometry, along with other techniques, for modeling and understanding how β-arrestin and the β_2-adrenergic receptor interact at the molecular level. Their data suggest that a β-arrestin protein first grasps onto its partner β_2-adrenergic receptor from one end, and then swings around to engage the receptor across a larger segment. In the resulting configuration, the β-arrestin is positioned as a barricade across the part of the β_2-adrenergic receptor necessary for sending its characteristic G-protein-mediated signals. The detailed structural knowledge gained from this research may help scientists develop additional medications that modulate the signaling of GPCRs to improve health.

Shukla AK, Westfield GH, Xiao K,...Lefkowitz RJ. Visualization of arrestin recruitment by a G-protein-coupled receptor. Nature 512: 218-222, 2014.

INVESTIGATING GENE REGULATION

The Role of Chromosome Loops in Turning On Genes: By studying a protein involved in "turning on" a red blood cell gene, NIDDK intramural scientists learned more about this process and provided insight into a major question in biology. To turn on genes, a process called transcription, a cell first copies ("transcribes") genetic information from the DNA into RNA, and then uses the RNA versions to direct production of proteins encoded by the genes. Red blood cells produce globin proteins, key components of hemoglobin, which carry oxygen in red blood cells from the lungs to the rest of the body. Globin genes are tightly regulated (turned on and off), ensuring that the subsequent production of globin proteins occurs at appropriate times during the development of red blood cells from their precursors in the bone marrow.

The mammalian *β-globin* genes were among the first gene clusters to provide insight into how gene regulation is influenced by long-range chromosomal interactions between DNA sequences far from and near to the protein-coding segment of a gene. Specifically, these interactions occur between a powerful element called an enhancer that helps turn on the *β-globin* gene, also referred to as the gene's locus control region

(LCR), and a DNA element called a promoter, which is immediately adjacent to the gene and helps regulate whether it is on or off. Scientists continue to study enhancers such as the LCR in order to understand more fully their role in regulation of gene transcription.

Scientists previously demonstrated that the formation of a loop of chromosomal DNA, created by the interaction between the *β-globin* gene promoter and LCR, played an important role in *β-globin* gene transcription, and that this required the protein LDB1. To determine how looping was associated with turning on the *β-globin* gene, these scientists dissected the LDB1 protein to test the roles of different parts of the protein in this process. They added different fragments of the LDB1 protein back to mouse blood cells engineered to lack LDB1. In the cells lacking LDB1, both looping and transcription were significantly reduced. However, in adding back fragments, they identified one portion of the LDB1 protein that restored the looping, but was unable to restore activation of transcription. Their findings demonstrated that the processes of looping and transcription activation are separate, that loops can be formed in the absence of transcriptional activation, and that LDB1 plays roles in both processes, addressing fundamental issues that have been poorly understood. These results suggest that looping occurs before transcriptional activation, though the exact role of looping in the ultimate activity of genes remains to be defined.

Further insight into the regulation of gene transcription, a fundamental process in biology, is central to understanding many disease processes. The detailed characterization of the *β-globin* gene locus in particular may help the development of new ways to treat hematologic diseases, such as sickle cell disease, by reactivating dormant hemoglobin genes.

Krivega I, Dale RK, and Dean A. Role of LDB1 in the transition from chromatin looping to transcription activation. Genes Dev 28: 1278-1290, 2014.

Understanding Gene Silencing in Fragile X Syndrome: New NIDDK intramural research has provided important clues in the quest to understand the biology of fragile X syndrome, the most common cause of inherited intellectual disability and autism spectrum disorders, and has identified a key hurdle in finding a method to treat the underlying cause of the disease. People with fragile X do not create enough of a protein called FMRP, which is needed for normal brain development and is encoded by the gene *FMR1*. The first step in making FMRP—like the first step in making any other protein in a cell—is to create a "transcript" of the *FMR1* gene. (The transcript is then processed, and delivered to another part of the cell, where it essentially becomes a blueprint for making the FMRP protein.) For most people with fragile X, the reason they do not make enough FMRP is not that the *FMR1* gene itself is missing or damaged, but rather because there is extra DNA adjacent to *FMR1* that causes it to be "silenced." That is, the extra DNA contributes to interference with that first step in the FMRP production process, the gene's transcription. Scientists studying fragile X have long understood that silencing depends on methylation, a chemical modification of the extra DNA, and also on methylation of certain proteins that are bound to DNA in the area. Drugs that reverse the methylation either of the DNA or the proteins can at least partially undo the silencing, allowing cells to make FMRP. Unfortunately, the improvement is temporary, and the drugs available today are too toxic for use in long-term treatment of people with the disease. The new study is part of an effort to better understand how and why *FMR1* silencing occurs in fragile X, with the hope of developing approaches to achieve more practical, long-term reactivation of the gene.

The researchers used a drug that reverses the DNA methylation in cells from men with fragile X—since the disease is much more common in men than in women—and found that methylation of the DNA-bound proteins was not reduced, even as the cells began to transcribe *FMR1*. In addition, they found that silencing recurred before the DNA was re-methylated. These observations suggest that DNA methylation stabilizes the silenced state of the gene, but is not absolutely required to initiate it, and also that the protein methylation occurs first. Importantly, the scientists also found that the transcript of *FMR1*—

produced after treatment with the drug—is one of the key factors responsible for bringing the enzymes that methylate DNA and protein into proximity with the gene. Thus, the *FMR1* transcript itself contributes to *FMR1* re-silencing. As a result of this study, it is now known that a successful approach to treating fragile X syndrome will need not only to reactivate *FMR1* transcription, but also to break the link between transcription and re-silencing.

Kumari D and Usdin K. Polycomb group complexes are recruited to reactivated FMR1 *alleles in Fragile X syndrome in response to* FMR1 *transcription. Hum Mol Genet 23: 6575-6583, 2014.*

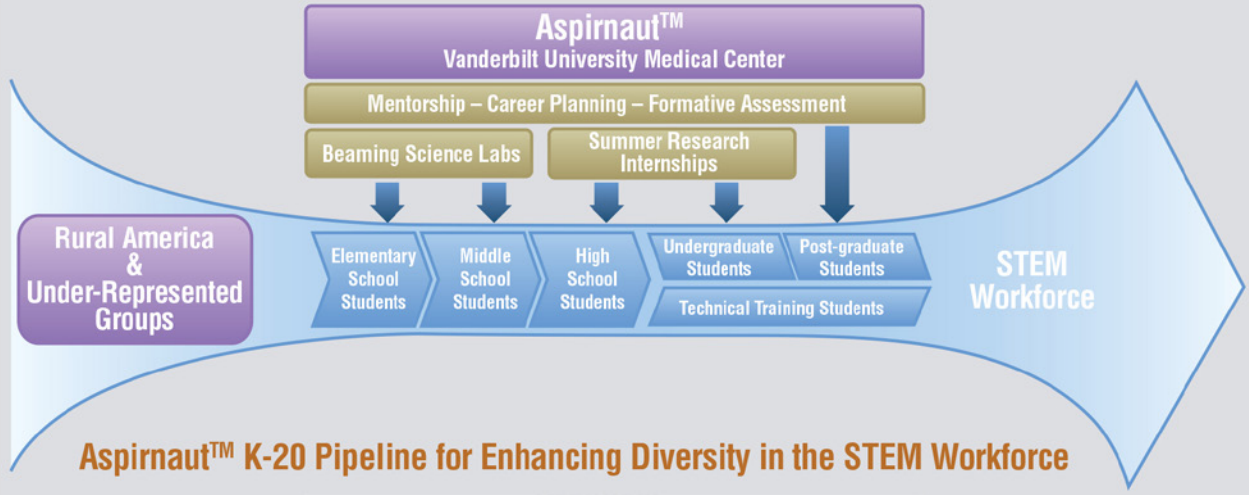
NIDDK-supported Research Training Programs for Underserved and Underrepresented Student Groups

THE ASPIRNAUT INITIATIVE

In 2007, Drs. Julie and Billy Hudson co-founded the Aspirnaut Initiative at Vanderbilt University to engage K-12 and undergraduate students from rural and underrepresented groups in STEM fields—science, technology, engineering, and mathematics. Their vision is to enhance and accelerate STEM education in order to provide talented rural and other underrepresented students the opportunity to choose and excel on a STEM-related career path. Their founding project provided Wi-Fi on school buses in rural Arkansas, where students were equipped with laptop computers, turning long bus rides into productive learning time for STEM subjects. The 3-year pilot project demonstrated feasibility and gained national publicity on *NBC Nightly News*,[1] in the *New York Times*, *Wall Street Journal*, and many other TV and news agencies.

The project has now emerged as the Aspirnaut K-20 Pipeline for Enhancing the Diversity of the STEM Workforce. The program leverages the STEM resources of faculty, students, and facilities of a research university to partner with teachers in rural America. The pipeline consists of: "beaming" of live, hands-on, inquiry-based science labs by university faculty and students to remote schools; sponsoring summer research internships; and continual mentorship and formative assessment of achievement. The project has affected over 120 high school and undergraduate interns and over 2500 elementary and middle school students from 26 states over the past 7 years (for more information, please visit www.aspirnaut.org). The Aspirnauts coauthored a recent publication in the *Proceedings of the National Academy of Sciences*: 6 were middle school students when the study was conducted, 42 were high school students, 30 were college undergraduates, and 5 were graduate students.[2] Specifically, the Aspirnauts researched the evolutionary origin of a specific chemical bond and determined that it traced to a common ancestor dating back more than 500 million years ago.

Dr. Billy Hudson, the Elliot V. Newman Professor of Medicine, Biochemistry, Pathology, Immunology, and Microbiology, and Director of the Center for Matrix Biology at Vanderbilt, grew up in rural south central Arkansas. As a high school dropout, he overcame poverty and childhood abuse by taking advantage of educational opportunities provided by mentors. "Bringing STEM opportunities to the 'forgotten' students



Aspirnaut "pipeline" figure courtesy of Drs. Julie and Billy Hudson, Vanderbilt University

in rural America can greatly increase the number and diversity of students entering STEM careers. Given the recent revolutionary advances in biology, it is essential that scientists partner with K-12 teachers to bring the excitement of discovery and career pathways to America's youth," Dr. Billy Hudson said.

Dr. Julie Hudson is Assistant Vice Chancellor of Health Affairs and Clinical Associate Professor of Anesthesiology and Pediatrics at Vanderbilt University Medical Center. She is a pediatric anesthesiologist with a background that includes development work, governmental relations, biomedical research, and science education. She and her husband have tirelessly spearheaded the Aspirnaut effort—writing grants, establishing cooperative efforts with institutions and businesses, and doing whatever is necessary for the children in the program in order to support the students' efforts to pursue careers in math and science.

Beaming Science Labs to Rural America: The Interactive Videoconference Program

Partnering with elementary and middle school teachers in rural Arkansas, Maine, and Tennessee, Vanderbilt University's undergraduate and graduate students, along with postdoctoral researchers and faculty, produce weekly live, interactive, and inquiry-based STEM lab videoconferences for students. The Hudsons welcome the opportunity to share this program with others in the hope that it can be replicated in other rural communities. The live sessions help participants learn STEM concepts and acquire critical thinking skills. "Engaging students early and often is key to increasing achievement in STEM and the number and diversity of students entering the STEM pipeline," said Dr. Julie Hudson.

The Summer Research Internships

The Aspirnaut pipeline offers summer research internships for rural and underrepresented high school and undergraduate students. Interns are given the opportunity to work side-by-side with scientists and are expected to learn and contribute to the ongoing pace of research advancements. The students conduct research in several areas, including basic science underlying the biology and physiology of kidney function and the basis of kidney diseases such as diabetic nephropathy and hypertension.

The NIDDK began supporting the summer research programs in 2009 when Dr. Billy Hudson applied for and was awarded an American Recovery and Reinvestment Act (ARRA) supplement to his existing grant. The 2009-2010 supplemental award included funds for the Aspirnaut initiative that provided stipends to 20 "Aspirnauts" during an 8-week summer research program. Demographic data of students in the Aspirnaut summer programs for years 2009–2014 illustrates tracked diversity categories (geographic, economic, racial/ethnic, family level of education) of the 69 interns in the cohort: 33 percent met one diversity category while 26 percent met three diversity categories.[3]

Undergraduate Research Program

Drs. Billy and Julie Hudson and their colleagues have been able to leverage the ARRA supplement award into a successful new NIDDK award: an education project grant, entitled "Undergraduate Research Internships in Pathobiology of Diabetic Nephropathy," to help recruit, prepare, and increase the numbers of undergraduate students who pursue STEM disciplines relevant to the NIDDK mission. The 5-year award, which began in 2012, supports 10 undergraduate students each summer; 40 percent of the participants are from underrepresented racial and ethnic groups, including American Indians, and 70 percent from geographically and economically disadvantaged backgrounds. As of May 2014, 38 of 57 interns have graduated from a college degree program, and none have dropped out. Notably, 44 percent of college graduates have enrolled in STEM-related graduate education programs (D.D.S., M.D., Health Administration, M.P.H., Nurse Practitioner, Ph.D.).[3] For more on the journey of two summer high school Aspirnaut students through their undergraduate years at Vanderbilt University, please see *www.youtube.com/watch?v=J07gNLZ8rsg*[4]

Efforts to inspire and elevate STEM achievement in rural K-12 and undergraduate students continue under the Hudsons' leadership and are aligned with the NIH's Recruitment and Retention Plan to Enhance Diversity (please see: http://grants.nih.gov/training/faq_diversity.htm) and the NIH's *Physician-Scientist Workforce Working Group Report* (http://acd.od.nih.gov/reports/PSW_Report_ACD_Executive_Summary_06042014.pdf).

[1] www.nbcnews.com/video/nightly-news/23526146

[2] *Fidler AL, et al. A unique covalent bond in basement membrane is a primordial innovation for tissue evolution. Proc Natl Acad Sci USA 111: 331-336, 2014.*

[3] *Data provided by Dr. Julie Hudson, Vanderbilt University.*

[4] *This video has been nominated for the Mid-South Emmy Award, and winners will be announced January 31, 2015.*

STEP-UP

These high school students participated in STEP-UP. (*Photo credit: Bill Branson, NIH Record*)

The NIDDK's Short-Term Research Experience for Underrepresented Persons (STEP-UP) provides summer research opportunities to talented high school and undergraduate students underrepresented in biomedical research, including students with disabilities, those from a disadvantaged background, and certain racial and ethnic minorities. The overall goal of STEP-UP is to build and sustain a biomedical, behavioral, clinical, and social science pipeline focused on NIDDK mission areas.

To accomplish this goal, STEP-UP provides research education grants to seven institutions to coordinate four high school STEP-UP Programs and three undergraduate STEP-UP Programs that provide eligible students with 10 to 12 weeks of summer research experience and training opportunities that include exposure to the scientific method, the fundamentals of laboratory research, and production of written and oral scientific presentations of their research accomplishments. The sites engaging high school students are located at the University of Hawaii-Manoa, University of Nevada-Las Vegas, Charles R. Drew University-Los Angeles, and Stanford University. The sites engaging undergraduate students are located at Pennsylvania State University-Hershey, Children's Hospital-Los Angeles, and the American Physiological Society.

Dr. Lawrence Agodoa, Director of the NIDDK's Office of Minority Health Research Coordination, which manages the program, has said that having a diverse pool of researchers to tackle some of science's most pressing issues is crucial. "People of all walks of life need to come together and think about how to solve these problems," he said. "Many chronic diseases such as diabetes affect minority communities disproportionately. Having friends and family who are affected by a disease often gives people extra motivation to pursue biomedical research."

STEP-UP also provides research opportunities for high school students who live in the Pacific region. "Students in the Pacific region often live thousands of miles away from facilities that can support cutting-edge

research," said Dr. Agodoa. To overcome this barrier, STEP-UP labs have opened in America Samoa, the Commonwealth of the Northern Mariana Islands, the Republic of the Marshall Islands, the Federated States of Micronesia, and the Republic of Palau. "By providing laboratories and training local science teachers as mentors, we expose students to the newest biomedical research techniques without them needing to travel far from home," Dr. Agodoa noted.

At the end of the summer, the STEP-UP Summer Research Symposium is held on the NIH Bethesda campus. This culminating event provides students the opportunity to showcase their summer research in a formal oral and poster presentation. In addition, the Symposium engages students in a true scientific, convivial manner and exposes them to students from diverse backgrounds.

STEP-UP lab opens in Palau. Local science teachers are being trained to use the equipment.
(Photo credit: Dr. George Hui, University of Hawaii)

dkNET: One-stop Shopping for Biomedical Resources

Want a one-stop Web-based "shop" for finding biomedical resources such as data, reagents, organisms, and tools? The NIDDK has created one: the NIDDK Information Network (dkNET), which allows users to simultaneously search multiple databases for information that may be difficult to find using regular search engines. The information in dkNET is relevant to anyone interested in doing research on kidney, urologic, hematologic, digestive, metabolic, and endocrine diseases; diabetes; or nutrition.

Scientists generate enormous amounts of data, but there is often no good way to track and search all of the existing digital resources for information specific to an important question. dkNET helps solve this problem by providing selected NIDDK-supported data in an online "data-mart" that allows users to take advantage of advanced informatics tools.

Launched in April 2014, dkNET was created by the NIDDK, along with researchers at the University of California, San Diego, and elsewhere, as a catalog of NIDDK-supported online resources. dkNET contains information on an increasing number of resources, currently exceeding 1900, including links to such digital networks as the Nuclear Receptor Signaling Atlas, the GenitoUrinary Development Molecular Anatomy Project, and the Diabetic Complications Consortium. Links to related sources of data take the user to the broader universe of online scientific networks such as the Antibody Registry and Addgene, among others.

The dkNET site is built upon the Web structure SciCrunch, which allows researchers to cost-effectively build and share data repositories. As the online connectivity of research increases, dkNET will gradually scale to serve as a vehicle for future NIDDK-supported data and resources.

The Institute's intent is that sharing data and tools will enrich the research landscape in serendipitous ways. For instance, researchers can use dkNET to search for resources, compare their data with existing data sets, add to the existing store of knowledge, and generate new ideas.

The dkNET site is easy to navigate, includes tutorials, and even has online "office hours" to answer user questions. To find out more, go to *www.dknet.org*

(Adapted from a piece originally published in the summer 2014 NIDDK Director's Update.)

NIH Seeking Balanced Approach to Representation of Both Males and Females in Animal and Cell Studies

Recent research has indicated that biological sex—determined in humans and many animals by the complement of X and Y chromosomes—can have profound and sometimes unexpected effects on health and disease. Some progress has been made in identifying sex and gender[1] differences in clinical research studies, such as finding that certain drugs require different dosing or have different side effects or efficacy in women and men, and that certain diseases and conditions progress differently in women than in men. However, knowledge gaps still exist that could be remedied by expanding consideration of biological sex in pre-clinical research studies conducted in animal models and cells. The NIH is taking steps to ensure that pre-clinical studies it supports include assessment of the impact of biological sex in ways most relevant and beneficial to human health.

In the May 15, 2014, issue of the scientific journal, *Nature*, the Director of the NIH Office of Research on Women's Health (ORWH) and Associate Director for Research on Women's Health, Dr. Janine Clayton, and the Director of the NIH, Dr. Francis Collins, outlined some of the key scientific challenges posed by a lack of knowledge about sex-based differences and pointed out the need for change.[2] For example, while over half of NIH-funded clinical research participants are now women—a change wrought largely through the NIH Revitalization Act of 1993—in studies using animal models, such as mice, researchers have continued to use male animals predominantly even when the condition or question under study is not exclusive to males. In studies using cells, the biological sex of the original animal or human has also been largely overlooked as a factor that could affect experimental results.

These practices may not only have unintentionally led to gaps in knowledge, but also inadvertently hindered the hunt for treatments and cures. For example, by using only male animals, fundamental differences between males and females that could guide later clinical studies in humans may have been missed. Discovery of such differences earlier in the research pipeline may help avoid such costly and challenging problems as sex-based adverse drug reactions. Moreover, it is possible that by focusing on male animals, novel drug targets and treatment approaches that might be more effective in females have remained as yet undiscovered. Finally, inadequate analysis and reporting of sex differences in scientific journals could be contributing to some of the irreproducibility of published research results that has been observed in recent years and which the NIH is actively working to address.

The NIH is now developing policies that will require NIH applicants to consider sex as a scientific variable in research involving animals, tissues, or cells. This change is being pursued through a deliberate and thoughtful approach, spearheaded by a trans-NIH working group, and will be implemented in phases. For example, to ensure that the views and concerns of the public and scientific community were considered in development of these policies, the NIH solicited comments on "Consideration of Sex as a Biological Variable in Biomedical Research" through a request for information in September 2014. Other efforts are informing policy development, as well. For example, on October 20, 2014, the ORWH hosted a public workshop to discuss scientific concepts, methods, challenges, techniques, and strategies for integrating biological sex as a variable in pre-clinical research. The ORWH also continues to offer, in partnership with the U.S. Food and Drug Administration, online training in the science of sex and gender differences, as well as other online resources that will help to support this change toward studying basic biology and disease processes in both males and females to strengthen science overall.

To facilitate the phasing in of the new policies, the NIH will be issuing notices in the *NIH Guide for Grants and Contracts* that will explain what new information should be included in applications and progress reports to

address sex differences and the timing of these new requirements. The NIH is also developing guidelines for how grant application reviewers should consider information given about the sex of animals when they evaluate proposals. In addition, the NIH is working with others in the scientific community to encourage consideration of the importance of sex differences in research efforts it supports. For example, the NIH is working with editors of scientific journals to encourage the reporting of analyses of both males and females in animal and human studies when these studies are published. For more details and updates about NIH policy development, as well as links to resources in this research area, see the ORWH webpage "Studying Sex to Strengthen Science (S4)" at *http://orwh.od.nih.gov/sexinscience/*

The NIDDK fully supports these efforts to enhance our knowledge of human health. Reflecting the spirit of these new policies, the NIDDK has updated its approach to reporting on research it supports within this annual publication. Already, summaries of research studies conducted in people usually note whether studies were performed in men, women, or both. Now, whenever the information is provided in the original research article, summaries of studies using mice, rats, and certain other animal models will indicate whether male, female, or both sexes were used. The results of any sex differences analyses that were performed will also be captured. In this way, the Institute hopes to model—and encourage—the routine reporting of biological sex as a scientific variable just as significant as whether an experiment was performed in the liver versus the pancreas.

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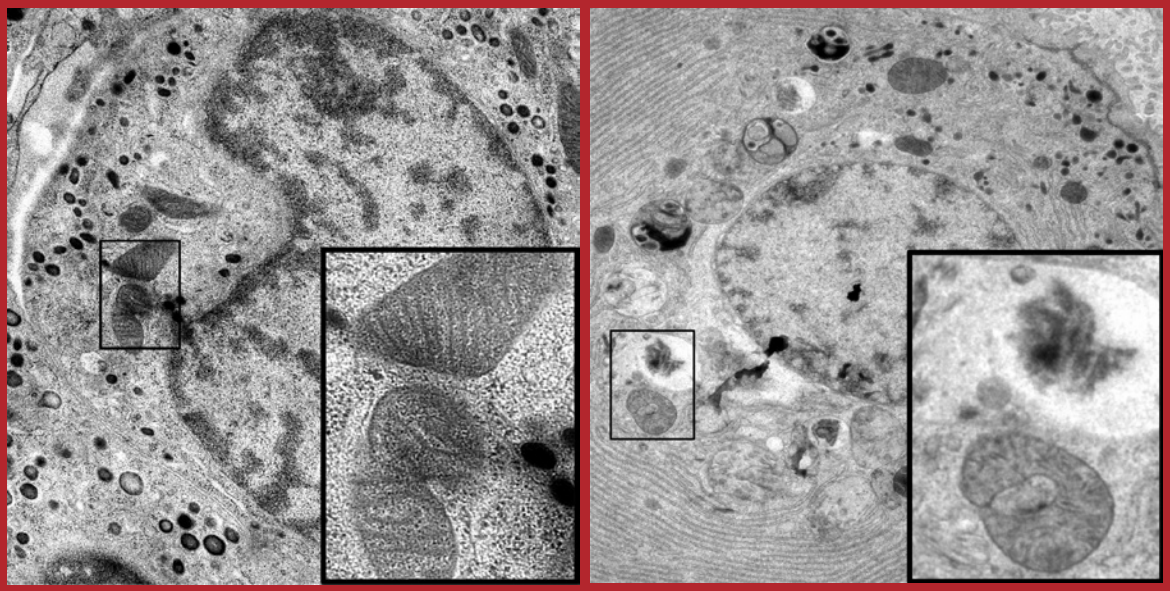
[1] *The terms "sex" and "gender" are often used interchangeably when referring to males and females. However, according to definitions recommended by the Institute of Medicine, biological sex differs from gender in that gender (*e.g.*, "woman" or "man") refers to how a person presents themselves to others as male or female, or how that person is responded to by social institutions on the basis of the individual's gender presentation. Gender is thus a human term rooted in biology and shaped by environment and experience, whereas sex is simply an innate biological feature shared by many organisms, including humans.*

[2] *Clayton JA and Collins FC. Policy: NIH to balance sex in cell and animal studies. Nature 509: 282-283, 2014.*



Healthy mitochondria, the “powerhouses” of the cell, are necessary for insulin-producing β (beta) cells of the pancreas to function normally. This set of high magnification transmission electron micrograph images shows how loss of the protein Clec16a in mouse β cells affects mitochondria. As shown in detail in the insets, normal, healthy mouse β cell mitochondria (left) exhibit highly ordered structural patterns, while mitochondria in β cells lacking Clec16a protein (right) appear amorphous and unhealthy. As described in this chapter, Clec16a appears to play an important role in cellular mechanisms exerting “quality control” on mitochondria. The gene encoding the human version of Clec16a is known to affect risk for type 1 diabetes, so findings from this study may help further understanding of the protein’s role in human disease.

Image courtesy of Dr. Scott A. Soleimanpour, University of Michigan, and Dr. Doris A. Stoffers, University of Pennsylvania School of Medicine. Reprinted from Cell, Vol 157, Soleimanpour SA, Gupta A, Bakay M, Ferrari AM, Groff DN, Fadista J, Spruce LA, Kushner JA, Groop L, Seeholzer SH, Kaufman BA, Hakonarson H, Stoffers DA, The diabetes susceptibility gene Clec16a *regulates mitophagy, Pages 1577-1590, Copyright 2014, with permission from Elsevier.*

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.

Diabetes is a debilitating disease that affects an estimated 29.1 million people in the United States—or 9.3 percent of the total population—and is the seventh leading cause of death.[1] Compared with people of similar age without the disease, overall rates of death are about 1.5 times higher in people with diabetes, and rates of death from cardiovascular disease are 1.7 times higher.[1] Although rates of diabetes-related complications have declined substantially in the past 2 decades, disease burden remains significant as the number of people with diabetes continues to increase.[2] 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 diabetes in the United States in 2012—including costs of medical care, disability, and premature death—was $245 billion.[3] Effective therapy can prevent or delay diabetic complications, but approximately one-quarter of Americans with diabetes are undiagnosed and therefore not receiving therapy.

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 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.[1] 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

[1] *Centers for Disease Control and Prevention. National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014. Atlanta, GA: U.S. Department of Health and Human Services, 2014.*

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

[3] *American Diabetes Association. Diabetes Care 36: 1033-1046, 2013.*

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 patients to control blood glucose levels to levels achieved by functional β cells. Thus, researchers are actively seeking new methods to improve blood glucose monitoring and insulin delivery. This includes continued development and testing of "artificial pancreas" technologies in real-world settings, as well as 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.[1] 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. 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, and Native Hawaiians and Pacific Islanders.[1] Gestational diabetes is also a risk factor: shortly after pregnancy, 5 to 10 percent of women with gestational diabetes continue to have high blood glucose levels and are diagnosed with diabetes, usually type 2.[1]

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 86 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.[1] This population is at high risk of developing 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. Moreover, follow-up research has shown that this benefit of reduced diabetes risk can persist for at least 10 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, the NIDDK-supported 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. 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 greater risk with respect to complications than those who develop the disease later in life. 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 lead 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.

UNDERSTANDING RISING DIABETES RATES IN AMERICAN YOUTH

Rates of Diabetes Increasing in U.S. Youth: The SEARCH for Diabetes in Youth study has provided new data on the prevalence (proportion of the population with the disease) and incidence (proportion of the population who develop the disease each year) of both type 1 and type 2 diabetes in a geographically and racially/ethnically diverse group of children and teens. This information will help researchers identify trends and potential causes of the disease, which is a significant health problem in the United States.

SEARCH researchers found that between 2001 and 2009, both types of diabetes became increasingly prevalent in youth under 20 years of age. Type 1 diabetes remains the predominant form in youth, with about four times as many youth affected with type 1 diabetes than with type 2 diabetes. Except in American Indians, type 1 diabetes accounts for nearly all diabetes in children under the age of 10. However, prevalence of type 2 diabetes is increasing more rapidly. Overall, the proportion of youth with type 2 diabetes rose by 30.5 percent while the proportion with type 1 diabetes rose by about 21 percent.

The increase in type 1 diabetes prevalence was seen in both sexes and in White, Black, Hispanic, and Asian Pacific Islander youth. Historically, type 1 diabetes has been considered to affect primarily non-Hispanic White youth; the new data demonstrate that it is also an increasing burden for minority youth. Additionally, SEARCH found that rates of new cases of type 1 diabetes increased by about 2.7 percent per year in non-Hispanic White youth, with the increase seen in both males and females and in all age groups except the youngest (0- to 4-year-olds).

Once considered a disease of adults, type 2 diabetes has emerged as a significant health issue among U.S. youth, spurred by the prevalence of obesity, a strong risk factor for this form of diabetes. SEARCH found significant increases in type 2 diabetes prevalence between 2001 and 2009 in both sexes, all age groups, and in White, Hispanic, and Black youth. While there were no significant changes for Asian Pacific Islanders and American Indians, American Indians had rates 10-fold greater than in White youth. Overall, the highest prevalence was found in American Indians, followed by Black, Hispanic, and Asian Pacific Islander youth, with lowest prevalence in White youth. These results demonstrate the increasing burden of type 2 diabetes on youth of minority racial/ethnic groups in the United States.

Increases in both type 1 and type 2 diabetes prevalence and incidence in youth are worrying because these populations face unique challenges in managing their diabetes and may be at greater risk of diabetic complications later in life due to their long disease duration. Collectively, the SEARCH findings provide critical information on recent trends in diabetes in U.S. youth and will help inform future planning, research, and policies aimed at relieving the burden of diabetes.

Dabelea D, Mayer-Davis EJ, Saydah S,…Hamman RF; SEARCH for Diabetes in Youth Study. Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. JAMA 311: 1778-1786, 2014.

Lawrence JM, Imperatore G, Dabelea D,…D'Agostino RB Jr.; for the SEARCH For Diabetes In Youth Study Group. Trends in incidence of type 1 diabetes among non-Hispanic white youth in the United States, 2002-2009. Diabetes 63: 3938-3945, 2014.

GENETICS AND GENETIC REGULATION OF TYPE 2 DIABETES

Rare Genetic Mutations That Protect Against Type 2 Diabetes Suggest Possible Approach for Treating the Disease: Researchers examining multiple different ethnic populations have identified

rare mutations in the gene *SLC30A8* that appear to significantly reduce risk for type 2 diabetes. Most mutations have little or no impact on health, and some have detrimental health effects, particularly if they interfere with the proper function of an important gene; but occasionally mutations may be found that reduce the function of a gene (referred to as loss-of-function mutations), yet provide a detectable health benefit. Such mutations offer a particularly alluring possibility: the potential to design medicines that specifically reduce the activity of the gene, or of the protein it encodes, in a way that may be similarly beneficial for people who do not have the protective mutation. To identify possible loss-of-function mutations that might protect against type 2 diabetes, the research team focused on genes already thought to be involved in diabetes because common variations in or near the genes are known to be associated with modest differences in risk for the disease. Among nearly 14,000 people, almost half of whom had type 2 diabetes, they found a mutation that truncates the protein encoded by the *SLC30A8* gene in 21 people without diabetes, but in only 7 people who had the disease. Expanding their analysis to include about 150,000 people of multiple racial/ethnic backgrounds (African American, European, South Asian, and East Asian), the researchers found more protein-truncating mutations in *SLC30A8*. Overall, they found that these rare mutations were about three times as likely to occur in people who did not have type 2 diabetes as in those who did, strongly suggesting that *SLC30A8* loss-of-function mutations protect against type 2 diabetes.

Surprisingly, this was just the opposite of the previous scientific consensus about the gene and its function. *SLC30A8* encodes a protein called ZnT8 that helps bring zinc into cells. ZnT8 is produced at high levels in the insulin-producing β (beta) cells of the pancreas, where zinc is known to play an important role: it stabilizes insulin stored within the cells prior to secretion. In humans, there are two common *SLC30A8* variants that both encode full-length ZnT8 proteins, although the ZnT8 proteins produced by the two variants are slightly different from one another. One of these two common variants was previously associated with a modest increase in risk for type 2 diabetes, and was also thought to reduce zinc transport. Taken together, the results of the newer and older studies represent something of a puzzle: rare mutations that completely inactivate one of a person's two copies of *SLC30A8* lower risk of type 2 diabetes, while a much more common version of *SLC30A8* that encodes a lower-activity form of ZnT8 raises disease risk. Further research is needed to explain this paradox and to determine whether type 2 diabetes can be better treated or prevented through therapeutic modulation of ZnT8 function.

Flannick J, Thorleifsson G, Beer NL,...Altshuler D. Loss-of-function mutations in SLC30A8 *protect against type 2 diabetes. Nat Genet 46: 357-363, 2014.*

Micro Molecules May Have Big Role in Type 2 Diabetes: Researchers identified microRNAs that may be factors in type 2 diabetes. MicroRNAs (miRNAs) are molecules that specifically regulate gene expression—whether a gene is "turned on"—and, in many cases, leads to the production of a protein. Specifically, miRNAs block the production of proteins by interfering after the gene is turned on. Not all genes, however, encode proteins. For example, some genes encode miRNAs. Because each type of miRNA is present in numerous copies in a cell and can specifically regulate expression of multiple different protein-encoding genes, cells can use miRNAs to downregulate several "targets" simultaneously. miRNAs have been shown to affect the development and progression of several diseases and, therefore, are speculated to have a role in type 2 diabetes. In this study, scientists aimed to identify miRNAs that are involved in type 2 diabetes. They catalogued all the miRNAs found in pancreatic islet cells from people with type 2 diabetes and from people without the disease, and found that some miRNAs are produced in greater abundance in the islets of people with type 2 diabetes, and some are produced at lower levels. Interestingly, several of the miRNAs produced at lower levels are encoded by genes in the same region of the genome.

By studying this cluster of genes that encode the miRNAs, the scientists gathered information about how these miRNAs might influence development of type 2 diabetes. Looking at different types of

islet cells, they discovered that the miRNAs in this cluster are highly and specifically produced in normal β (beta) cells and repressed in β cells from people with type 2 diabetes. The repression, the researchers discovered, correlated with the increased presence of an "epigenetic" mark near the cluster. Epigenetics refers to a phenomenon in which there are changes in gene activity without alteration of the genome sequence. This phenomenon can be temporary or fixed, and can even be passed from generation to generation in families. Thus, along with genetic factors that increase type 2 diabetes risk due to genome sequence variants, epigenetic marks may also increase risk for developing this disease and may explain, in part, why type 2 diabetes runs in families. Therefore, the increased presence of the epigenetic mark near this cluster of miRNA genes could be a heritable risk factor for type 2 diabetes.

Additional experiments showed that the miRNAs in this cluster regulate gene expression and, consequently, the production of proteins involved in many key biological processes, including cell death, a process that can contribute to the development of type 2 diabetes. These results suggest that, in people with type 2 diabetes, the miRNA cluster is "turned off," leading to decreased production of the miRNAs and, as a consequence, increased production of proteins that cause β cell dysfunction and cell death. This early study indicates that miRNAs may play a role in type 2 diabetes development, progression, or both. Further research is needed to elucidate the detailed role of miRNAs in type 2 diabetes and to determine whether increasing activity of specific miRNAs could be a therapeutic strategy to protect against the disease and its progression.

Kameswaran V, Bramswig NC, McKenna LB,...Kaestner KH. Epigenetic regulation of the DLK1-MEG3 *microRNA cluster in human type 2 diabetic islets. Cell Metab 19: 135-145, 2014.*

A Genetic Mutation That Affects Fat Metabolism Can Bring on Type 2 Diabetes: Recent research suggests that a protein involved in the metabolism of fat may play a critical role in helping prevent type 2 diabetes. Although overweight and obesity are clearly associated with an elevation in risk for type 2 diabetes, the precise way or ways in which excess body fat contributes to development of the disease remain a subject of considerable scientific debate and investigation. According to one major hypothesis, defects in fat metabolism and increased levels of metabolic intermediates in the fat utilization process may adversely affect insulin sensitivity and/or production. To explore this possibility, researchers examined genes encoding many of the proteins involved in fat metabolism in Amish families in which some family members had type 2 diabetes. An advantage of conducting this kind of study among the Amish is that they have a relatively homogenous, non-mechanized lifestyle. As a result, differences in metabolic health between Amish individuals are more likely to be due to genetic factors than to diet or exercise. Working with this unique group of people, researchers identified a mutation present in about 5.2 percent of Amish people tested that was strongly associated with type 2 diabetes risk. This mutation affects a gene that encodes a protein called hormone-sensitive lipase (HSL). The mutation is also found in about 0.2 percent of non-Amish people of European descent.

HSL plays a vital role in liberating triglycerides—the primary type of fat stored by the body in adipose (fat) tissue—when they are needed for energy. Thus, one might expect that having insufficient HSL would lead to an accumulation of excess fat throughout the body, promoting obesity. Surprisingly, however, the researchers found that having one mutated copy of the HSL gene and one normal copy of the gene (effectively cutting levels of the HSL protein in half) was associated specifically with an increase of fat stored in the liver, though not elsewhere, and with unhealthy levels of triglycerides and other fats in the blood. Importantly, the mutation promoted significant insulin resistance, and almost doubled the risk for type 2 diabetes. In addition, the researchers identified four individuals who lacked any normal copy of the gene, making it possible to examine health effects of not being able to make any HSL protein at all. All four developed type 2 diabetes before the age of 50, and had sharply elevated fasting triglyceride levels and slow triglyceride clearance after a high-fat meal, compared to people with functional

HSL protein. These findings mark HSL as an important metabolic regulator. Further research will be needed to determine how the protein helps prevent type 2 diabetes, and whether its activity can be safely modulated to help treat or prevent the disease.

Albert JS, Yerges-Armstrong LM, Horenstein RB,...Damcott CM. Null mutation in hormone-sensitive lipase gene and risk of type 2 diabetes. N Engl J Med 370: 2307-2315, 2014.

INSIGHTS INTO TYPE 2 DIABETES TREATMENTS

Comparing Surgical and Non-surgical Treatments for Type 2 Diabetes in Adults Who Have Mild or Moderate Levels of Obesity: Two small clinical trials found that after 1 year of treatment, bariatric surgery may be more effective than non-surgical approaches for treating type 2 diabetes in adults who have mild or moderate levels of obesity. They also identified factors to be considered in planning further research. Previous studies demonstrated the benefits of bariatric surgical procedures for weight loss and for ameliorating type 2 diabetes, at least in the short-term, in individuals with either extreme obesity (defined as a "body mass index," or BMI, of 40 or more) or somewhat lower levels of obesity (BMI 35 to 40). However, there has been only limited research on this surgery in people with milder levels of obesity (BMI 30 to 35). Thus, investigators recently conducted small clinical trials to gain preliminary insights into the risks and benefits of bariatric surgery, compared to non-surgical interventions, for type 2 diabetes in people with various levels of obesity. The trials also aimed to elucidate the challenges to be addressed in designing a more extensive study with larger numbers of participants.

In one of the clinical trials, investigators randomly assigned 69 volunteers to receive either bariatric surgery or an intensive lifestyle intervention for weight loss, and then compared health outcomes after a year. All of the participants had type 2 diabetes and mild to moderate levels of obesity (BMI 30 to 40); most were women. Those assigned to surgery received either a Roux-en-Y gastric bypass (RYGB) procedure or laparoscopic adjustable gastric banding (LAGB). The intensive lifestyle intervention involved both diet and exercise and was delivered in an individualized format. After 1 year, about 50 percent of the individuals in the RYGB group and 27 percent of those in the LAGB group had partial diabetes remission. Their blood glucose (sugar) levels, although not quite normal, were no longer in the range of diabetes; and they were able to discontinue their diabetes medications. Several individuals in each surgical group achieved complete remission of their diabetes—normal blood glucose levels without need for diabetes medications. None of the people in the lifestyle intervention group had partial or complete diabetes remission. Participants in all of the groups lost weight; those in the RYGB group had the greatest weight loss. However, several individuals in the RYGB and LAGB groups experienced complications, including a surgery-related ulcer and dehydration.

In the other clinical trial, investigators randomly assigned volunteers with type 2 diabetes and obesity (BMI 30 to 42) to receive either RYGB surgery or a lifestyle and medical intervention of diet and exercise, delivered in group sessions, with a weekly medication adjustment plan. Of the 38 participants analyzed in the study, a majority were women. After 1 year, 58 percent of the individuals in the RYGB group, but only 16 percent of those in the lifestyle and medical therapy group, saw their blood glucose levels improve to the target for glucose control chosen as the study outcome. Weight loss was greater in the RYGB group as well. Assessing the safety of the interventions, the researchers noted several serious adverse events among participants in the RYGB surgery group, including ischemic heart disease requiring coronary artery bypass surgery and depression with attempted suicide. In the non-surgical group, a few individuals experienced near-fainting.

The researchers also described important "lessons learned" from these clinical trials. A major challenge was recruitment. Each research team screened hundreds of potential volunteers, but only about 1 of every 10 individuals screened for one study and 1 of 20 screened for the other study were both eligible and interested in participating. In each study, several individuals stopped

participating after being assigned to a group, but before receiving the intervention. Costs also posed a challenge because insurers do not currently cover bariatric surgery for people with lower levels of obesity. For these trials, the investigators obtained some funding for the surgical procedures either from their academic medical center or industry. The research aspects were supported by the NIH. Finally, the types of bariatric surgery used in clinical practice continue to change over time; it may thus be valuable to study different surgical procedures in the future.

In summary, these small clinical trials add to preliminary evidence that bariatric surgery may be more effective than current non-surgical treatments for type 2 diabetes in individuals with mild to moderate levels of obesity. This research also highlights challenges to be met in designing larger and longer-term studies in the future, to gain more definitive data on risks and benefits.

Courcoulas AP, Goodpaster BH, Eagleton JK,...Jakicic JM. Surgical vs medical treatments for type 2 diabetes mellitus: a randomized clinical trial. JAMA Surg 149: 707-715, 2014.

Halperin F, Ding SA, Simonson DC,...Goldfine AB. Roux-en-Y gastric bypass surgery or lifestyle with intensive medical management in patients with type 2 diabetes: feasibility and 1-year results of a randomized clinical trial. JAMA Surg 149: 716-726, 2014.

For more information on bariatric surgery, please see the Obesity chapter.

New Explanation for the Glucose-lowering Effect of the Diabetes Medication Metformin: New research suggests that metformin, the first-line therapy for type 2 diabetes, works by directly inhibiting the activity of a key metabolic enzyme. In addition to its role as a diabetes medication, metformin is a safe and effective treatment for prediabetes and polycystic ovarian disease, and is being studied as a potential therapy for cardiovascular disease and some cancers. While safe, inexpensive, and highly effective for type 2 diabetes, its use is limited due to gastrointestinal side effects in some people, genetic variation causing inter-individual differences in response, and safety concerns in people with chronic kidney disease. For these reasons, there is considerable interest in finding alternative treatments that act in the same way as metformin. However, although metformin has been widely used for decades, its molecular mechanism has remained elusive and is a subject of considerable scientific debate.

Metformin is known to lower blood glucose (sugar) by reducing the amount of glucose produced by the liver and released into the blood. While it is agreed upon that energy metabolism is at the center of metformin's mechanism of action, the precise target of metformin within the complex processes that take place in cells to convert energy from nutrients into forms needed to fuel cellular activity has not been established. Now, a new mode of action has been suggested by the finding that metformin directly inhibits the action of mitochondrial glycerophosphate dehydrogenase (mGPD), an enzyme that helps make it possible to move molecules derived from stored fat into the mitochondria for conversion into glucose. This enzyme is not among those previously studied in connection with metformin action, although mice deficient in this enzyme do not develop elevated glucose levels on a high-carbohydrate diet. Importantly, researchers found that experimentally reducing the amount of mGPD in cells lowered blood glucose levels at a speed and to an extent similar to what is observed with metformin treatment. Further, they found that in male rats lacking mGPD, metformin did not result in any further lowering of blood glucose, suggesting that metformin's effect on this enzyme may account for much of the medicine's glucose-lowering properties. Because these experiments were performed in rodents, the researchers tested the effect of metformin on a purified human form of the enzyme and found similar inhibition.

These findings identify mGPD as a key facilitator of liver glucose production and suggest that if safe new medicines that target the same enzyme in people can be identified, they might represent effective

glucose-lowering treatments for individuals who cannot tolerate metformin, or in whom the drug is not effective.

Madiraju AK, Erion DM, Rahimi Y,...Shulman GI. Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. Nature 510: 542-546, 2014.

ADVANCING TECHNOLOGY IN DIABETES MANAGEMENT

Artificial Pancreas Technologies Excel in Real-world Tests: Two studies propelled progress toward development of artificial pancreas technologies, a promising treatment for people with type 1 diabetes. People with this disease do not produce insulin, a hormone made by the pancreas that regulates the level of glucose (sugar) in the blood and delivers glucose to the cells of the body. Therefore, they have to receive injections of insulin on a daily basis or wear an insulin pump. Too little insulin can lead to high blood glucose, which increases the risk of diabetic complications. Too much insulin, however, is dangerous as well, resulting in low blood glucose (hypoglycemia) which can lead to coma or death, a particular concern during sleep. People with type 1 diabetes walk a tightrope to keep their blood glucose levels within a healthy range and continually must check their levels with fingerstick tests or a continuous glucose monitor. With these burdensome methods it is difficult to achieve recommended levels of blood glucose control. An artificial pancreas, or a closed-loop system, could help people achieve these recommended levels, as well as alleviate patient burden, by linking three technologies: a glucose-sensing component, an insulin delivery device, and a computer that calculates the amount of insulin needed in response to the blood glucose level.

Early artificial pancreas clinical trials took place in hospital settings and used laptop computers to run the technology, restricting the activities of participants. Recent trials have built on the success of the inpatient trials, testing ambulatory devices in real-world settings, with some of the challenges of everyday life—such as eating a variety of foods, which raise blood glucose to different levels, and participating in various forms of physical activity, which lower blood glucose. In one study, scientists achieved exciting results testing unsupervised overnight home use of a closed-loop system in 16 adolescents with type 1 diabetes for 21 nights. During the day, the participants used standard glucose sensor and pump therapy and there were no restrictions placed on their daytime activities—they participated in school and other activities, including sports, and ate a regular diet. At night, they used the closed-loop system, controlling it on their own, with minimal supervision on only the first night. Compared to a control period during which the standard glucose sensor and pump therapy were used both day and night, unsupervised closed-loop control at night improved participants' glucose control during the day and night, and also reduced the number of episodes of nighttime hypoglycemia. The success of this study illustrates the high potential for closed-loop technology to be translated into clinical care.

In another study, researchers tested a wearable, automated, bihormonal "bionic" pancreas—one that releases both insulin and its counteracting hormone, glucagon. By including both of these hormones, the scientists hope to replicate more closely the sophisticated glucose control of the biological pancreas. They tested the bionic pancreas in two scenarios, one with adults with type 1 diabetes in Boston and one with adolescents with type 1 diabetes at diabetes summer camp. Twenty adults wore this device, which was controlled by a cell phone, around Boston for 5 days and nights, unrestricted in their activities. They ate in restaurants, exercised at gyms, and stayed in a hotel and were accompanied by study staff. Thirty-two adolescents at diabetes summer camp wore the same device for 5 days. In both scenarios, compared to usual care (insulin pump), participants had lower mean glucose levels and reduced episodes of hypoglycemia. In fact, the bionic pancreas allowed nearly all participants to achieve recommended levels of blood glucose control.

With the encouraging results of these two studies, additional, larger trials of artificial pancreas technologies could pave the way toward conducting pivotal trials needed for U.S. Food and Drug

Administration approval of these technologies. Further development of this technology will also improve efficacy and usability, but this research highlights the ability of artificial pancreas technologies to help people with type 1 diabetes achieve good blood glucose control and lead freer, healthier lives.

Hovorka R, Elleri D, Thabit H,...Dunger DB. Overnight closed-loop insulin delivery in young people with type 1 diabetes: a free-living, randomized clinical trial. Diabetes Care 37: 1204-1211, 2014.

Russell SJ, El-Khatib FH, Sinha M,...Damiano ER. Outpatient glycemic control with a bionic pancreas in type 1 diabetes. N Engl J Med 371: 313-325, 2014.

BETA CELLS AND DIABETES

Discovery of New Ways To Produce Beta Cells: Two different research groups in the NIDDK's Beta Cell Biology Consortium have made important steps forward with research on generating insulin-producing β (beta) cells from other types of cells, accelerating progress toward potential new therapies for type 1 diabetes. In people with type 1 diabetes, the β cells are destroyed erroneously by the immune system, resulting in people with the disease having to rely on administration of insulin. Although administration of insulin is a life-saving therapy, it does not replicate the biology of the β cells. Therefore, a goal of type 1 diabetes research is to generate ways to replace the lost β cells. Years of research into β cell biology has focused on ways of producing β cells in the laboratory or of regenerating β cells within a person's pancreas.

One research group determined a method to produce β cells from human stem cells in the laboratory, an advance that could bolster islet transplantation as a therapy for type 1 diabetes. Islet transplantation—during which pancreatic islets, including β cells, are transplanted into people whose own β cells are destroyed—is a promising experimental treatment for type 1 diabetes. However, islet transplantation has been hindered, in part, by the limited quantities of donor islets and the side effects of immunosuppression. The field of stem cell biology has offered hope that islets could be produced in the laboratory. Stem cells are pluripotent, meaning they are able to produce any type of cell in the body, and induced pluripotent stem cells can be made by "reprogramming" adult cells. Previous attempts to produce islets in the lab by differentiating, or maturing, human stem cells into β cells have generated cells that produce insulin, but which lack several important β cell-like qualities, such as a finely tuned response to changing glucose (sugar) levels.

Drawing on this previous research, scientists sought to improve on these results by testing over 70 chemical compounds in over 150 combinations. They developed an optimized, multistep process utilizing 11 of these compounds in a precise sequence over the course of 4 to 5 weeks. By the end of this process, the researchers had coaxed large numbers of both human embryonic stem cells[1] and induced pluripotent stem cells (which can be made from adult cells, including cells from those with type 1 diabetes) into a state that closely resembles naturally occurring β cells. Importantly, these "stem-cell-derived β (SC-β) cells" are similar to pancreatic β cells and respond to fluctuating glucose levels by increasing or decreasing secretion of insulin, as appropriate. To test whether they might be therapeutically useful, the researchers transplanted human embryonic SC-β cells into mice genetically engineered to display type 1 diabetes-like symptoms. After 2 weeks, the SC-β cells were producing significant amounts of insulin in response to glucose and prevented the mice from developing dangerously high blood glucose levels.

Although the process will need to be adapted for large-scale manufacturing, and further tests must be conducted to determine if SC-β cells can be a long-term replacement for β cells in people, this dramatically improved process for making large amounts of β cells is a promising step toward developing therapeutic stem cell therapies. SC-β cell technology may lead to advances in treating diabetes and in artificial organ

[1] *The NIH supports research using human embryonic stem cells within the* NIH Guidelines for Human Stem Cell Research.

development, especially if ways to protect newly transplanted β cells from the autoimmune attack are developed. Additionally, SC-β cells offer a valuable new resource for investigating β cell biology and disease modeling, as well as opportunities for drug screening and testing novel potential therapies.

The second research team discovered that δ (delta) cells within the pancreas are capable of being reprogrammed into β cells, another potential way to restore the β cells lost in people with type 1 diabetes. Pancreatic islets are composed of several types of hormone-producing cells: insulin-producing β cells, glucagon-producing α (alpha) cells, and somatostatin-producing δ cells. Previous research in mice demonstrated that, following targeted destruction of the β cells, the pancreas can recover the ability to produce insulin. How this recovery occurs has not been well-understood, but the biological processes potentially could be adapted to restore insulin production in people with type 1 diabetes.

The researchers had previously demonstrated that, following β cell injury, α cells were able to convert directly to "β-like" cells and produce insulin in adult male mice. In this new advance, scientists discovered a novel pathway for the generation of "β-like" cells following a β cell injury in juvenile male mice, which differs from the α cell-dependent pathway in adult male mice. They found that a different pathway occurs in juvenile mice than in adults: δ cells revert to an uncommitted precursor-like state, replicate, and then some convert to β-like cells and acquire the ability to produce insulin. Further research will determine whether either or both the adult and juvenile β cell regeneration processes are active in humans and whether these processes can be adapted to regenerate lost β cells in people with type 1 diabetes. As these experiments showing δ cell conversion to β cells were performed in male mice, future studies may show if this process also occurs in females, and new ways to protect regenerated β cells from immune attack will need to be developed, as well.

These two studies are important steps forward in β cell biology, biotechnology, and the treatment of type 1 diabetes. Future studies will be necessary to determine whether these new ways of producing β cells will be useful in developing human therapies. Together, these findings offer hope that new β cells can be generated to replace those lost in type 1 diabetes and suggest exciting opportunities to improve the lives of people with this disease.

Pagliuca FW, Millman JR, Gürtler M,...Melton DA. Generation of functional human pancreatic β cells in vitro. *Cell 159: 428-439, 2014.*

Chera S, Baronnier D, Ghila L,...Herrera PL. Diabetes recovery by age-dependent conversion of pancreatic δ-cells into insulin producers. Nature 514: 503-507, 2014.

NEW INSIGHTS FROM TYPE 1 DIABETES GENETICS

Gene Affecting Risk for Type 1 Diabetes Involved in Maintaining Healthy "Power Plants" in Insulin-producing Cells: Researchers have found that a gene known to affect risk for type 1 diabetes encodes a protein involved in quality control of mitochondria—the cell's "power plants." Found in cells throughout the body, mitochondria are specialized metabolic structures that have the critical job of converting the energy that is ingested (sugars and fats, for example) into a chemical form that is more readily usable by cells. Over time, mitochondria may develop problems, and require recycling and replacement to keep cells going strong. A protein called Parkin initiates this multi-step recycling process, which marks the mitochondria for death and eventually results in their destruction. Working with mice, the researchers found that *Clec16a*—a gene linked to risk for type 1 diabetes in human studies—encodes a protein involved in targeting Parkin for destruction. They found that reducing the amount of Clec16a protein resulted in an increase in Parkin levels, causing mitochondrial recycling to be initiated too frequently. Interestingly, the researchers found that pancreatic islets lacking Clec16a had more mitochondria, not fewer, suggesting that Clec16a not only acts as a check on mitochondrial recycling, but also is critically involved in completing mitochondrial destruction once the process is initiated. Further, although insulin-producing β (beta) cells that lack Clec16a have more mitochondria, they

are less able to process energy for the cell and produce less insulin in response to rising blood glucose (sugar) than normal β cells do. These data suggest that initiation of the recycling process at least partially incapacitates the mitochondria and weakens the insulin response. Notably, people with a common mutation of *CLEC16a* that is linked to type 1 diabetes also have lower cellular levels of the Clec16a protein and poorer insulin response than people with other variants of the gene, suggesting the protein's role in humans is similar to its role in mice. Although it is uncertain precisely how a defect in mitochondrial recycling might lead to type 1 diabetes, the researchers found that mouse β cells lacking Clec16a showed signs of cellular stress that might make them more susceptible to a disease-initiating autoimmune attack. Further research is needed to verify the role of Clec16a in maintaining β cell health, and to determine whether modulating the mitochondrial recycling program might help prevent the disease.

Soleimanpour SA, Gupta A, Bakay M,...Stoffers DA. The diabetes susceptibility gene Clec16a *regulates mitophagy. Cell 157: 1577-1590, 2014.*

Study of Children at Genetic Risk of Developing Type 1 Diabetes Sheds New Light on Celiac Disease: A study found that more than one quarter of children with two copies of a specific genetic variant develop an early sign of celiac disease, called celiac disease autoimmunity (CDA), by age 5. Results also showed that children's risk of developing CDA was different depending on where they lived. These results are from The Environmental Determinants of Diabetes in the Young (TEDDY) study, an international study that is investigating both celiac disease and type 1 diabetes, because both are autoimmune diseases sharing some of the same genetic risk factors. Although the primary aim of TEDDY is to understand what environmental factors trigger or protect against type 1 diabetes in children, researchers are able to mine the data for insights into celiac disease. Celiac disease stems from an immune reaction to gluten, a protein found in wheat, rye, and barley. Over time, celiac disease can damage the small intestine and cause other health problems. People with celiac disease or CDA must follow a gluten-free diet to prevent or reduce this damage, so it is important—but currently challenging—to identify people early in the course of the disease so that they can implement dietary changes before intestinal damage occurs.

TEDDY researchers followed 6,403 newborns with one of two high-risk genetic variants (*HLA-DR3-DQ2* or *HLA-DR4-DQ8*) that are known to increase susceptibility to celiac disease to see who would develop celiac disease or CDA. Overall, 12 percent of the children had CDA at 5 years of age. However, youth with two copies of *HLA-DR3-DQ2* had the highest likelihood of disease development by age 5. Of this group, 26 percent developed CDA by age 5, and 11 percent developed celiac disease. The study also found that girls were at greater risk than boys for CDA. Additionally, Swedish children had higher rates of CDA and celiac disease than participants in the United States, Finland, and Germany, even when matched for the same genetic risk. In fact, Swedish children had nearly double the risk of CDA compared to American children with the same genetic risk variants; the extra risk faced by the Swedish children may come from environmental or other factors. These results could help inform future recommendations for celiac disease screening in young children and pave the way to early personalized prevention and treatment approaches based on genetic risk. They also suggest the need for further research to understand the environmental factors that influence the development of celiac disease in those who are genetically susceptible, which the TEDDY study is also addressing.

Liu E, Lee H-S, Aronsson CA,...Agardh D; TEDDY Study Group. Risk of pediatric celiac disease according to HLA haplotype and country. N Engl J Med 371: 42-49, 2014.

METABOLIC REGULATORS OF HEALTH AND DISEASE

Understanding How Nerve Cells Detect and Respond to Available Glucose: Researchers identified cellular factors that monitor the availability of glucose (sugar), the primary nutrient source for nerve cells (neurons), and enable these cells to use these sources to generate energy. Fuel sources are used to make

energy by specialized structures in the cell called mitochondria. Mitochondria are dynamic and move around within neurons to generate energy in response to local energy needs in these elongated, complex cells. Therefore, the mechanisms that sense energy status and regulate mitochondrial movement are important for cell function. Thus, researchers were interested in understanding those cellular mechanisms.

Glucose is a key fuel source for neurons, but these long cells can traverse considerable distances through the brain, and glucose concentrations are unlikely to be the same everywhere along the nerve cell. How do the energy-generating mitochondria, which migrate throughout the neuron, know to pause at the regions of high nutrients where they are most needed? The researchers hypothesized that mitochondrial movement may be regulated by local glucose levels outside of the cell. To test this hypothesis, they examined mitochondrial movement in rat neurons grown in laboratory culture in either low or high glucose levels. They found that mitochondrial movement decreased when glucose levels were high. The researchers then examined the mechanisms by which cells sensed glucose to regulate mitochondrial movement. They zeroed in on a protein called O-GlcNAc Transferase (OGT). OGT catalyzes the addition of special types of activated glucose molecules to proteins. OGT's activity depends on the availability of glucose—making it a "metabolic sensor" for glucose. The scientists found that inducing high levels of OGT experimentally in cultured neurons decreased mitochondrial movement, mimicking the effect seen with high glucose levels and suggesting that glucose may be working through OGT to reduce mitochondrial movement.

Next, to understand how OGT may be exerting these effects, the researchers studied another protein called Milton. Milton binds to a protein on mitochondria, as well as to proteins on intracellular structures (microtubules) that are involved in transporting mitochondria and other organelles around cells. By way of analogy, Milton connects the train car (mitochondria) to the wheels on the track (proteins on microtubules). Through a series of cell culture experiments, the scientists discovered that, in the presence of glucose, OGT was activated and added sugar molecules to Milton, and this modification was necessary to decrease mitochondrial movement. These results suggest that, in the presence of glucose, OGT is activated and modifies Milton, and modified Milton serves as a "brake" to keep the mitochondria in place so they can metabolize the available glucose. In contrast, when glucose is scarce, OGT is not activated, Milton is unmodified, there is no brake, and mitochondria move to another part of the cell.

This research shows that mitochondrial movement is regulated by glucose through OGT modification of Milton. It also suggests that OGT is a key nutrient sensor for neurons and promotes local energy production near where fuel is abundant. Although most of the experiments were conducted in neurons, the proteins are found in other cell types, suggesting that glucose, through OGT, may be a global regulator of mitochondrial movement.

Pekkurnaz G, Trinidad JC, Wang X, Kong D, and Schwarz TL. Glucose regulates mitochondrial motility via *Milton modification by O-GlcNAc transferase. Cell 158: 54-68, 2014.*

FGF1 as a Possible Novel Therapeutic for Type 2 Diabetes: Scientists demonstrated that a protein called FGF1 has potential as a new therapeutic for type 2 diabetes. In people with type 2 diabetes, cells in muscle, fat, and liver tissue lose their ability to respond to insulin, a hormone necessary for the body to use glucose (sugar). This condition is referred to as "insulin resistance" and is often the first step in development of type 2 diabetes. One approach to treating type 2 diabetes is to develop strategies to improve the body's sensitivity to insulin. A class of type 2 diabetes medications—thiazolidinediones—act as insulin sensitizers; however, they also have significant side effects like weight gain, bone loss, and increased risk for heart failure. Scientists, therefore, continue to search for agents that improve insulin sensitivity without leading to unwanted side effects.

A group of researchers hypothesized that FGF1 had a role in regulating blood glucose levels based on previous results linking FGF1 deficiency to

diet-induced insulin resistance in rodent models. FGF1 normally acts locally; it does not circulate throughout the body. To evaluate the potential of FGF1 to modulate glucose levels when distributed more broadly in the body, the researchers injected FGF1 in male mice that had insulin resistance resulting from genetic mutations or from diet-induced obesity, and measured the effect of this treatment on blood glucose levels. They found that a single administration of FGF1 normalized blood glucose levels within 18 to 24 hours, and that chronic administration of FGF1 every other day for 35 days resulted in a sustained reduction in blood glucose levels.

Administration of FGF1 did not affect blood glucose levels in normal insulin-sensitive mice, and did not cause insulin levels to rise, suggesting that FGF1's effects were not mediated by increasing insulin production in the body. Further research demonstrated that insulin was required for FGF1 to exert its effects on glucose levels. These results indicated that, under these circumstances, FGF1 does not replace insulin, but instead acts in concert with insulin to regulate blood glucose. Additional experiments suggested that FGF1 acted as an insulin sensitizer, as FGF1 increased the mice's responsiveness to insulin.

Importantly, FGF1-treated mice did not exhibit side effects associated with thiazolidinediones, such as weight gain or reduced bone density. However, FGF1 can induce cell proliferation, a property raising safety concerns that could limit its usefulness as a therapy. To address this potential problem, the researchers tested a modified version of FGF1 that did not stimulate cell proliferation. The modified version retained the ability of FGF1 to improve insulin sensitivity, demonstrating that FGF1's action to reduce glucose can be separated from its proliferative activity. These promising results indicate that FGF1 may have potential as a new therapeutic for type 2 diabetes, if similar results are observed in human studies.

Suh JM, Jonker JW, Ahmadian M,…Evans RM. Endocrinization of FGF1 produces a neomorphic and potent insulin sensitizer. Nature 513: 436-439, 2014.

RESEARCH ON CYSTIC FIBROSIS AND OTHER RARE DISEASES

Understanding Why a Promising Therapeutic Approach for Cystic Fibrosis Is Not Working as Hoped May Lead to Improved Treatment Options: New research helps explain why a therapeutic approach currently being tested for cystic fibrosis (CF) is providing little therapeutic benefit, but also suggests alternative approaches that might be more successful. CF is caused by mutations in the *CFTR* gene. Many such disease-causing mutations have been identified. Some mutations yield a version of the CFTR protein that is present in normal amounts and at the right location in the cell, but unable to do its job: letting chloride ions into or out of the cell.

Recently, a medication called VX-770 (ivacaftor; marketed as Kalydeco™) was approved for use by the U.S. Food and Drug Administration. This drug is able to open the chloride channel in people with *CFTR* mutations that produce reasonably stable but inactive CFTR protein, an enormous therapeutic advance for people with this type of mutation. Other *CFTR* mutations, however, make the CFTR protein unstable, causing cells to degrade it before it can fulfill its cellular role. The most common CF-causing mutation, called *ΔF508*, does both of these things: it destabilizes the protein and also inactivates its chloride channel function. Thus, for the majority of people with CF, VX-770 alone is inadequate, and another drug is needed to stabilize the CFTR protein. Researchers previously found that treating cells from people with *ΔF508* with either of two promising CFTR-stabilizing medications led to a meaningful increase in the amount of CFTR protein reaching the cell surface, where it is needed. Subsequent treatment of the cells with VX-770 led to a measurable boost in chloride transport, raising hopes that "combination therapy" with VX-770 and one or the other of the stabilizing medicines would yield clinical benefits for people with *ΔF508*. Surprisingly, however, initial results from clinical trials testing this approach have been disappointing.

In new studies from two different research groups, scientists sought to understand why combination

therapy seems so much less effective in clinical trials than it does in cells grown in the laboratory. The two groups both noted that the preliminary cell experiments involved ongoing treatment with the CFTR-stabilizing medications, followed by a shorter-duration treatment with VX-770. For this reason, they repeated the cell-based experiment, but administered VX-770 continuously, to more closely parallel its administration in the clinical trials. Both groups found that, in cells expressing the *ΔF508* version of the CFTR protein, chronic VX-770 administration had the effect of further destabilizing the protein—effectively undoing the beneficial action of the candidate stabilizing drugs. One of the research groups noted that this finding suggests it might be beneficial to fine-tune dosing of VX-770 and candidate stabilizing drugs to optimize benefit for people with the *ΔF508* mutation. The other group tested a panel of other candidate channel-opening medications in cells with the mutation, and found one called P5 that did not exhibit the same destabilizing effect as VX-770. P5 is still in pre-clinical stages of development, so it is not yet clear whether it would be safe and effective in people with *ΔF508*. Even if not, however, the observation suggests it might be possible to find a drug combination that provides sustained benefit for most people with CF.

Cholon DM, Quinney NL, Fulcher ML,...Gentzsch M. Potentiator ivacaftor abrogates pharmacological correction of ΔF508 *CFTR in cystic fibrosis.* Sci Transl Med *6: 246ra96, 2014.*

Veit G, Avramescu RG, Perdomo D,...Lukacs GL. Some gating potentiators, including VX-770, diminish ΔF508-CFTR *functional expression.* Sci Transl Med *6: 246ra97, 2014.*

Study of a Rare Genetic Disorder of Metabolism Leads to a Promising Therapeutic Approach: A recent analysis has clarified understanding of the metabolic disorder glycogenesis type XIV, and found that dietary supplementation with the sugar galactose may be a practical approach to alleviate some of the health effects of the disease, which can damage the heart, liver, and other organs. People with glycogenesis type XIV are unable to make phosphoglucomutase 1, an enzyme that helps supply some of the specific carbohydrate (sugar) "building blocks" used to make glycoproteins, an important group of molecules with diverse roles in the body. Phosphoglucomutase 1 is also involved in the storage of carbohydrates in liver and muscle, as well as in the utilization of that stored energy. The study began with a pair of brothers with an unusual combination of symptoms: muscle weakness, liver disease, short stature, cleft palate, and recurring hypoglycemia (low blood glucose). Genetic testing revealed that both boys had mutations in each of their two copies of the gene for phosphoglucomutase 1, with the effect that they produced none of the enzyme. The researchers identified 17 more individuals who were similarly unable to produce active phosphoglucomutase 1 and experienced a similar (though somewhat variable) constellation of symptoms.

To investigate how the lack of phosphoglucomutase 1 might cause the signs and symptoms of glycogenesis type XIV, the researchers compared the array of sugars available for making glycoproteins in cells from people with and without the enzyme. They found that people without the enzyme are unable to make significant amounts of a compound called UDP-galactose. Although the body normally produces UDP-galactose *via* a process utilizing phosphoglucomutase 1, an alternative path exists whereby the body can make UDP-galactose from the naturally occurring sugar, galactose. Galactose by itself is not normally a significant part of a person's diet, although it is bonded to another sugar, glucose, in the milk sugar, lactose. The scientists found that adding a small amount of galactose to the diet of six of the people lacking phosphoglucomutase 1 significantly improved their ability to make glycoproteins, helped improve heart function, and alleviated some of the other impacts of the disease. Supplemental lactose, on the other hand, had no effect. In addition, the researchers developed a blood test that may help diagnose glycogenesis type XIV. Long-term studies will be needed to determine whether galactose is a safe and effective treatment for people with this rare genetic disease.

Tegtmeyer LC, Rust S, van Scherpenzeel M,...Marquardt T. Multiple phenotypes in phosphoglucomutase 1 deficiency. N Engl J Med *370: 533-542, 2014.*

The National Diabetes Education Program Provides Resources To Support Improved Care for People with or At Risk for Diabetes

The National Diabetes Education Program (NDEP)—a joint effort of the NIH and the Centers for Disease Control and Prevention—shares model programs and resources to help health care providers and community-based organizations support their constituents to develop and sustain healthy lifestyles to delay or prevent type 2 diabetes, or effectively manage diabetes and improve outcomes.

Established in 1997, the NDEP is a federally funded program that includes over 200 partners at the federal, state, and local levels. NDEP partners work together to improve the treatment and outcomes for people with diabetes, promote early diagnosis, and prevent or delay the onset of type 2 diabetes.

Support for Behavior Change

Working with its partners, the NDEP developed the Diabetes HealthSense website to meet the need for resources and tools to promote behavior change and to address the psychosocial and lifestyle-change challenges associated with diabetes self-management. Diabetes HealthSense provides health care professionals and their patients with easy access to resources for making lifestyle changes and coping with stress and emotional health issues. Health care professionals can gain access to patient tools as well as a complete library of review articles, landmark studies, and meta-analyses to facilitate behavior change in clinical practice settings. Resources have been reviewed by leading independent experts on psychosocial issues with specific expertise on how to make and sustain lifestyle changes. This Web resource can be found at *www.YourDiabetesInfo.org/HealthSense*

New Guiding Principles

A newly published set of 10 guiding principles highlights areas of agreement for diabetes care that could be clinically useful in diabetes management and prevention. Presented by the NDEP, *Guiding Principles for the Care of People With or at Risk for Diabetes* is aimed at assisting with identification and management of the disease, self-management support for patients, physical activity, and blood glucose (sugar) control, among other topics. More than a dozen federal agencies and professional organizations support the document, which can be found at *http://ndep.nih.gov/hcp-businesses-and-schools/guiding-principles/*

To find more resources from the NDEP, please visit *www.YourDiabetesInfo.org*

(Adapted from a piece originally published in the fall 2014 NIDDK Director's Update.)

Accelerating Medicines Partnership

The NIH, 10 biopharmaceutical companies, the U.S. Food and Drug Administration, and several non-profit organizations have designed an unprecedented new partnership. Managed through the Foundation for the NIH (FNIH), the Accelerating Medicines Partnership (AMP) seeks to identify and validate the most promising biological targets of disease for new diagnostic and drug development. The partners have designed a milestone-driven research plan to tackle this challenge for type 2 diabetes, as well as for Alzheimer's disease and the autoimmune disorders rheumatoid arthritis and systemic lupus erythematosus (lupus). A key feature of this public-private partnership that makes it unique is that AMP data will be considered precompetitive and made publicly accessible to the broad biomedical community for further research.

The AMP Approach to Finding New Type 2 Diabetes Therapies

The AMP type 2 diabetes partnership will be a 5-year initiative, from mid-2014 through mid-2019. The partnership steering committee will ensure substantial scientific and logistical interaction among the partners to catalyze diabetes drug development by taking advantage of a major existing asset in the field of type 2 diabetes research: the tremendous volume of genetic data on the disease in diverse populations—made possible primarily through NIH-supported research—which is unmatched in most other diseases. Much of the information on gene variation can be linked to clinical information and knowledge about how and in what parts of the body the genes function, making the combined data a potentially rich resource for research aimed at better understanding and treating this complex disease. The AMP plans to leverage that great strength, using and supplementing the genetic data to identify and validate novel molecules and pathways as targets for therapeutic development.

To speed analysis, researchers will assemble a "Knowledge Portal"—a database of gene sequences, known variants, functions, regulatory information, and associated clinical data from studies on type 2 diabetes and its cardiac and renal complications, involving 100,000 to 150,000 individuals. The data set and analytical tools will be accessible to academic and industry researchers to identify and validate changes in DNA that spur the onset of diabetes, alter disease severity, speed or slow disease progression, or have a protective effect. The NIDDK-led Multiethnic Study of Type 2 Diabetes Genes Consortium (T2D-GENES) is independently developing and testing a database and Web utility that may serve as a prototype for the AMP Knowledge Portal. This resource will bring together data in a way that is useful not only to geneticists, but also to drug discoverers and investigators in many other specialties of type 2 diabetes research.

Generating New Genomic Data

As the Knowledge Portal is being mined and analyzed, the partnership will seek to fill in data gaps and

investigate targets of particular interest by sequencing key genes in specific populations, hoping to find, for example, a genetic variant that occurs in few individuals but has a significant effect on disease risk or progression. Such discoveries can provide clues about the biological processes underlying the disease and uncover possible therapeutic targets.

A case study in how this might work comes from recent discoveries about the gene *SLC30A8*, which encodes the protein ZnT8. The gene first came to the attention of diabetes researchers when they discovered that common *SLC30A8* genetic variants cause a modest increase in risk for type 2 diabetes. New research recently identified much rarer mutations in the gene that appear to have the opposite effect, providing significant protection from the disease. These rare mutations inactivate one copy of the gene, effectively cutting the amount of ZnT8 a person produces in half. This finding suggests a potential strategy for pharmacologic prevention or treatment of type 2 diabetes might target reducing the amount of this protein.

By combining resources to investigate many such genetic opportunities, the AMP partners hope to open a new era of treatment and prevention for people with or at risk for type 2 diabetes. For more information on this program, please see:
www.nih.gov/science/amp/type2diabetes.htm

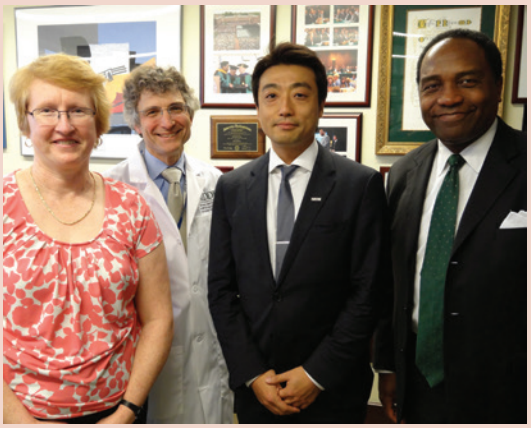
Dr. Shingo Kajimura Receives Prestigious Presidential Award

NIDDK-supported scientist Dr. Shingo Kajimura has received the Presidential Early Career Award for Scientists and Engineers (PECASE).

The PECASE is awarded annually to scientists and engineers who, while early in their research careers, have demonstrated the pursuit of innovative research and outstanding scientific leadership. Dr. Kajimura was honored for his important contributions to understanding the development of brown adipose tissue, also known as brown fat. Unlike white adipose tissue, which is used by the body to store excess energy, brown fat burns energy to produce heat and help protect the body from cold exposure. Because brown fat is capable of burning many calories quickly, it is hoped that learning how to regulate its development and activity may one day become therapeutically valuable for the prevention and treatment of obesity and related metabolic conditions.

Elucidating the Path Toward Brown Fat Development in Humans

Adults vary considerably in the amount of brown fat they possess, although people who are thinner tend to have more than individuals who are overweight or obese. Prolonged and repeated exposure to cold is known to help trigger development of brown fat in adults, but there is great interest in better understanding the way this occurs, to help people produce this calorie-burning type of cell without enduring such discomfort. Brown adipose cells are known to form from precursor cells that also have potential to develop into skeletal muscle. Dr. Kajimura's work helped establish that precursor cells become brown fat if they are making a protein called PRDM16, while those that do not make this protein become skeletal muscle. Recently, Dr. Kajimura and colleagues identified another key piece of the puzzle, by studying brown fat development in mice. They showed that PRDM16 must work in concert with a different protein, called EHMT1, to drive the precursor cells to become brown fat cells. Further, they found that a lack of EHMT1 in male mice led to a severe loss of potential to form brown fat cells, and to obesity. Rare mutations that reduce EHMT1 in humans also result in obesity, among other consequences, although it is currently unknown whether humans lacking the protein also have a diminished capacity to produce brown fat.



From left, Dr. Carol Haft, NIDDK program director for adipocyte biology; NIDDK Deputy Director Dr. Gregory Germino; Dr. Shingo Kajimura; and NIDDK Director Dr. Griffin Rodgers. The group met on the NIH campus during Dr. Kajimura's trip to Washington, D.C., to accept his award from the White House. (*Photo Credit: Jen Rymaruk*)

By contributing to the understanding of how the body controls formation of brown fat, Dr. Kajimura's research may lead to the development of therapeutic approaches to stimulate human brown fat production, potentially helping people to maintain a healthier weight and metabolism.

The PECASE awards support the continued professional development of awardees, promote careers and foster innovation in science and technology, and recognize the scientific missions of participating agencies. A list of NIH scientists who have received this prestigious award is available at *www.grants.nih.gov/grants/policy/pecase.htm*

STORY OF DISCOVERY

Leptin as a Treatment for Generalized Lipodystrophy: A Translational Success Story

In 1949, researchers identified a new mouse model that was extremely obese. Little did they know that research on that obese mouse would lead—65 years later—to an approved medical treatment for people who lack fat tissue altogether.

But, that is exactly what happened, after many decades of research that included the discovery of a hormone called leptin. The translational success was a result of collaborations among many scientists, including NIDDK-supported scientists at universities, scientists in the NIDDK Intramural Research Program, industry researchers, and many others. This story demonstrates how exciting discoveries in the laboratory provide the foundation for improving the health of people.

The Obese Mouse and the Discovery of Leptin

Scientists who identified the obese mouse model in 1949 called the unknown gene causing the obesity "*ob*." By the 1980s, the identity of the *ob* gene was still a mystery, but it was becoming more and more apparent that research on genetic contributors to obesity was critically important to pursue. Therefore, the NIDDK sought to support research to identify obesity-related genes in rodents, including the *ob* gene. The Institute sponsored a workshop on this topic and developed an initiative to solicit research applications.

In 1989, the NIDDK awarded a grant to Dr. Jeffrey Friedman through this initiative. Dr. Friedman's subsequent pioneering research led to the 1994 discovery of the mouse *ob* gene. The hormone produced by this gene was named "leptin," a term that derives from a Greek word meaning thin. Because the *ob* mutant mouse was obese, the scientists realized that the normal *ob* gene—and the hormone it encodes—must contribute to leanness.

The landmark discovery of leptin unleashed a wave of new research advances in fat biology and metabolism. Researchers found that leptin is secreted by fat cells and released in proportion to the amount of fat. These observations drastically altered the prevailing view of normal fat tissue as simply a metabolically passive "fat storehouse." Research fueled by this 1994 discovery also led to the identification of a number of other substances that, like leptin, are secreted by fat cells and influence appetite and metabolism.

Studies demonstrated that obese animals deficient in leptin, including mice carrying the mutant form of the *ob* gene, lost weight when given the hormone. Therefore, researchers postulated that leptin treatment might also be useful for human obesity. There are, in fact, very rare instances of complete deficiency of leptin in humans that result in morbid obesity from infancy. Leptin treatment in these individuals caused substantial weight loss, providing hope for improved quality of life and longevity.

Unfortunately, in clinical studies done at that time, leptin administration was not effective in treating the vast majority of cases of human obesity, which are not due to leptin deficiency. In most cases, obesity results from a complex interaction among an individual's

genes and the environment. Obese individuals, in fact, usually have very high levels of leptin, probably a consequence of the many fat cells secreting it. The inability of the high levels of leptin to decrease body weight suggests that the more common forms of obesity are associated with a resistance to leptin's actions. Although these results were disappointing, scientists did not give up in their quest to use this new knowledge to benefit people.

Testing Leptin as a Treatment for Lipodystrophy

Scientists in the NIDDK's Intramural Research Program had broad experience with respect to studying people with various forms of insulin resistance. Using this experience and knowledge, they identified a patient population—people with lipodystrophy—who could potentially benefit from leptin treatment.

Lipodystrophy is actually a group of disorders with disparate origins but with a common set of metabolic consequences. Lipodystrophy can either be genetic or acquired, and can be generalized (near total lack of fat) or partial (fat loss in certain parts of the body). While lipodystrophy is characterized by the loss of fatty tissue in certain areas of the body, tissues such as liver and muscle exhibit significant abnormal accumulation of fat, which impairs metabolic activity. People with the disorder also exhibit resistance to the effects of insulin and are thus at high risk of developing diabetes. They may also have a range of lipid abnormalities.

Treatment of lipodystrophy has included the administration of insulin, oral hypoglycemic (blood glucose [sugar]-lowering) agents, and lipid-lowering drugs. Even with treatment, people with lipodystrophy continue to have severely high levels of triglycerides, leading to recurrent attacks of acute inflammation of the pancreas; severe problems controlling blood glucose levels, posing risks of developing diabetic complications; and fat accumulation in the liver, which can result in cirrhosis and liver failure.

Because many people with lipodystrophy have low leptin levels due to the lack of fat cells that produce the hormone, and because research had demonstrated beneficial effects of leptin on insulin sensitivity and fat metabolism in a number of tissues, researchers in the NIDDK Intramural Research Program and their collaborators, including major collaborators at the University of Texas Southwestern and Yale University, investigated whether leptin treatment could ameliorate conditions associated with lipodystrophy.

Results from two small clinical studies testing this hypothesis were published in 2002. The studies showed that short-term leptin therapy (3 to 8 months) had dramatic benefits in individuals with lipodystrophy. In one study of females with different forms of lipodystrophy, most of whom also had diabetes, leptin therapy improved blood glucose levels, lowered triglyceride levels, and decreased liver fat content. In another study, leptin therapy markedly improved insulin sensitivity, lowered lipid levels, and decreased liver fat content in individuals with severe lipodystrophy who also had poorly controlled diabetes. Participants in these studies were able to reduce or discontinue their diabetes medications.

Seeing such dramatic results, researchers next examined the effect of longer-term leptin therapy. In results published in 2005, the researchers found that in 15 people with severe forms of lipodystrophy and poorly controlled diabetes, 12 months of leptin treatment led to improved blood glucose and blood lipid levels, and decreased fat in their livers. Participants also reported a dramatic reduction in their appetite, which led to moderate reductions in their

weight. In addition, they were able to discontinue or reduce their diabetes medications. In 2010, the scientists reported similarly remarkable results in 35 participants treated with leptin for 12 months.

The scientists also examined the effect of leptin on other metabolic abnormalities associated with lipodystrophy. For example, females often have irregular or absent menstrual cycles; leptin treatment was found to correct that condition. Leptin treatment was also highly effective in treating people with lipodystrophy and nonalcoholic steatohepatitis (NASH), a progressive metabolic liver disease. In a study of 25 people, researchers found that a surprisingly high number had some form of kidney disease; leptin treatment was found to improve their kidney function. Thus, leptin corrected a broad range of metabolic defects associated with lipodystrophy.

Because lipodystrophy is a chronic condition, it was important for the researchers to study whether leptin treatment was safe and effective to use as a long-term treatment. In 2011, they reported the results of a study of 55 people with lipodystrophy who were treated with leptin for 3 years: the participants had robust and sustained improvements in their blood glucose and blood lipid levels, and also had improvements in markers of liver function, a sign that the excess fat in their livers had likely diminished. Importantly, there were few adverse reactions to leptin during the study. Together, these data suggest that leptin is a safe and highly effective treatment for people with lipodystrophy.

Leptin Is Approved as a Treatment for People with Generalized Lipodystrophy

As a result of the clinical studies described above, in 2010, the industry collaborator that provided the leptin used in the studies—Amylin Pharmaceuticals/Bristol-Myers Squibb/AstraZeneca—began the process to submit a "biological license application" to the U.S. Food and Drug Administration (FDA) seeking approval to use leptin (marketed as Myalept™) as a treatment for people with lipodystrophy; the application was completed in 2012. The primary data used in the application were directly from the NIDDK's clinical studies.

In February 2014, the FDA made the exciting announcement that it approved Myalept™ for treating people with generalized lipodystrophy—whether genetic or acquired—in addition to following a healthful diet. Of note, leptin treatment helps people with lipodystrophy follow a healthful diet. Without leptin, they are always hungry; leptin treatment dramatically reduces their appetite and thus also decreases their food intake.

Leptin is the first approved therapy that is indicated for people with generalized lipodystrophy. People may still need to take conventional medicines (*e.g.*, lipid-lowering drugs or insulin), but required dosages of those medicines are markedly lower while taking leptin. In particular, people are often able to discontinue insulin use.

The FDA approval of leptin represents a much-needed treatment for people with generalized lipodystrophy—a rare and life-threatening disorder for which available therapies were only partially effective and did not address the underlying cause of the metabolic abnormalities, leptin deficiency.

A Team Effort Leads to a Research Success Story

The clinical studies testing leptin therapy for lipodystrophy conducted by the NIDDK Intramural

Research Program—and used as the primary scientific basis for FDA approval—required numerous collaborators and spawned new collaborations. Leading this effort was Dr. Phillip Gorden, a former NIDDK Director who returned to the laboratory to continue his research. Because the leptin used in the research was manufactured by industry, the Intramural Research Program and the NIDDK Technology Advancement Office worked with industry to obtain the leptin needed for the studies. In addition, because lipodystrophy affects the liver and kidneys, scientists in the Intramural Research Program with expertise studying those organs were valuable contributors to the studies. Furthermore, collaborators external to the NIDDK have studied the underpinnings of different forms of genetic lipodystrophy; several genes have now been identified. Finally, many of the study participants were evaluated and treated at the NIDDK's Metabolic Clinical Research Unit, a facility in the NIH Clinical Center that enables scientists to make precise metabolic measurements. It was only through the contributions of all of these collaborators that this translational success story came to fruition.

Looking to the Future

As described in this story, knowledge gained from studying a common condition, obesity, led to the discovery of leptin and a treatment for a very rare disease, generalized lipodystrophy. Scientists are now coming full circle by building on the successful clinical studies with leptin in lipodystrophy and applying that knowledge to research on common diseases. For example, by studying women with lipodystrophy, scientists in the NIDDK Intramural Research Program are also gaining insights into a more common condition: polycystic ovarian syndrome (PCOS). PCOS is a set of symptoms that results from a hormonal imbalance; it affects females of childbearing age and is the most common cause of anovulatory (absence of ovulation) infertility. Women with lipodystrophy have features of PCOS, which are improved by leptin therapy. This observation suggests that knowledge gained by studying women with lipodystrophy may provide understanding of the more complex and common PCOS and shed light on improved ways to treat it.

NIDDK-supported scientists are also gaining important insights into leptin's biological functions, which are informing future research. For example, NIDDK-supported extramural researchers found that after people lost 10 percent of their body weight, their leptin levels and metabolic rate decreased, resulting in a metabolic state that favors weight regain. Researchers discovered that leptin replacement after weight loss increases people's metabolic rate to the pre-weight loss level. Although it is not yet known whether giving people leptin could help maintain weight loss, the findings suggest the need for future research to develop novel therapies to support weight loss maintenance.

Other NIDDK-supported researchers are exploring leptin's use in treating other diseases and disorders. For instance, NIDDK-supported extramural researchers found that leptin treatment improved the health of women with a condition called hypothalamic amenorrhea (absence of a menstrual cycle due to excessive exercise or stress, or inadequate food intake), which can result in infertility and bone loss. In women with this condition, leptin treatment restored menses and ovulation, independent of weight gain. In addition, researchers in the NIDDK Intramural Research Program studied people with a rare genetic syndrome caused by mutations in the gene encoding the insulin receptor, which results in extreme insulin

resistance; people with this syndrome have a very difficult time controlling their blood glucose levels. In a small study, the researchers found that leptin treatment improved patients' blood glucose levels. These studies have identified other populations who may possibly benefit from leptin treatment.

Through future research, it is also important to identify safe and effective treatments for people with other forms of lipodystrophy, including rare forms of partial lipodystrophy, as well as a more common form of lipodystrophy that is acquired from taking certain types of medications for human immunodeficiency virus (HIV). While leptin was approved for a rare form of lipodystrophy, partial forms of the disorder are more common and additional research is needed to identify others who might benefit. Thus, future research is needed to identify therapies to improve the health of all people with lipodystrophy.

Conclusion

The FDA approval of leptin for generalized lipodystrophy is a culmination of decades of research—NIDDK-supported basic research that led to the discovery of leptin, as well as clinical research conducted by scientists in the NIDDK Intramural Research Program and their collaborators testing leptin in people. People with lipodystrophy were not originally envisioned as a group who would benefit from leptin treatment, as leptin was first thought to be a promising treatment for common forms of obesity. But, because of the dedication of numerous scientists and clinical trial volunteers, people with generalized lipodystrophy have a new FDA-approved treatment. It is a translational success story representing the ultimate goal of NIDDK-supported research—to improve people's health and quality of life.

PATIENT PROFILE

Edward Augustin

A Life with Type 1 Diabetes Turned Around by Islet Transplantation

Ed Augustin and his daughter Jill

December 4, 2011, 7:04 am. It's a date that Edward (Ed) Augustin won't forget. That morning, Ed woke up at the University of Illinois Medical Center in Chicago a changed man. For the first time he could remember, Ed didn't have to worry about his type 1 diabetes. He had just received a transplant of insulin-producing islet cells. "I'm ecstatic," he exclaims, "I'm the luckiest guy in the whole universe. There are just not words that I can say to tell you how happy I am."

Ed wasn't always so happy. When he was a young child, he explains, "My mom noticed that something was wrong. I was thirsty and going to the bathroom all of the time. Finally, one day, she said we're going to go get a blood test." He was diagnosed with type 1 diabetes when he was only 5 years old. Ed spent a week in the hospital learning about his new life of diets and insulin shots. "It was horrible," he recalls. "The only time I've ever seen my dad cry was when they told me you've got to go to the hospital."

Type 1 diabetes is an autoimmune disease in which a person's immune system destroys the cells that make insulin. These cells are found in the pancreas in clusters called islets. People with the disease must carefully monitor their blood sugar (glucose) levels and administer insulin. Without insulin, the cells of the body starve, while the excess sugar in the blood can, over time, lead to devastating complications of the eyes, kidneys, nerves, and heart. Too much insulin, however, can cause blood sugar levels to fall dangerously low, a condition called hypoglycemia, which can lead to confusion, difficulty in awakening, loss of consciousness, seizures, and death. It is very difficult for people with type 1 diabetes to achieve a balance between too much sugar in the blood and too little; it was even more difficult when Ed was diagnosed over 50 years ago, before the development of advanced medical technologies.

Life with Type 1 Diabetes

In 1961, life for people with type 1 diabetes was different from the way it is now. Ed's parents were told he would only live for another 5 years. Fortunately, he was too young to understand—and he defied those odds. He went on a strict, measured diet. "Never again was I to have sugar. When I went into the hospital for the first time, my mom bought me a fudge sundae and said: 'This is your last fudge sundae.'"

PATIENT PROFILE

Ed's sugar levels were measured with urine tests, a procedure that, to this day, has been etched in his memory because he didn't like doing them.

His family was vigilant in administering his shots of insulin, which at that time were given in glass syringes with insulin that had to be kept cold. "Mom watched me like a hawk," he remembers. His parents also tried to teach him to give himself insulin shots, but "I didn't want to learn because then I'd have to give myself a shot, and I didn't want to take the shot," he shares. So, Ed's parents taught his siblings how to give insulin shots. "They taught my sister, and she'd give me a shot. They taught my brother, and he nailed me with a shot. And he liked it," laughs Ed, "so I didn't like it when he did it."

When Ed had a hypoglycemic reaction, he'd lose control. He couldn't think straight, couldn't talk, couldn't put words together. He would be completely disoriented.

Despite his family's watchful care, Ed's diabetes was particularly difficult to control, meaning that, even with his strict diet and insulin administration, Ed's blood sugar levels varied wildly between very high and very low. Ed had hypoglycemic reactions, also known as "hypoglycemic episodes," when his blood sugar got dangerously low. When Ed had a hypoglycemic reaction, he'd lose control. He couldn't think straight, couldn't talk, couldn't put words together. He would be completely disoriented.

These episodes were especially difficult to manage when he wasn't at home. As a child, he'd "know I was going down for the count because I'd sweat or see double." But when he was playing in the schoolyard and began experiencing these symptoms, he'd need to eat something to raise his blood sugar levels, even sweets that weren't part of his normal diet. "I'd just hope I was close to a candy bar. If I was close to a candy bar, that was good. Sometimes, you had to get on your bike and go ride home to get the candy bar," he remembers. Ed was the only kid with type 1 diabetes in his school and neighborhood, and he never told his classmates and friends what was going on. "You didn't tell any of your friends because they'd think you're weird," he shares. "No one could understand how a kid can fall to the ground and not be able to run with the football anymore."

Then, when Ed was about 13, those symptoms of low blood sugar disappeared. "One day it all just was gone. I never felt or saw them coming." Ed began suffering from frequent periods of "hypoglycemia unawareness," when people don't realize that their blood sugar levels are dangerously low, preventing them from eating sugar or taking medicine in response. He recalls an example while playing a football game in high school: "We were going to be undefeated, and we lost that game because I let a guy go 96 yards right down the sideline. It was an easy tackle, but I couldn't even see him I was so blind." This lack of awareness meant that Ed had no warning that he was in a dangerous situation. While many people with type 1 diabetes suffer from hypoglycemia, Ed's condition was particularly severe. He was in a minority of people with type 1 diabetes who, for reasons not fully understood, have frequent severe hypoglycemia and hypoglycemia unawareness and for whom standard blood sugar control, even with today's technology, is not sufficient.

As Ed became an adult and began work in construction, things got even worse. He'd be on a job, working fine, and then "all of a sudden I couldn't lay out a stair. I

couldn't cut a roof. I couldn't cut a sheet of plywood. I'd sit there and look at my tape measure. I'd have the measurements, but I just couldn't find them on my tape measure," he recalls. "I'd be walking on top of a 28-foot wall, and I wouldn't know where I was." Or he would go to the hardware store for something, but then couldn't figure out what he was doing there. One time he found himself out of gas and parked in a stranger's front yard with no recollection of how he got there. Even keeping a supply of sugar tablets nearby didn't always help because he couldn't get to them, couldn't open them, or couldn't remember what he was trying to do.

"My family was always scared," he says. "They were always afraid that I was going to die."

All of this took a toll on Ed's family and co-workers. "My family was always scared," he says. "They were always afraid that I was going to die. It got to where my wife and daughter would know [he was having a low sugar reaction] just by looking in my eyes or by the way I was talking on the phone." They'd tell him: "Check your blood. Check your blood." Ed shares, "You get tired of hearing 'check your blood.'"

It wasn't only recognizing Ed's hypoglycemic reactions that affected his family and co-workers, but treating them as well. For example, when a low blood sugar reaction would strike "in the middle of the night, I'd be thrashing about in my bed," Ed recounts, "and my wife—who has to go to work in the morning—would try to wake me up." She would try to give him food or medicine to raise his blood sugar levels, but if she couldn't, "she'd have to call the paramedics," he says. At work Ed's crew would also look out for him. "They were relying on me, and they'd walk into my office. I'd be out of it, and they'd have to tell me: 'Ed, eat a candy bar.'" Despite all this, he remembers thinking: "It's just life, and that's how it's going to be."

Islet Transplantation: A Promising Treatment

Unbeknownst to Ed at the time, researchers at the nearby University of Illinois at Chicago were working with other researchers around the world on islet transplantation, a procedure that has the potential to restore blood sugar control to a person with type 1 diabetes, leading to insulin independence and reductions in episodes of hypoglycemia. In islet transplantation, the insulin-producing islets from a deceased organ donor are purified from the other cells in the pancreas, processed to maintain their viability and promote engraftment, and infused into the liver of the recipient. Once implanted, the islets begin to make and release insulin in response to the body's needs. This procedure may be preferable, for some people, to whole pancreas transplantation as it is less invasive, but the procedure is still considered experimental in the United States.

World experts in the field of islet transplantation, including researchers at the University of Illinois at Chicago, joined together to form the Clinical Islet Transplantation (CIT) consortium to move the field forward with innovative approaches and toward a more consistent procedure that could be approved by the U.S. Food and Drug Administration (FDA). This collaborative research group is led by the NIDDK and the National Institute of Allergy and Infectious Disease and is funded by the *Special Statutory Funding Program for Type 1 Diabetes Research*.

Ed was driving in his car when he heard a radio advertisement recruiting participants for CIT studies at the University of Illinois at Chicago. "I was listening

to the sports scores…and [the ad said]: 'If you want to be cured of diabetes, give us a call.' And I figured, yeah right. I've had this for 48 years. There's no way," remembers Ed. But 5 minutes later he got a call from his brother who had heard the same ad and encouraged him to call.

Ed had to go through a rigorous screening process before being selected for the procedure. The doctors needed to confirm that Ed's diabetes was so uncontrollable with standard insulin therapy that the benefits of the transplant would outweigh the potential risks. The risks include those associated with the transplant procedure (*e.g.*, bleeding and blood clots) as well as those associated with the immunosuppressive medications that transplant recipients must take to stop the immune system from rejecting the transplanted islets. Immunosuppressive medications have significant side effects, and their long-term effects are still not fully known. Immediate side effects may include mouth sores and gastrointestinal problems, decreased kidney function, and increased susceptibility to bacterial and viral infections and cancer. In addition, there is currently no guarantee that the transplanted islets will work long-term; previous transplant recipients have lost insulin independence over time.

For the majority of people with type 1 diabetes, whose diabetes can be controlled with the ever-improving technologies that are available for managing their disease (such as advanced glucose monitors and improved forms of insulin), the risk of side effects from the immunosuppressive medications may outweigh the benefits of the transplant. Therefore, most people with type 1 diabetes are not candidates for islet transplantation. But, for people like Ed who have frequent severe hypoglycemia and hypoglycemia unawareness, the benefits of the transplant may outweigh the risks of having to take immunosuppressive medicines. The benefits might also outweigh the risks for people who have received, or who will receive, a kidney transplant and thus will need to take the immunosuppressive medications for that transplant. Islet transplantation could also be beneficial for some people who don't have type 1 diabetes—people who have had their pancreas removed due to severe pancreatitis. In this case, people could receive their own islets, rescued from any remaining healthy tissue, and not need immunosuppressive medication.

After his transplant, Ed says: "It's so magnificent, a miracle, fantastic. Never in my lifetime did I see this coming....Not only do I feel great, but my family, co-workers, friends…they're feeling better because they don't have to worry about me anymore."

The researchers determined that the benefits of the transplant outweighed the risks for Ed, so he was eligible to enroll in the trial. For him, it was an easy decision to participate. "I didn't even think about it," he confesses, "I was getting cured. This is a huge gift. How could I ever turn that down? I was thinking about no shots, no passing out. Where do I sign?"

Then began the wait for a deceased donor pancreas that would be a match for Ed. He was told that the call could come at any time, so he would need to be ready to go at a moment's notice and have a bag packed with everything he'd need for the hospital. "When you're first doing it," he remembers, "you think about it every day and night. I've got to have this, I've got to have that." But, the suitcase sat in Ed's bedroom for a

year. He remembers that, after waiting for a while, he stopped thinking about it as much and began to assume that he wouldn't get the call. But then, one night that changed: "All of sudden I got a call, and [the study staff said] 'NOW. You must be here now,'" says Ed. The researchers had islets ready to transplant into him.

A New Life

Two months after the first transplant, Ed had a second islet transplant, which is not unusual. Since then, he has been insulin free, meaning that he has not needed to take insulin shots, and he no longer suffers from hypoglycemia unawareness. His life has been forever changed. "You guys don't know what you've got," he says. "I never knew what I could have had. I was 5 years old. All I remember was shots and shots, and lows and highs, and passing out here, and walking until I dropped." And now, after his transplant, Ed says: "It's so magnificent, a miracle, fantastic. Never in my lifetime did I see this coming. I don't have to worry about not waking up. I can drive, and I don't have to test my blood at stop lights. [This] turned my life around."

Ed's participation in the CIT study has not only changed his life, but also the lives of those around him. "Not only do I feel great, but my family, co-workers, friends … they're feeling better because they don't have to worry about me anymore. Everyone was so relieved. They don't have to watch me. They're not afraid anymore. My co-workers have meetings through lunchtime now," he laughs, grateful that he no longer has to schedule meetings around checking his blood sugar, taking an insulin shot, or eating.

Being part of a clinical trial is work, Ed says, "A lot of records, a lot of paperwork, and you have to show up at the times they tell you." But, he is quick to encourage others to consider participating. He is eager to give back and help others with type 1 diabetes, having seen adult friends with the disease suffer from complications. "I'm not an angel diabetic," he reflects, knowing firsthand how hard it is to check blood sugar levels continually, take insulin shots regularly, and watch what you eat diligently. "But I think I'm really like most people out there. I'd love to help other people like that and get them to understand that there's a way we can get over the hump."

To everyone involved with the islet transplantation procedures, Ed says, "You've given me a great, big gift. I can't thank you enough. My family and co-workers can't thank you enough."

For now, islet transplantation is still an experimental procedure, only allowed by the FDA as part of research trials. By developing a standardized procedure for the production of the islets for transplantation and working closely with the FDA to determine whether that procedure conducted by researchers at multiple medical centers can lead to improved blood sugar control in people with type 1 diabetes, the CIT consortium is paving the way toward making this procedure more widely available. If the research conducted by CIT is successful and leads to FDA approval, then islet transplantation would no longer be considered experimental. There will still be challenges, however, including one that Ed is hopeful will be overcome: the shortage of donated pancreata. "I hope that more people donate their organs," he shares. Beyond that, Ed is hopeful for a future without type 1 diabetes, one in which the disease is prevented outright and the devastating hypoglycemia and complications don't occur. "It would be huge," he says of preventing the disease.

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Ed is so grateful and thankful to everyone involved in the trial, especially the families and friends of the deceased donors who provided the pancreata. "I think about them all the time," he shares. "I say a prayer, and I thank those people whoever they are. I thank them, their families, and their friends, every morning and every night. I want to tell them how it saved my life. I want to give them a hug. I want to take them out to dinner." He'd like to tell them: "You've given me a great, big gift. I can't thank you enough. My family and co-workers can't thank you enough." Ed feels very fortunate to have participated in the CIT study and to have received this "gift." "I want to thank everyone—the people who do this: the researchers, those people who you don't even see—the ones breaking the pancreas apart and taking the cells— the nurses who work really hard and care, and the doctors. And my family. I'd like to thank all of them."

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Hailey Jeter

Treatment for Rare Genetic Disorder Provides Health Today and Hope for the Future

Hailey Jeter

Hailey Jeter is a mature, smart 17-year-old high school senior who is an avid reader, likes all her classes in school—especially math—and loves spending time with her three best friends. She has also bravely tackled more than most other teenagers—she has a very rare disorder called generalized lipodystrophy. "There aren't very many of us," Hailey says. It's so rare, in fact, that she's never met another person with the disorder. She and her family didn't find out that she had lipodystrophy until she was 14 years old, even though she started having health problems as a baby. But, thanks to a loving and supportive family, as well as her participation in an NIDDK clinical trial, her health is better today than it's ever been.

A Baby Who Wouldn't "Plump Up"

"It all started not long after she was born," says Hailey's mom, Kelli, remembering back to the first signs that her baby daughter was having health problems. Already an experienced mother of two other daughters, Kelli says that about a month after Hailey was born, "my husband and I noticed that she wasn't turning into the tubby little infant that our other daughters had turned into. She was eating, but she just wasn't plumping up." By the second month of Hailey's life, Kelli and her husband knew that something was wrong when their baby was losing weight and had no visible fat under her skin. Their pediatrician ordered blood work on baby Hailey and found that her sugar (glucose) and triglyceride levels were extremely high.

"It all started not long after she was born," says Hailey's mom, Kelli. "My husband and I noticed that she wasn't turning into the tubby little infant that our other daughters had turned into. She was eating, but she just wasn't plumping up."

After getting the results of the blood work and not knowing what could be causing Hailey's symptoms, the pediatrician referred the Jeters to several medical experts to determine the underlying cause. But, "no one could give us an answer," says Kelli. "At that point, all they really knew to do was to change her diet." Thus, she was told to stop nursing Hailey and to give her a special formula to help her gain weight. "And it worked," she reports, "she was starting to fill out." Even so, "her blood work was still the same. So it was a frustrating thing, and this went on for years." They

were told to keep Hailey on a high-calorie, low-fat diet, and "that's how we walked through life for the first 6 years," says Kelli.

The family came close to getting a diagnosis when Hailey was 6 years old. A nutritionist who treated her as a baby transferred to a new job and got involved in research on lipodystrophy. Even though he hadn't seen Hailey since she was a baby, he remembered her and thought that she might have the disorder. However, test results were inconclusive, so the family still didn't have any answers. "So we continued to go forward with the [high-calorie, low-fat] diet," says Kelli, "and that continued for another 6 years until she was 12." But, the diet wasn't working, and Hailey's health was getting worse.

More Health Problems…But Still No Answers

When Hailey was 12 years old, she had a series of new health problems. First, she developed xanthomas, a skin condition in which fats build up under the skin. "When your triglycerides get so high, you actually break out in lesions and little bumps," explains Hailey. But, even the xanthomas were misdiagnosed—she was given cream for a viral rash, which "wasn't doing anything," she remembers. She then started having other symptoms, such as a stomach ache and fever, so her mother took her to the emergency room. There, they were told that she had pancreatitis—inflammation of the pancreas that could be life-threatening. The next blow came after Hailey went to her endocrinologist because of the recent health problems and found out that she had diabetes. She was put on insulin shots to treat the diabetes.

However, the doctors still didn't know the underlying cause of all of these health problems, so they were treating the problems as they arose. "And that's how it's been for us," explains Kelli. "It's always been 'treat the side symptoms.' It's been so frustrating."

"I was hungry all the time," says Hailey. "After I would eat, I would think about what I'd be eating next."

Having diabetes was particularly difficult for Hailey. "This is where life got hard for her," her mother states. "She's 12 years old, and she's taking 11 [insulin] injections per day." She was also taking other medications, such as drugs to lower her triglycerides, which were still very high. Hailey recalls that when she first found out that she had diabetes, "it was kind of fun to me. It was learning the shots, and I like to learn stuff." But the initial enthusiasm didn't last long, and the insulin wasn't making her feel better. "After a while, especially once we were doing all the work and weren't seeing results, that's when it got hard because I felt that I didn't want to do it [take insulin shots] anymore, but I had to," she explains.

Compounding the health problems was the fact that Hailey found out she had diabetes in 7th grade, an age when not being able to do what the other kids do can be challenging. For example, Hailey remembers that, before lunch, she'd have to leave early to go to the nurse's office for an insulin shot rather than just go to lunch with the other kids, making her stand out. And the xanthomas were getting worse, too. "It was hard on her psychologically," says her mother.

Hailey didn't have any energy and "I was hungry all the time," she says. "After I would eat, I would think about what I'd be eating next." She and her family had no idea why she was hungry all the time, and it was difficult to control her diabetes when she was constantly eating. "They would tell me to try to eat smaller portions, and I was thinking 'ok,' but once

it was time to eat, I couldn't do it," she remembers. Her mother knew how difficult this was for Hailey. "Everyone was trying to get her to do something that she just didn't want to do."

A Chance Meeting Leads to an Answer

When Hailey was 13 years old, the family heard from the same nutritionist who had contacted her doctor when she was 6. All those years later, he still thought that Hailey had lipodystrophy. He told them about a clinical trial enrolling people with lipodystrophy near their home state of Ohio. However, the trial had only been going on for a short time; Kelli didn't have much information about it; and her daughter had never been diagnosed with lipodystrophy. Thus, Kelli was hesitant. She said that, with all the uncertainty and not knowing the risks, "it just didn't feel right." Still, it put lipodystrophy on the family's radar screen once again.

Meanwhile, the insulin was having less and less of an effect, so Hailey's endocrinologist kept increasing her insulin dose and, at age 14, put her on a very concentrated form of insulin. "Now here's the miracle part," says Kelli. After Hailey had been on that new insulin for a few months, a nurse at their endocrinologist's office attended a scientific conference. There, she heard a talk given by Elaine Cochran, a pediatric nurse practitioner in the NIDDK's Intramural Research Program. Hailey's nurse approached Elaine after the talk to ask her some questions. According to Kelli, "Once our nurse started explaining to Elaine some of the symptoms that Hailey was having, she [Elaine] knew right away. She said: 'I think she is one of ours.'"

About Lipodystrophy

What Elaine suspected was that Hailey had lipodystrophy—as their former nutritionist had suggested, but that had never been confirmed by their doctors. People with lipodystrophy lack fatty tissue. Depending on the extent of fat loss, lipodystrophy can be generalized (near total lack of fat) or partial (fat loss in certain parts of the body); Hailey has a genetic generalized form. Because lipodystrophy is very rare, many doctors are unfamiliar with it, so people may not be diagnosed for years.

While lipodystrophy is characterized by the loss of fatty tissue in certain areas of the body, fat accumulates in other more dangerous places, like liver and muscle, where it impairs metabolic activity. People with lipodystrophy may have very high triglyceride levels in their blood, which can lead to attacks of pancreatitis. They also have a condition called insulin resistance and thus have elevated blood sugar levels, putting them at high risk of developing diabetes.

An important consequence of lipodystrophy is a deficiency in leptin, a hormone made by fat cells. One function of leptin is to travel to a key control center in the brain to report on the body's energy stores and reduce appetite. Without adequate levels of leptin, the brain doesn't get that important signal, resulting in constant hunger, as Hailey experienced.

Leptin was first identified in 1994 by NIDDK grantee Dr. Jeffrey Friedman. Administering the hormone to obese animals lacking leptin led to weight loss, so scientists thought that leptin may be a useful therapeutic for common forms of obesity. Unfortunately, clinical trials showed that it was not effective in treating the vast majority of cases of human obesity, which are not due to leptin deficiency. Obese individuals actually have high levels of leptin, suggesting that common forms of obesity are associated with a resistance to leptin's actions. However, there are rare forms of obesity and other

conditions—like lipodystrophy—in which people are leptin deficient, so researchers have examined using leptin as a therapeutic in such diseases and disorders.

Elaine's comment that Hailey could be "one of ours" stemmed from her involvement in a long-term NIDDK clinical trial being conducted at the NIH Clinical Center in Bethesda, Maryland, under the leadership of scientists in the NIDDK Intramural Research Program: former NIDDK Director Dr. Phillip Gorden and Dr. Rebecca Brown. The trial was investigating whether leptin treatment—to correct the leptin deficiency—could ameliorate conditions associated with lipodystrophy. Before leptin, people with lipodystrophy were given medicines to treat the associated health problems, but those medicines were only marginally effective—as had been Hailey's experience. The NIDDK's trial had been ongoing for over a decade and was still enrolling patients to gather more data; researchers were also publishing trial results periodically to report on the progress. Thus, by the time that the Jeters found out about the trial, published results showed that leptin treatment led to dramatic health benefits, including improved blood sugar and triglyceride levels, and reduced or discontinued need for diabetes medications.

When Kelli heard about the nurses' conversation at the scientific conference, she contacted Elaine immediately, and Elaine sent her information on research being conducted on lipodystrophy. "And there were pictures of people who actually looked like me physically," recalls Hailey, "I'd never seen anyone who looked like me." What convinced Kelli that this could finally be the answer they had been looking for was when Elaine told her about a Facebook page for people with lipodystrophy. Kelli saw photos of babies who looked exactly like Hailey had looked as a baby. "I thought: this is it, this is what she has," she remembers, "it just felt right." Elaine told Kelli about the NIDDK's clinical trial testing leptin and that Hailey could be eligible to enroll. Within 2 months, mother and daughter were at the NIH Clinical Center.

Kelli emphasizes that enrolling Hailey in the NIDDK's clinical trial "literally saved and changed her life for the better. Everything is different. Her outlook on life is different. Her confidence is different. She's happy."

A Life Changed for the Better by Participating in an NIDDK Clinical Trial

Tests at the NIH Clinical Center confirmed the suspicions that Hailey had generalized lipodystrophy. As part of the clinical trial, she was started on a treatment regimen of twice-daily leptin injections and was told she would see results in about 3 months. It didn't take that long. "In 3 weeks, we were taking her off insulin," reports her happy mother, because the leptin treatment improved her blood sugar levels without the need for insulin. Hailey remembers having a lot more energy, not being hungry all the time—and being happy. And even though she still has to take other medicines along with leptin, it is nothing like the 11 insulin shots each day that she once endured. She administers the leptin at home, so it doesn't affect her school day—she no longer needs to leave early for lunch to take medicine. "School is completely normal now," Hailey states happily.

As part of the trial, they came back to the NIH Clinical Center after 6 months and saw huge improvements in all of Hailey's test results. They came back another 6 months later, and Hailey was doing so well that the doctors said she didn't have to come back for an entire year. During that year—and based on the

positive results seen in Hailey and in other participants enrolled in the NIDDK's clinical trial—the U.S. Food and Drug Administration approved the use of leptin for treating people with generalized lipodystrophy.

Although Hailey and her family were happy about the approval and being an important part of it, it was bittersweet for them, because they would only have one more visit to the NIH before responsibility for her care was transferred to her local doctor—and Hailey loves coming to the NIH. Talking about the NIDDK's doctors and nurses who have cared for Hailey, Kelli says, "They've made us feel like we're part of their family, so we treat them that way, and we feel that way about them....we just feel like they're so much a part of her life and how she's changed." Also important to Kelli was that, "Elaine took the time to explain to me what was going on in her [Hailey's] body metabolically ... and that had never been answered before." That personal attention helped make Kelli feel comfortable with enrolling her daughter in the NIDDK's trial.

At their final visit to the NIH, there was more exciting news—Hailey was doing great. Her triglyceride and blood sugar levels were both in the normal range, and she no longer had conditions that once affected her, like diabetes, fatty liver, and xanthomas. The progress motivates Hailey to keep taking her medicines. Compared to past experiences of taking medicines and not feeling better, "It's a lot easier to take the medicine when you know it's working," she says.

Kelli emphasizes that enrolling Hailey in the NIDDK's clinical trial "literally saved and changed her life for the better. Everything is different. Her outlook on life is different. Her confidence is different. She's happy. It was just a chance meeting between Elaine and our nurse, and her whole life has changed for that reason."

Moving Forward with the Support of Family and Friends

Hailey's future is bright now that she is on medicine that is improving her health so dramatically. And Kelli has a positive outlook on the future, even though "there was a time it wasn't," she recalls, "when Hailey was younger and I thought there were just no answers." Because her daughter is doing so well, she says, "Now we can laugh. Now we can share. We have a positive outlook that we didn't have before."

And the family is looking forward. On one of their long drives from their home in Ohio to the NIH Clinical Center in Maryland, Kelli told her daughter that she felt so bad that she had to go through all that she did, and that they didn't get a diagnosis of lipodystrophy sooner. Hailey responded, "I think I needed to go through that because it makes me appreciate more the way that it ended up. I have an appreciation now for my medicine, the way that it works, for taking care of myself." Kelli was amazed by her daughter's maturity and knew right then that they would be able move forward and concentrate on the future.

Looking to that future, Hailey wants to be a nurse—being inspired by the wonderful nurses, like Elaine, who have cared for her over the years. There's no question that she will be able to draw on her intellect, maturity, family support, and personal experiences and be caring, compassionate, and empathetic toward her future patients. "She's such an outstanding person," says her proud mom.

Hailey continues to appreciate the support of her entire family, including her dad, Eric, and two older sisters, Amber and Taylor. Her mother has always been extremely supportive and continues to take some of the burden off her daughter by readying

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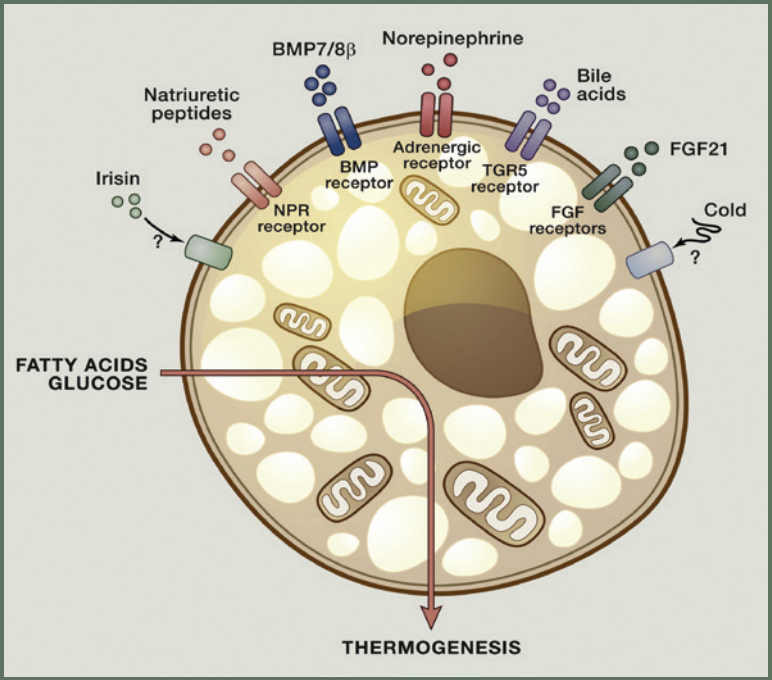
Hailey's leptin injections and putting her other medicines in a pill box. Hailey's three best friends have also been a huge support, both at home and at school, including waiting for her to take insulin before lunch so they could eat together. As Kelli explains, "Our recipe is to do what the doctors say and mix that with a lot of support from our family and her friends." She has always given her daughter an important message: "You're not different. You're special," and Hailey knows that to be true.

Hailey decided to share her personal story because she wants people to know about lipodystrophy, how serious it is, and how people with the condition should be treated with respect and compassion. "I go through a lot with kids at school," Hailey says, remembering times when her classmates didn't understand that she was living with a serious medical condition and would say unkind things to her. "I feel that if the disease was something that everybody was aware of, and at least had heard about and [understood] what we go through, then they would be more sensitive to what they say."

Hailey Jeter (center) with her sisters Taylor (left) and Amber (right)

When asked what she would say to the scientists who made leptin treatment possible, Hailey responds, "I can't say a 'thank you' that's big enough. There aren't enough words." Thinking back to all she went through, she can't imagine having to cope with those serious health problems for the rest of her life. And now, because of the support of her family and friends, and "because of NIH," she says, she doesn't have to.



The body has different types of fat, or adipose, tissue. This figure illustrates various biological molecules that can increase fat burning and heat production by activating brown adipose tissue or by inducing the formation of fat-burning cells within white adipose tissue—normally a storage depot for fat—in a process known as "browning." Cold temperatures can also increase fat burning by adipose tissue. As described in this chapter, these research discoveries have implications for strategies to improve metabolic health.

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Obesity

Obesity has risen to epidemic levels in the United States. Individuals who are obese 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. More than one-third of U.S. adults are considered obese based on body mass index (BMI), a measure of weight relative to height.[1] Nearly 17 percent of children and teens ages 2 through 19 are also obese, and thus 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, schools, health care, and other settings using a variety of approaches and technologies; medical and surgical interventions; and combinations of these 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. These represent examples of the NIDDK's broad spectrum of research efforts toward reducing the burden of obesity so that people can look forward to healthier lives.

OBESITY RISK FACTORS

In the Loop—A New Obesity-associated Gene, *IRX3*, and a Strategy To Track Down Other Genes That Affect Health: Researchers discovered that the *IRX3* gene is associated with obesity by finding that it interacts with a segment of DNA in another gene, *FTO*, that contains genetic variants previously implicated in obesity. The *IRX3* and *FTO* genes reside some distance from each other along a stretch of DNA, and the

[1] *Ogden CL, et al. JAMA 311: 806-814, 2014.*

[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*

intervening DNA loops around to connect *IRX3* to the variant-containing region of *FTO*.

The scientists began with a conundrum from prior research: variants in the *FTO* gene's DNA sequence are associated with risk for obesity, but these variants are not in the part of *FTO* that codes for its protein product. Rather, they are in other regions of the gene (called introns). Moreover, although "non-coding" regions in or near genes can serve regulatory functions, these variants do not appear to regulate *FTO* gene activity. With a hunch that these perplexing variants might control the activity of another gene, the scientists widened their search for clues across genomic terrain both near and far from *FTO*. The researchers thought that they could trap the variants interacting with another gene because DNA strands bend and loop, bringing distant segments of the genome together so that regulatory factors can dock at one point and grasp onto a gene farther away. Using a technique devised by other scientists to capture these loops, they found that the variant-containing region of *FTO* connects with the *IRX3* gene in mice. Further study showed similar interactions in human cells. They also found that this part of *FTO* helps increase levels of *IRX3* gene activity, and that obesity-associated variants in this region correlate with differences in *IRX3* activity in the human brain. To determine more directly whether *IRX3* plays a role in weight and metabolism, the researchers investigated whether removing it from mice would have any effect. Compared to normal mice, the *IRX3*-deficient mice weighed less, had less body fat overall, and, interestingly, had more active "brown fat"—a type of fat tissue that burns rather than stores calories. Even within the calorie-storing fat tissue of these mice, some cells had taken on brown fat traits. Mice without *IRX3* were also protected from adverse metabolic consequences of a high-fat diet, including elevated blood glucose (sugar) and excess liver fat. The effects on diet were found in an experiment with male mice; future study could determine whether or not *IRX3* deficiency confers similar protection from an unhealthy diet in females.

This research yields novel insights into obesity and metabolism, which may spark new ideas for therapeutic development. Like the variants in the non-coding part of *FTO* that led scientists to the *IRX3* gene, there are numerous other genome variants that are associated with health conditions but that do not disrupt protein-encoding parts of genes. This study thus also demonstrates the value of research to explore whether such variants might regulate gene activity—even of a distant gene.

Smemo S, Tena JJ, Kim KH,…Nóbrega MA. Obesity-associated variants within FTO *form long-range functional connections with* IRX3. *Nature 507: 371-375, 2014.*

Nourishing Body and Mind—Effects of Maternal Diet on Brain Wiring and Metabolism in Offspring: Seeking to understand how a mother's health and diet during pregnancy can affect the children, researchers discovered, in mice, that a maternal high-fat diet disrupts the wiring of brain circuitry and fuels the development of excess body fat and other metabolic conditions in the offspring.

The researchers first pinpointed a critical time for the dietary effects by feeding mice either standard chow or a high-fat diet during pregnancy and lactation. A high-fat diet was most detrimental to the mouse pups when given to their mothers exclusively during lactation, a stage similar to the third trimester of human pregnancy. Within a few weeks, mice born to mothers on this unhealthy diet developed excess body fat along with signs of prediabetes and type 2 diabetes (insulin resistance and glucose intolerance). Based on clues from prior studies, the scientists then traced the connections among cells in a region of the brain called the hypothalamus, which plays a key role in regulating metabolism. In the offspring of mothers fed the high-fat diet during this stage of development, brain cells did not branch out normally to build a dense network of connections. Instead, these mice had many fewer fibers linking different areas of the hypothalamus. From additional experiments, the scientists found that the adverse dietary effects result, in part, from elevated signaling by the hormone insulin in the brains of the young mice. Other biologic pathways, yet to be defined, also play a role.

Because diabetes and obesity in women during pregnancy are known to increase risk for diabetes and obesity in their children, this study of a high-fat diet in mice may have implications for human health. Further research may lead to improved strategies for healthy eating and other interventions during pregnancy, to benefit women and their children.

Vogt MC, Paeger L, Hess S,...Brüning JC. Neonatal insulin action impairs hypothalamic neurocircuit formation in response to maternal high-fat feeding. Cell 156: 495-509, 2014.

MICROBIOME, DEVELOPMENT, AND OBESITY

Antibiotic Exposure in Early Life Changes Gut Microbes, Long-term Metabolism, and Immune Function: Researchers have used a mouse model to show that even short-term exposure to antibiotics early in life can transiently alter the gut microbial community, or "microbiome," resulting in lasting effects on metabolism, weight gain, and immune function. The first few years of life are a critical time for healthy growth and development, including establishment of a healthy gut microbial community. Children raised in countries such as the United States routinely have their microbial communities altered by receiving antibiotics, which have been associated with a greater risk of obesity later in life. Also, based on decades of agricultural practice, antibiotics are known to increase weight gain in livestock, particularly if used from a young age. However, antibiotics do not cause weight gain in animals lacking gut microbes, suggesting the microbes play a key role in altered metabolism following antibiotic treatment.

One research group investigated in a mouse model the alterations occurring with antibiotic treatment in early life. Using both male and female mice, the researchers studied timing of antibiotic exposure and gut microbial changes, altered metabolic and immune indicators, and interactions with diet and resulting obesity risk. They first looked at mouse pups whose mothers had been given penicillin in drinking water right before birth and prior to weaning. Both male and female pups whose mothers received the antibiotic were bigger and had more fat mass than the controls as adults, including in the liver, where their fat cell production genes were more active. Mice treated directly with antibiotics from a young age also showed altered shifts in the maturing gut microbial composition as they grew, compared to untreated mice. To look at how gut microbes interact with diet in mice given antibiotics, the researchers fed some of the antibiotic-treated mice a high-fat diet after weaning. In both the male and female mice, the combination of early antibiotics and high-fat diet led to a large increase in weight gain by 30 weeks (adulthood in mice), particularly in the female mice, who doubled their fat mass. When the researchers looked at metabolic changes throughout the body in these mice, they found fatty livers, especially in the male mice, as well as changes in the activity of genes associated with carbohydrate and fat metabolism. Similar effects on weight gain were found in mice given just a limited course of antibiotic for 4 to 8 weeks during early life and fed the high-fat diet. These mice also had reduced activity of cells and genes involved in intestinal immunity. Surprisingly, following the short-term antibiotic exposure, the mice's gut microbe populations slowly returned to a normal mix of bacterial species, even though the metabolic effects persisted. Finally, the researchers were able to transfer this altered metabolism between individuals by transplanting the antibiotic-treated mice's intestinal contents into the sterile guts of germ-free mice. This showed that it was changes in the gut microbes themselves, not direct effects of the antibiotic, causing the altered metabolism.

These studies show that changes early in life in the structure of the gut microbial community, due to factors such as antibiotics, could have life-long consequences by "programming" an organism's future metabolism and immune function. If these results in mice hold true for humans, they would provide compelling reasons for devising ways to restore normal gut microbial composition in children exposed to antibiotics, both to reduce risk of metabolic disorders and establish healthy immunity.

Cox LM, Yamanishi S, Sohn J,...Blaser MJ. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell 158: 705-721, 2014.

BARIATRIC SURGERY—SAFETY AND EFFICACY

Bariatric Surgery Yields Significant but Variable Weight Loss and Health Improvements After Three Years: A new study has found that adults with severe obesity had substantial weight loss 3 years after bariatric surgery, with significant improvements in diabetes, high blood pressure, and cholesterol outcomes. The researchers also found that weight loss and other outcomes varied among the study participants. People with severe obesity often do not gain sufficient health benefits from lifestyle interventions alone, and thus may turn to bariatric surgery, in addition to lifestyle changes, to help them lose weight and reduce their risk for obesity-associated health conditions. However, the generalizability of previous reports assessing the medium- and long-term risks and benefits of bariatric surgery has been limited. The multi-center Longitudinal Assessment of Bariatric Surgery (LABS) Consortium was launched to assess the safety and efficacy of bariatric surgery procedures with standardized, detailed data collection from a geographically diverse cohort of study participants across the country to provide evidence that can be broadly applicable to clinical practice.

In a new report from the LABS Consortium, more than 2,000 adults underwent either Roux-en-Y gastric bypass (RYGB) or laparoscopic adjustable gastric banding (LAGB)—two different commonly performed bariatric surgery procedures. About 80 percent of the initial group of study participants were women. Significant weight loss was observed 3 years after surgery, with the majority of participants losing the most weight during the first year. There were, however, differences in the extent of weight loss between the two procedures. There was also substantial variation among individuals who had the same procedure; for each procedure, the weight loss trajectories of the study participants were not uniform, but fell into five distinct groups. Some lost more weight during the study and experienced only a small amount of weight regain, while others lost less and gained more of their weight back more quickly. Overall, the median weight loss for individuals who underwent RYGB was 31.5 percent of the body weight they had before surgery, compared with 15.9 percent weight loss for those who had LAGB surgery. The scientists also observed significant improvements in obesity-associated health conditions, but again with differences between the two procedures. Many participants had at least partial remission of type 2 diabetes (67.5 percent for those who had RYGB and 28.6 percent for those who had LAGB, respectively), improvements in high blood pressure (38.2 percent and 17.4 percent, respectively), and a reduction in excess fats in the bloodstream (61.9 percent and 27.1 percent, respectively). Thus, RYGB, and to a lesser degree LAGB, leads to significant weight loss and reduction in obesity-related health conditions, but the extent of these improvements is variable. Factors such as the large number of individuals enrolled in the study, standardized data collection, and diversity of geographic locations where the surgeries took place add to the generalizability of the findings from this report. To determine the durability of these results, LABS researchers are conducting longer-term follow-up analyses of participants' health and weight.

Courcoulas AP, Christian NJ, Belle SH,...Longitudinal Assessment of Bariatric Surgery (LABS) Consortium. Weight change and health outcomes at 3 years after bariatric surgery among individuals with severe obesity. JAMA 310: 2416-2425, 2013.

Bariatric Surgery in Teens Leads to Relatively Few Short-term Complications: In a new study in adolescents with severe obesity and weight-related health problems who underwent bariatric surgery, researchers found few incidents of major complications in the first 30 days after the surgery. Children and teens who are obese—and particularly those with severe obesity—are at increased risk for developing serious diseases both during their youth and later in adulthood. In youth, obesity is often accompanied by other adverse health conditions, such as sleep apnea, joint pain, hypertension, and nonalcoholic fatty liver disease. For adolescents who are severely obese, lifestyle changes are important, but when lifestyle interventions alone do not reduce obesity and ameliorate its associated health conditions, additional therapies may be considered, such as surgery. However, despite the use of this surgery in clinical practice in adolescents,

there has been limited data on its outcomes. Thus, the Teen-Longitudinal Assessment of Bariatric Surgery (Teen-LABS) study was launched in 2007 to assess the short- and long-term risks and benefits of bariatric surgery among teens with severe obesity. This is an observational study that enrolled teens who were already planning to have bariatric surgery.

Researchers from five U.S. Teen-LABS centers have now analyzed and reported short-term outcomes of three bariatric surgery techniques: Roux-en-Y gastric bypass, sleeve gastrectomy, and gastric banding. A cohort of 242 study participants, aged 19 and under, was evaluated for major, or life-threatening, complications (*e.g.*, bowel obstruction/bleeding, gastrointestinal leaking, deep vein thrombosis, splenectomy), as well as for minor (non-life-threatening) complications (*e.g.*, pneumonia, urinary tract infections or other complications, bowel injury, hypertension). At 30 days after surgery, there were no deaths; 8 percent of the participants experienced major complications; and 15 percent experienced minor complications. Thus, over the short-term, bariatric surgery led to relatively few complications. While the study is limited by a lack of ethnic diversity, the standardized data collection procedures, multi-site enrollment, and comprehensive study design provide valuable information that could help inform health care providers. Importantly, Teen-LABS investigators will continue to study the participants to determine longer-term safety, health, and weight outcomes of bariatric surgery in teens.

Inge TH, Zeller MH, Jenkins TM,...Buncher R; Teen-LABS Consortium. Perioperative outcomes of adolescents undergoing bariatric surgery: the Teen-Longitudinal Assessment of Bariatric Surgery (Teen-LABS) study. JAMA Pediatr 168: 47-53, 2014.

For more information on bariatric surgery, please see the Story of Discovery later in this chapter and also "Comparing Surgical and Non-surgical Treatments for Type 2 Diabetes in Adults Who Have Mild to Moderate Levels of Obesity" on page 20.

FUNCTION OF BROWN FAT

Cool Temperature Alters Human Brown Fat and Improves Insulin Sensitivity: New research shows that, in men, prolonged exposure to a cool environment increases brown fat volume and energy expenditure. Mammals harbor different types of fat tissue: calorie-storing white adipose tissue (WAT) is the most abundant; brown adipose tissue (BAT), which burns calories to generate heat; and beige fat tissue, which exhibits some characteristics of classic BAT cells but also has distinct properties, and can appear within WAT depots in response to various triggers. Because of its calorie-burning properties, scientists have considered BAT a promising target tissue for the development of treatment strategies for obesity. However, how BAT is regulated in humans, whether its volume can change dynamically, and its effects on metabolism remain poorly understood.

A team of scientists has now explored the effects of ambient temperature on brown fat and metabolism in five healthy men with an average age of 21 years old. The study participants resided for 4 months in a clinical research unit in the NIH Clinical Center in Bethesda, Maryland. The men engaged in regular activities during the day and then returned to their private room each evening. The temperature of the room was set to 75 degrees Fahrenheit during the first month, 66 degrees the second month, 75 degrees again for the third month, and 81 degrees the remaining month. The participants were exposed to the temperature for at least 10 hours each night. They wore standard hospital clothing and had only bed sheets. All meals were provided, with calorie and nutrient content carefully controlled and all consumption monitored. At the end of each month, the men underwent extensive evaluations, including energy expenditure testing, muscle and fat biopsies, and imaging of an area of the neck and upper back region to measure BAT volume and activity. After a month of exposure to mild cold (*i.e.*, 66 degrees), the participants had a 42 percent increase in brown fat volume and a 10 percent increase in fat metabolic activity. These alterations returned to near baseline during the following month of neutral temperature,

and then were completely reversed during the final month of warm exposure. All the changes occurred independently of seasonal changes. The increase in brown fat following a month of cold exposure was accompanied by improved insulin sensitivity after a meal during which volunteers were exposed to mild cold. Prolonged exposure to mild cold also resulted in significant changes in metabolic hormones. There were no changes in body composition or calorie intake. While this study included a relatively small number of people, and only men, these proof-of-concept findings suggest that humans may acclimate to cool temperature by increasing brown fat, which in turn may lead to improvements in metabolism—changes that can be dampened or reversed following exposure to warmer temperatures.

Lee P, Smith S, Linderman J,…Celi FS. Temperature-acclimated brown adipose tissue modulates insulin sensitivity in humans. Diabetes 63: 3686-3698, 2014.

(Information adapted from original article by Dr. Carol Torgan, published on July 8, 2014 in NIH Research Matters.)

To Beige or Not To Beige: Novel Molecular Insights into the Induction of a Calorie-burning Fat Tissue

Several recent studies supported by the NIDDK revealed the roles of various biological molecules in turning one type of body fat tissue into another type of fat in mice and people. This research has implications for strategies to improve metabolic health.

Mammals harbor different kinds of adipose (fat) tissue in various regions of the body. Calorie-storing white adipose tissue (WAT) is the most abundant type of fat tissue. In contrast to WAT, brown adipose tissue (BAT) burns calories to generate heat. Research has shown that another type of brown fat cells—called beige fat cells—exhibits some characteristics of classic BAT cells, such as an active program of genes involved in generating heat, but also have distinct properties. These beige fat cells appear within WAT depots (a process referred to as the "browning" of WAT) in response to cold, other nervous system triggers, and muscle activity. The metabolic potential of beige fat has led many researchers to believe that it could serve as a target for treatment strategies in humans, but the molecular control of beige fat induction remains poorly understood. A series of new studies has shed light on the molecules and pathways that regulate beige fat.

In one report, researchers genetically modified mice to lack the protein PRDM16 in adipose tissues. The resulting mice lost the ability to induce beige fat cells specifically in subcutaneous WAT, which is found just under the skin. These mice developed obesity, insulin resistance, and elevated fat accumulation in the liver. In addition, the subcutaneous fat tissue shifted form to resemble visceral fat tissue, which is the type of fat that surrounds internal organs and is associated with risk for type 2 diabetes. These findings demonstrate that, in mice, beige fat contributes to the overall metabolic health of the animal and PRDM16 in WAT is required for the induction of beige fat cells.

Previous research has shown that exercise can induce beige fat production, but what molecular signals trigger this "browning" of WAT? In a recent study, scientists identified a metabolite, called β-aminoisobutyric acid, or "BAIBA," that is produced by muscle tissue following exercise and can induce the "browning" of WAT. When mice were either put on an exercise program or genetically modified to resemble mice that have undergone exercise, BAIBA levels were elevated in muscle tissue and in circulating blood compared to normal mice. Mice fed BAIBA exhibited decreased body fat, increased energy expenditure (calorie burning), improved glucose (sugar) tolerance, and a slight, but significant, reduction in weight. To see if humans similarly produce BAIBA, the scientists examined participants in the HERITAGE Family Study and found that, after a 20-week exercise program, blood BAIBA levels increased by 17 percent—a response similar to that observed in rodents. The researchers also examined levels of the metabolite in blood samples of participants in the landmark Framingham Heart Study, which has followed participants for long periods of time to identify cardiometabolic risk factors. They found that higher levels of circulating BAIBA were associated with reduced cardiometabolic risk, including reduced insulin resistance and lower levels of fasting glucose, insulin, triglycerides, and total cholesterol. These findings reveal a novel link between an exercise-induced, secreted metabolite from muscle tissue and beige fat production and improved metabolic health.

Another molecular trigger of beige fat induction was also recently identified, that links exercise to the activation of immune cells within WAT. Scientists searched in mice for signals that were being sent from muscle cells engineered to contain high levels of PGC-1α4—a protein in muscle that induces a signal capable of triggering the "browning" of WAT. The researchers identified the little-known protein hormone Meteorin-like, or "Metrnl," as an important mediator of this communication. Interestingly, the scientists found that in mice, as well as in male

human study participants, exercise leads to the production of Metrnl in the muscle and its release into the bloodstream. In mice, mild exposure to cold temperatures induces production of Metrnl in fat tissue. To better understand Metrnl's function, the researchers generated experimentally modified male mice that, in the absence of exercise, could produce the hormone—not in their muscles, but in their livers—and secrete it into the bloodstream. In these mice, they observed characteristic "browning" of WAT, similar to that seen in mice with high levels of PGC-1α4. The mice with liver-produced Metrnl also were protected from some of the negative effects of a high-fat diet: they exhibited improved glucose tolerance and increased energy expenditure, a physiological feature that is associated with thermogenesis. However, Metrnl did not appear to act directly on white fat cells; rather, it appeared to trigger the stepwise activation of a number of types of immune cells that normally reside within WAT, some of which have been previously implicated in the "browning" of WAT. One type of immune cell, called an eosinophil, produces proteins, called IL-4 and IL-13 (referred to collectively as "IL-4/IL-13"), in response to Metrnl. The researchers found that exposure to cold temperatures increases the levels of *IL-4/IL-13* gene activity, and blocking the action of IL-4/IL-13 protein prevents thermogenic genes from being induced in WAT in response to Metrnl. IL-4/IL-13 may increase thermogenesis by inducing another type of immune cell, called an "alternatively activated" macrophage. These results provide a link between exercise and the "browning" of WAT through activation of immune cells.

These results were supported by a separate study, in which scientists exposed male mice that were genetically modified to lack the *IL-4/IL-13* genes to mild cold stimulation and examined their ability to induce "browning" of WAT—that is, the induction of beige fat. In contrast to normal mice, the mutant mice failed to stimulate beige fat cell production within subcutaneous WAT in response to cold exposure, and the scientists did not detect the accompanying characteristic robust energy expenditure. The researchers observed similar results with mice in which eosinophils were genetically ablated from WAT. Other experiments demonstrated that IL-4/IL-13 may promote alternative activation of macrophages, which in turn induce the browning of WAT. When the scientists administered IL-4 to normal male mice that were not exposed to cold temperature, they observed the induction of thermogenic genes in WAT and increased energy expenditure; these mice, when fed a high-fat diet, were also protected from the metabolic dysfunction seen in untreated mice. Taken together, these results strongly support a role for eosinophils, IL-4, and alternatively activated macrophages in cold-induced "browning" of WAT—cells and signaling molecules also involved in exercise-induced browning of WAT, as reported by the other researchers.

Understanding the molecules and processes contributing to beige fat development and function could also lead to previously unforeseen health benefits for other diseases. Cachexia is a dramatic loss of skeletal muscle mass and adipose tissue often accompanied by substantial weight loss and frailty, and is commonly associated with sepsis and cancer. Previous research implicated BAT induction and activation in the increased resting energy expenditure associated with cachexia in animals. Scientists have now found that in a mouse model for lung cancer, tumors send a protein signal, called PTHrP, which induces and activates beige and brown fat. In male mice with lung cancer, blocking the activity of PTHrP led to reduced "browning" of WAT, decreased heat production, and improvements to the cachectic condition. Thus, although increased beige fat mass and activity may provide benefit in the setting of obesity and diabetes, in the case of cachexia the induction of brown or beige fat by PTHrP is associated with adverse effects. The researchers also examined 47 human lung or colon cancer patients with cachexia, and found that elevated levels of PTHrP in the blood were associated with less muscle mass and increased resting energy expenditure. These results suggest that PTHrP may be playing a similar role in people and could be a target for cancer-related cachexia.

These studies provide important new insights into the mechanisms regulating beige fat induction, as

well as its associated metabolic effects, both in normal physiology and in disease progression. Most of these experiments were performed in animal models, but some initial human studies support a role for beige and brown fat in improved metabolic health. (For one example, please see the advance entitled "Cool Temperature Alters Human Brown Fat and Improves Insulin Sensitivity," also in the Obesity chapter.) While further investigation in humans is needed, these promising findings could aid in the development of therapeutic strategies for obesity and associated metabolic diseases that promote the "browning" of WAT.

Cohen P, Levy JD, Zhang Y,...Spiegelman BM. Ablation of PRDM16 and beige adipose causes metabolic dysfunction and a subcutaneous to visceral fat switch. Cell 156: 304-316, 2014.

Kir S, White JP, Kleiner S,...Spiegelman BM. Tumour-derived PTH-related protein triggers adipose tissue browning and cancer cachexia. Nature 513: 100-104, 2014.

Qiu Y, Nguyen KD, Odegaard JI,...Chawla A. Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. Cell 157: 1292-1308, 2014.

Rao RR, Long JZ, White JP,...Spiegelman BM. Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. Cell 157: 1279-1291, 2014.

Roberts LD, Boström P, O'Sullivan JF,...Gerszten RE. β-aminoisobutyric acid induces browning of white fat and hepatic β-oxidation and is inversely correlated with cardiometabolic risk factors. Cell Metab 19: 96-108, 2014.

Another study, not supported by the NIDDK, showed substantial "browning" of intestinal fat tissue and fat surrounding the liver, kidney, and pancreas in patients with cachexia, secondary to a variety of cancers—findings that complement those from the study described in the feature above (Cell Metab 20: 433-447, 2014).

STORY OF DISCOVERY

Understanding the Health Benefits and Risks of Bariatric Surgery

Severe obesity is a chronic condition that, for many people, is difficult to treat with diet or exercise alone, and increases risks for type 2 diabetes, cardiovascular disease, fatty liver disease, and many other devastating health conditions. Bariatric surgical procedures, which restrict stomach size and/or alter the intestinal tract, have been increasingly performed to treat severe obesity when other interventions have not worked. Additionally, bariatric surgery is used in clinical practice for people who have milder levels of obesity along with type 2 diabetes or other serious obesity-related disease. These surgical procedures can have dramatic benefits—such as significant and sustained weight loss, improved control of blood glucose (sugar) levels, or even reversal of type 2 diabetes—especially when accompanied by exercise and a healthy diet. They also carry substantial risks, and researchers have been evaluating the benefits and risks of different procedures.

Despite the increasing popularity of bariatric surgery, crucial questions still remain, such as how best to select candidates for surgery, based on improved definition of specific benefits versus short- and longer-term complications and survival rates, and the effects of different procedures on specific co-morbidities in people with lesser degrees of obesity. While the surgical modifications of the stomach and intestines reduce food intake and the amount of nutrients—including calories—absorbed, emerging evidence is revealing potential additional mechanisms for the effects on weight and metabolism. Researchers would thus like to determine precisely how certain types of bariatric surgical procedures work to help patients lose a considerable amount of weight, maintain weight loss, and improve obesity-related diseases. Finally, there is as yet unexplained heterogeneity in the outcomes of bariatric surgery, ranging from dramatic weight loss and improvement in comorbidities to subsets of patients who fail to lose weight, or who regain weight, and do not have a satisfactory outcome.

Scientists supported by the NIDDK and other organizations have been studying the risks and benefits of bariatric surgery, to help individuals with obesity and their doctors make more informed decisions. Additionally, research on the underlying mechanisms for the effects of bariatric surgical procedures could lead to the development of novel, non-surgical treatments that confer the benefits without the risks of surgery.

Bariatric Surgical Procedures

The first surgery of this type used for severe obesity dates back 50 years and grew out of the results of operations for certain cancers or severe ulcers. Doctors became aware that their patients lost weight following surgeries that removed large portions of the stomach or small intestine. Some physicians began to use such operations to treat patients with severe obesity. Over time, these operations have been modified to improve patient safety and to incorporate technological advances in surgical procedure. There are several general problems involved in assessing the outcomes of bariatric surgery. One is that new surgical approaches and technologies evolve continuously, and are continuing to change at the present time. Secondly, the patient populations who receive bariatric surgery

also continue to evolve and may be quite different in individual studies. Together, these issues often make direct comparisons of research studies difficult.

There are several different surgical procedures performed that work through restricting food intake, changing the way in which food is absorbed or metabolized, or both. Physicians performing restrictive operations such as the laparoscopic adjustable gastric banding (LAGB) reduce the opening to the stomach or stomach size. Other procedures such as the biliopancreatic diversion, with or without duodenal switch, restrict the amount of calories and nutrients the body absorbs. The most commonly performed procedure at this time, which has both a restrictive and malabsorptive component, is the Roux-en-Y Gastric Bypass (RYGB). RYGB connects the upper stomach to the lower part of the small intestine, so that food bypasses a large portion of the gastrointestinal tract in which digestion and nutrient absorption normally take place. Increasingly, surgeons are performing a sleeve gastrectomy (SG) procedure in which a portion of the stomach is removed, leaving a sleeve or tube through which food can pass. Over the past several decades, researchers have sought to understand the benefits and risks of different bariatric surgery procedures.

The "Swedish Obese Subjects" Study of Bariatric Surgery

One early large-scale trial was the Swedish Obese Subjects (SOS) study, which remains the largest prospective study on bariatric surgery to date. The landmark SOS study was initiated in 1987. It included more than 2,000 participants who were undergoing bariatric surgery as part of their clinical care, and, as a control group, over 2,000 individuals who had similar health-related measures at the beginning of the study but had declined surgery and instead were receiving usual care. The researchers studied the participants over many years to compare the two groups for overall mortality, weight loss, and other important health outcomes, such as heart attacks, stroke, and type 2 diabetes. When the patients were recruited into the study (from 1987 to 2001), the most common procedure performed was vertical banded gastroplasty, a procedure which is infrequently performed today. RYGB, the most common procedure performed today, was only done in 13 percent of the SOS patients.

The SOS study now has 15 to 20 years of follow-up results. Bariatric surgery was associated with a 29 percent reduction in mortality, which was not correlated with the extent of weight loss. Type 2 diabetes remission after 2 years was 72 percent, and 36 percent after 10 years. Bariatric surgery was also associated with fewer cardiovascular events after more than 10 years. The SOS study found a reduced cancer incidence in women, but not in men, following bariatric surgery. Bariatric surgery lowered medication costs from years 7 to 20, but hospital days and outpatient visits were greater in the surgery group in the first 6 postoperative years. Although the SOS study has yielded important information about the long-term outcomes of bariatric surgery, most patients in the study underwent procedures that are no longer performed today.

The Longitudinal Assessment of Bariatric Surgery (LABS) Study

Many other bariatric surgery studies have been performed over the past few decades. However, the generalizability of some of these studies has been limited by a variety of factors, such as relatively small numbers of participants, lack of diversity in geographic locations or populations, non-standardized research practices, and short-term follow-up.

STORY OF DISCOVERY

Thus, in 2003, the NIDDK began a new research effort in bariatric surgery. The Institute partnered with researchers at multiple sites across the country to create the Longitudinal Assessment of Bariatric Surgery (LABS) consortium. The goal was to facilitate and accelerate clinical, epidemiological, and behavioral research to address key long-term outcomes of bariatric surgery, with a planned follow-up of at least 5 years. Between 2004 and 2009, the multi-center LABS consortium enrolled thousands of patients with severe obesity who were already planning to undergo bariatric surgery. The geographically diverse participants were evaluated at baseline and annually with standardized measures by trained personnel to address important questions about the comparative efficacy and safety of surgical procedures, as well as the durability of weight loss and health improvements. The overall goal of this observational research was to provide evidence that can be broadly applicable to clinical practice.

LABS has already provided critical insights into the risks and outcomes of bariatric surgical procedures. One early report followed 4,776 patients with severe obesity who had bariatric surgery, from before their surgery through the first 30 days following surgery, to evaluate death and complication rates. The study took place over 2 years at 10 U.S. medical centers, with one center coordinating data collection and analysis. Within 30 days of surgery, 4 percent of patients had at least one major adverse outcome, defined as development of blood clots in the deep veins of the legs or the pulmonary artery of the lungs, repeat surgeries, not being discharged from the hospital within 30 days, or death. Mortality rates were low: fewer than 1 percent of patients died within 30 days. This evaluation highlights the level of short-term risks associated with bariatric surgery.

An important goal of the LABS consortium is to determine longer-term outcomes of bariatric surgery. One study, reported in 2013, found that adults with severe obesity had substantial weight loss 3 years after bariatric surgery (RYGB or LAGB) with significant improvements in diabetes, high blood pressure, and cholesterol outcomes, although results varied among the study participants. A majority of the study participants lost the most weight during the first year. Overall, the median weight loss after 3 years for individuals who underwent RYGB was 31.5 percent of the body weight they had before surgery, compared with 15.9 percent weight loss for those who had LAGB surgery. Many participants had at least partial remission of type 2 diabetes, improvements in high blood pressure, and a reduction in excess fats in the blood. Although both procedures were effective, RYGB consistently led to greater health improvements than LAGB. For both procedures, fewer than 1 percent of study participants died within 3 years. Only 0.3 percent of patients who had RYGB required subsequent bariatric surgery, whereas 17.5 percent of individuals who received LAGB needed additional surgery, such as band replacement, band removal, or revision to another bariatric surgical procedure. Thus, RYGB, and to a lesser degree LAGB, leads to significant weight loss and reduction in obesity-related health conditions after 3 years, but the extent of these improvements is variable.

LABS investigators are also seeking to understand behavioral predictors and outcomes of bariatric surgery. For example, researchers found that adults who had RYGB were at significantly higher risk for alcohol use disorders (AUD) 2 years after surgery compared with before surgery. Among participants who had the RYGB procedure, there was no significant increase in AUD 1 year after surgery. By the second postoperative year, however, there was a relative increase in AUD of more than 50 percent in participants who had RYGB compared to pre-surgical rates. Patients who underwent gastric banding did

not report an increase in AUD symptoms. While a number of predictors for AUD were identified, these results suggest that clinicians should be aware of the importance of monitoring for signs and symptoms of AUD and consider counseling after bariatric surgery, particularly in patients who undergo RYGB.

In addition to funding research on outcomes in adults, the NIDDK also supports the Teen-Longitudinal Assessment of Bariatric Surgery (Teen-LABS) study. Although not common in adolescents, the use of bariatric surgery in this age group has been increasing in clinical practice. Thus, the Teen-LABS study was launched in 2007 to assess the short- and long-term risks and benefits of bariatric surgery among teens with severe obesity. This observational study enrolled 242 teens who were already planning to have bariatric surgery. The participants underwent either RYGB, SG, or gastric banding, and they were evaluated for major, life-threatening complications (*e.g.*, bowel obstruction/bleeding, gastrointestinal leaking, deep vein thrombosis, and splenectomy), as well as for minor, non-life-threatening, complications (*e.g.*, pneumonia, urinary tract infections or other complications, bowel injury, and hypertension). At 30 days after surgery, there were no deaths; 8 percent of the participants experienced major complications; and 15 percent experienced minor complications. Thus, over the short-term, bariatric surgery led to relatively few complications. Teen-LABS investigators will continue to study the participants to determine longer-term safety, health, and weight outcomes of bariatric surgery in teens.

Mining Health Databases to Evaluate Outcomes of Bariatric Surgery

In another type of effort to gain insights on bariatric surgery outcomes from relatively large numbers of patients, researchers have sought to use data from pre-existing databases to perform retrospective analyses. For example, one group of scientists, supported by the Agency for Healthcare Research and Quality, examined data from the electronic medical records of 1,395 adults with severe obesity and with type 2 diabetes, from 20 centers across the United States, who had bariatric surgery—most had RYGB, but a small percentage had other procedures—and more than 62,000 who did not. The goal of this study was, at 2 years after surgery, to compare various health outcomes, including diabetes remission and death. The researchers' analyses, published in 2012, showed that in this large, multi-center study, the patients who had bariatric surgery achieved higher rates of diabetes remission, with no increased risk of death, compared with patients who received usual care.

Scientists in another study, supported by the NIDDK, also used electronic medical records of 690 patients who had RYGB surgery to develop an algorithm, called the DiaRem score, that can predict the likelihood of postoperative type 2 diabetes remission. Sixty-three percent of the patients achieved partial or complete diabetes remission after the surgery. The researchers examined 259 different pre-operative clinical variables, and found that four standard measures could effectively predict type 2 diabetes remission: insulin use, age, circulating glucose levels, and type of anti-diabetes drugs. This predictive model could provide a tool for clinicians and patients to help consider whether bariatric surgery might be an appropriate treatment option, and to better manage type 2 diabetes after the operation.

Clinical Trials of Bariatric Surgery for Obesity and Type 2 Diabetes

While observational and retrospective studies can provide insights about risks and benefits of bariatric surgery, proving its health impact would require long-term randomized, controlled trials (RCTs), which are

difficult and costly to undertake. In the past few years, several relatively small RCTs enrolled participants with type 2 diabetes who either underwent bariatric surgery or were given one of a variety of non-surgical treatments for obesity and/or diabetes. After short-term follow-up, bariatric surgery procedures resulted in greater weight loss and greater remission of type 2 diabetes compared with the non-surgical options.

In addition to research focusing on people with severe obesity, recent clinical trials have been conducted to gain preliminary insights into the risks and benefits of bariatric surgery for type 2 diabetes in people with milder levels of obesity, as defined by body mass index (or "BMI," a measure of weight relative to height). For example, reports of two small, NIDDK-supported short-term trials published in 2014 found that bariatric surgery may be more effective than non-surgical approaches for treating type 2 diabetes in adults who have moderate (BMI 35 to 40) or mild (BMI 30 to 35) levels of obesity. In one trial, 69 volunteers with type 2 diabetes were randomly assigned to receive either bariatric surgery or an intensive lifestyle intervention for weight loss, and health outcomes were compared after 1 year. About one-half of the individuals who underwent RYGB and 27 percent of those who underwent LAGB had partial diabetes remission. Their blood glucose levels were no longer in the diabetes range, and they were able to discontinue their diabetes medications. None of the people in the lifestyle intervention group had partial or complete diabetes remission. However, several individuals in the RYGB and LAGB groups experienced complications, including a surgery-related ulcer and dehydration. In another trial, investigators assigned 43 volunteers to receive either RYGB surgery or a lifestyle and medical intervention of diet and exercise, delivered in group sessions, with a weekly medication adjustment plan. After 1 year, 58 percent of the individuals in the RYGB group, but only 16 percent of those in the lifestyle and medical therapy group, saw their blood glucose levels improve to the target for glucose control chosen as the study outcome. Weight loss was greater in the RYGB group as well. Importantly, while assessing the safety of the interventions, the researchers noted several adverse events among participants in the RYGB surgery group, including ischemic heart disease requiring coronary artery bypass surgery and depression with attempted suicide. In the non-surgical group, a few individuals experienced near-fainting.

The results of these studies were similar to other RCTs, which found improved weight and diabetes outcomes for the bariatric surgical groups when compared to those receiving non-surgical treatments for obesity or diabetes. However, RCTs are difficult to perform, generally small, and of short duration. Given these challenges, high-quality, carefully designed observational studies might be preferable to answer questions about the safety of bariatric surgery, as well as the durability of weight loss and health improvements.

Looking Forward

While a picture may be emerging regarding the safety and efficacy of bariatric surgery as a treatment for obesity in the short- and medium-term, further research is needed to better understand the long-term risks and durability of positive outcomes. Additional avenues of clinical research, such as efforts to elucidate the behavioral and psychological factors that influence the trajectory of weight loss after bariatric surgery, could also inform clinical decisions for the treatment of obesity and its complications.

Researchers are also investigating the molecular mechanisms behind physiological changes that occur following bariatric surgery. Emerging evidence

suggests that the beneficial effects of bariatric surgery may extend beyond the physical restrictions of the surgery, malabsorption, and the postoperative reduction of calorie intake. For example, scientists have observed near immediate improvements in type 2 diabetes and other metabolic complications prior to weight loss following bariatric surgery, but the reasons for the dramatic change in glycemic control are not yet well understood. Other physiological effects, such as alterations in the types of bacteria that normally reside in the gut (the gut microbiome) and changes in hormones, metabolic factors such as bile acids, and nervous system pathways controlling feeding behavior and metabolism are also under investigation. These and other studies may ultimately provide the foundation for non-surgical therapies, including medications and devices, to achieve health improvements similar to those following bariatric surgery, but perhaps without the associated risks.

SCIENTIFIC PRESENTATION

Brown and Beige Fat: Basic Biology and a Potential New Generation of Therapeutics

Dr. Bruce M. Spiegelman

Dr. Bruce M. Spiegelman is the Stanley J. Korsmeyer Professor of Cell Biology and Medicine at Harvard Medical School and Dana-Farber Cancer Institute. Dr. Spiegelman received a B.S. with highest honors from the College of William and Mary, his Ph.D. in Biochemistry from Princeton University, and completed postdoctoral work at the Massachusetts Institute of Technology. Dr. Spiegelman has been honored with many awards, including the Bristol-Myers Squibb Award for Distinguished Achievement in Metabolic Research; the Solomon Berson Award, American Physiological Society; the Rolf Luft Award in Endocrinology, Karolinska Institute (Sweden); The Elliot P. Joslin Medal; the Trans-Atlantic Medal, British Endocrine Society; and the Naomi Berrie Award for Outstanding Achievement in Diabetes Research, Columbia University. He won the Banting Medal for Scientific Achievement in 2012, the highest award of the American Diabetes Association. In 2002, Dr. Spiegelman was elected to the American Academy of Arts and Sciences and the National Academy of Sciences. He was elected to the Institute of Medicine of the National Academies in 2014.

Dr. Spiegelman's research focuses on fat cell biology, diabetes, and the regulation of energy homeostasis in mammals, primarily at the level of gene transcription. At the February 2014 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Spiegelman presented findings from his laboratory's research; the following are highlights from his presentation.

Distinct Mammalian Fat Tissues with Distinct Forms and Functions

Mammals harbor different kinds of adipose (fat) tissue in various regions of the body. Calorie-storing white adipose tissue (WAT) is the most abundant, and can be found surrounding internal organs and under the skin. In contrast to the fat-storing WAT, brown adipose tissue (BAT) burns calories to generate heat. The heat-generating activity of brown fat is induced by cold exposure, and contributes to a phenomenon known as "non-shivering thermogenesis," in which heat is produced as a by-product of specific biochemical processes that aid mammals in staying warm. Although human brown fat was initially thought to be present only in newborns, a series of studies have now confirmed its presence and function in adults. Recent research in rodents has identified a third type of fat cell, called beige fat (alternately called brite, for brown in white, or recruitable BAT cell), that is inducible and exhibits some of the characteristics of classic brown fat. Beige fat cells appear within portions of white fat in response to cold or other nervous system triggers.

Because brown and beige fat are capable of burning calories, Dr. Spiegelman has sought to understand whether these tissues could serve as suitable targets for the development of treatment strategies for obesity and metabolic disease. In his presentation, Dr. Spiegelman focused on two different molecules important for beige fat physiology and development that shed important new light into the molecular

characteristics of beige and brown fat cells—the only types of cells in adult mammalian animal models known to fight diabetes and obesity by directly increasing energy expenditure.

The Role of PRDM16 in Beige Fat Development and Activity

Several years ago, Dr. Spiegelman and colleagues identified a protein, called PRDM16, that is found in relatively high quantities within mouse brown fat cells, but at much lower levels in WAT. The team also showed that isolated mouse cells that ordinarily develop into brown fat cells, when depleted of PRDM16, did not develop into fat cells, as predicted, but rather became muscle cells. In the reverse experiment, mouse muscle cells converted to brown fat cells when forced (by molecular manipulation) to produce PRDM16. These studies also showed that brown fat and muscle cells have similar developmental origins, and that PRDM16 was critical for brown fat development and activity. Interestingly, Dr. Spiegelman's team characterized cells in adult human brown fat deposits, and found that these cells more closely resembled mouse beige fat than they did classical brown fat cells, highlighting the importance of understanding beige fat biology.

In mice, scientists had observed for many years brown-fat like cells embedded in WAT, but little had been known about their properties, development, and activity, primarily due to technical limitations. To better understand the role of PRDM16 in the development and activity of these cells, Dr. Spiegelman and his colleagues developed new methods to isolate and characterize beige fat cells. These techniques allowed the team to carefully define the program of genes that is turned on specifically in mouse beige fat cells. Dr. Spiegelman's group then genetically modified mice to lack PRDM16 in brown and white fat cells (while maintaining normal PRDM16 levels elsewhere in the body), and found that the resulting mice lost the ability to induce beige fat cells in subcutaneous WAT, which is found just under the skin. Importantly, in contrast to the effects seen in beige fat tissue, the BAT did not appear to be affected by loss of PRDM16 in this model, indicating that any physiological effects were due specifically to loss of beige fat.

Further examination of the subcutaneous WAT of mutant mice under these conditions revealed that the loss of induction of beige fat cells was accompanied by a reduction in overall energy expenditure. Mutant mice that were fed a high-fat diet gained more weight over time than did normal mice. Some regions of subcutaneous fat in these mutant mice were up to twice as large as those in their normal counterparts, but the mass of visceral WAT, which surrounds internal organs, was not altered. Mutant mice also developed insulin resistance and elevated fat accumulation in the liver. Together, these findings demonstrated that, in mice, PRDM16 in WAT is required for the induction of beige fat cells, and these cells are important for metabolic health; a loss of PRDM16 impairs the "browning" of subcutaneous WAT, leading to obesity, insulin resistance, and other harmful metabolic effects when mice are fed a high-fat diet. Researchers are currently working to develop the appropriate tools and techniques to determine if humans also possess beige fat cells, and if so, how to induce their presence for potential metabolic benefit.

Meteorin-like: Linking Exercise to Metabolism

Based on these and other studies, Dr. Spiegelman reasoned that activation of beige fat could improve metabolic health in human beings. However, relatively little had been known about the molecular triggers

that induce the production and activity of these calorie-burning cells. Therefore, Dr. Spiegelman and his team sought to identify molecular signals that could promote the induction of beige fat.

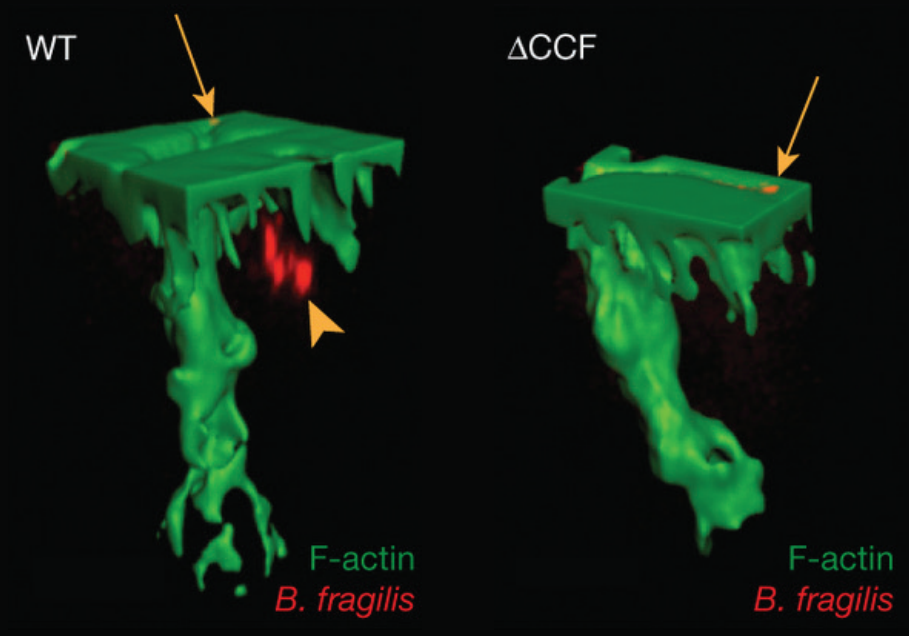
The protein PGC-1α is known to be critical in muscle tissue for mediating some of the molecular effects of exercise (*e.g.*, increased mitochondrial production, breakdown of fatty acids, promotion of blood vessel development). Dr. Spiegelman's group found that one specific version of this protein, called PGC-1α4, when experimentally turned on at high levels in muscle tissue, can cause mice to become lean and more muscular. Surprisingly, PGC-1α4 elevations in muscle also turned on beige genes in WAT, suggesting that there was some communication between muscle and fat tissues that resulted in this "browning" phenomenon. Molecular tools were developed and used to search for the signal that was being sent from the muscle cells containing high levels of PGC-1α4, and the researchers identified the little-known protein hormone Meteorin-like (or "Metrnl") as an important mediator of this communication. Interestingly, Dr. Spiegelman's team also found that, in both mice and humans, exercise leads to the production of Metrnl in the muscle and its release into the bloodstream.

To better understand Metrnl's function, the researchers generated experimentally modified mice that, in the absence of exercise, could produce the hormone—not in their muscles, but in their livers—and secrete the protein into the bloodstream. In these mice, they observed characteristic "browning" of WAT, similar to that seen in mice with high levels of PGC-1α4. The mice with liver-produced Metrnl also were protected from some of the negative effects of a high-fat diet: they exhibited improved glucose (sugar) tolerance and increased energy expenditure (a physiological feature that is associated with thermogenesis). These results showed that, in mice, Metrnl signals WAT to produce physiologically active beige fat cells. An important focus of Dr. Spiegelman's ongoing research is the development of a therapeutic form of the Metrnl hormone that, in humans, could help in weight loss or maintenance and improve metabolic health by triggering the "browning" of WAT.

Conclusions

Dr. Spiegelman's research has revealed mechanisms by which beige fat is induced in WAT, leading to the "browning" phenomenon that helps protect mice from obesity and type 2 diabetes. In his talk, he described how exercise can lead to the production in muscle tissue and secretion into the bloodstream of the protein Metrnl, a hormone that acts upon WAT to induce beige fat production. Dr. Spiegelman's group previously showed that a completely different hormone, called irisin, is similarly produced by muscle in response to exercise and can also promote the "browning" of WAT. However, the cellular processes by which these two hormones exert their effects on WAT are quite different, suggesting that multiple pathways work to trigger beige fat development. Once these signals from muscle tissue are received by WAT, proteins like PRDM16 are required in mice for the "browning" phenomenon; loss of PRDM16 hinders the production of beige fat cells, thereby preventing their ability to help protect the animal from metabolic disease.

Dr. Spiegelman's discoveries have illuminated multiple molecular pathways involved in brown and beige fat production. If similar pathways are employed in humans, they could be exploited for the development of potential new therapeutics to combat obesity, type 2 diabetes, and other metabolic diseases.



The gut is home to trillions of bacteria that play numerous roles in human health; understanding how the different bacterial species interact with each other and survive the harsh gut environment is important to therapeutics aimed at establishing healthy gut microbial communities. This image shows three-dimensional reconstructions of tiny pockets in the intestinal wall called colon crypts (green). (Left panel) While normal gut bacteria called *Bacteroides fragilis* (*B. fragilis*) (red) can be detected on the surface (arrow) and within the colon crypt (arrowhead) of mice, (Right panel) *B. fragilis* lacking "commensal colonization factors" can only be detected on the surface (arrow). As described in this chapter, these newly identified factors may help certain bacterial species establish themselves and survive in protective spaces within the gut.

Image courtesy of Dr. Sarkis Mazmanian, California Institute of Technology. Adapted by permission from Macmillan Publishers Ltd: Nature, Lee SM, Donaldson GP, Mikulski Z, Boyajian S, Ley K, Mazmanian SK. Bacterial colonization factors control specificity and stability of the gut microbiota. 501:426-429, copyright 2013.

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 a total of 72 million ambulatory care visits to physicians' offices, and hospital emergency and outpatient departments with a primary diagnosis of digestive diseases in the United States.[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 visits with a primary diagnosis of digestive diseases and a total charge of $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 cellular 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 people 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

[1] *Everhart JE and Ruhl CE. Burden of Digestive Diseases in the United States Part I: Overall and Upper Gastrointestinal Diseases. Gastroenterology 136: 376-386, 2009.*

[2] *Myer PA, et al. Clinical and Economic Burden of Emergency Department Visits Due to Gastrointestinal Diseases in the United States. Am J Gastroenterol 108:1496-1507, 2013.*

example, irritable bowel syndrome (IBS) causes pain and constipation or diarrhea. IBS more frequently affects women, who may display a different range of symptoms and respond differently from men to pharmacologic treatments for the disease. While diet and stress contribute to this disorder, its underlying causes are unknown. Gastroesophageal reflux disease, in which stomach acids rise up into the esophagus, is a common functional bowel disorder that can lead to a condition known as Barrett's esophagus. This condition, in which cells lining the esophagus turn into an intestinal type of cell, is associated with a heightened risk of esophageal cancer—one of the cancer types still on the rise in the United States. Gastroparesis, another type of functional bowel disorder, is characterized by delayed emptying of food from the stomach, resulting in nausea, vomiting, and abdominal discomfort. While many cases of gastroparesis are of unknown origin, a common cause is diabetes, which is thought to damage nerves leading to the stomach and controlling movement of food. Sphincter of Oddi dysfunction is a disorder marked by attacks of abdominal pain and is often found in individuals who have had their gallbladders surgically removed. Fecal incontinence, or impaired bowel control, is another bowel disorder that poses a major public health burden. Although fecal incontinence is more common in older adults, it can affect people of any age. Because it is difficult to talk about, many people suffer without seeking professional treatment for this surprisingly prevalent condition. Researchers thus aim both to examine barriers in addressing fecal incontinence and to develop improved treatment strategies.

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

The microorganisms that inhabit the GI tract, termed the gut "microbiome," are important factors in maintaining or tipping the balance between digestive health and disease. These microbes can affect long-term health and nutritional status in some surprising ways, depending on their interactions with each other, with intestinal cells, and with nutrients ingested by their human host. Scientists are gaining insights into the ways these GI microorganisms 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. 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 such as hepatitis B and C, or by genetic mutations such as alpha-1-antitrypsin deficiency; others arise from diverse factors such as autoimmune reactions, drug toxicity, and other triggers, some of which are unknown. Many liver diseases, such as chronic hepatitis B and C, place individuals at elevated risk for developing liver cancer. A healthy liver is necessary for life, and the only treatment for end-stage liver disease is a liver transplant. Because the number of livers available from deceased donors is limited, research is critical to identify liver disease early, find methods to preserve liver function in people with liver disease, and develop and further study new treatment options, including experimental, cell-based approaches to liver regeneration.

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

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

GUT MICROBIOME AND HEALTH

A "Who's Who" of the Gut Microbial Community: Their Origins and Health Effects: Researchers have used new and creative methods to identify specific bacterial strains from the microbial communities of humans, other organisms, and habitats on land and sea; learn how they colonize the gut; and determine their impacts on host health and disease. The human gut is home to an estimated 100 trillion bacterial cells, the composition of which varies greatly between individuals. Unraveling the mystery of which microbes are present in the gut, how they come to reside there, and their health implications for the host is the subject of recent investigations by one research group.

One study focused on the question of how microbes colonize the mammalian gut. Scientists used the inventive approach of testing how microbes from non-native sources compete for resources and space in the intestinal environment. The scientists transplanted male mice raised under sterile conditions with microbes from a wide range of sources, including the guts of humans, zebrafish, and termites; skin and tongue of humans; or from the soil or a marine estuary. They conducted successive stages of experimentation in which microbes were shared between the original transplanted mice and other mice, using transplantation of intestinal contents and/or co-housing in the same cage. Over a few weeks, they collected fecal samples from the mice and analyzed the genetic material to identify bacterial species present. In their primary set of experiments, they set up mixed groups of transplanted mice rooming together, with some surprising results. For example, one group living arrangement consisted of four mice—one colonized by soil microbes, a second by termite gut microbes, a third by zebrafish gut microbes, and a fourth without any microbes. In this setup, the gut microbes of the four mice quickly came to resemble one another, with species from the soil and zebrafish gut dominating early on; however, after a week, the soil sample-derived microbiome became predominant in all the cohabitating animals. A single type of soil bacteria, a previously unidentified *Ruminococcus*, proved particularly successful at seizing the opportunity to

colonize the mouse gut, likely aided by its ability to process multiple types of carbohydrates in the gut. In another experiment, mice were transplanted with gut microbes from humans living in three very different environments (urban United States, rural Malawi, and the Venezuelan state of Amazonas, with its large population of indigenous peoples). The mice were then co-housed to develop a roughly average mix of human microbes. The mice harboring a composite human gut microbial mix were then housed in a cage with both mice transplanted with a composite of mouse gut microbes and mice without any gut microbes. Early on, human gut microbes dominated, even in the guts of mice with native mouse gut microbes. After 4 days, the mouse microbes were starting to overtake the invaders from the human gut, though the human gut microbes remained detectable weeks later. These experiments help to define the "succession" of bacterial species as they come to colonize sequentially and compete with each other in the unique environment of the mammalian gut. In addition, the researchers also measured some of the molecules related to bacterial (and host) metabolism, such as carbohydrates, short chain fatty acids, and bile acids, which help these species succeed in outcompeting others in the gut.

Another research project took a new approach to the problem of conducting a census of gut bacteria and providing valuable insights into their health effects. The scientists used combinations of gut microbes harvested from human stool samples and tested them in male mice raised under sterile conditions to be free of any microbes. Two weeks after transplanting the human gut microbes into the mice, they measured increases in a type of immune cell that prevents inappropriate inflammation in the gut, but they also saw an increase in fat deposits (adiposity). Using one of the human donors' samples as a representative, the researchers sequenced the bacterial genomes present. They identified 17 unique bacterial strains that, when given to mice, showed effects on immune cells and adiposity similar to the effects of the initial bacterial transplants. To find out which specific combinations of bacterial strain subsets were responsible for these effects, the researchers gave 94 different combinations of the bacteria as well as single bacterial strains to the mice. They then measured immune cells, adiposity, and products of nutrient metabolism, such as bile acids, fatty acids, and amino acids, and compared the results to measurements of the same elements in control mice that remained bacteria-free. Through these experiments, they identified which bacteria, alone or in combination, promoted these immune and metabolic functions. For example, they found several bacterial strains were associated with increased adiposity, including five strains in the bacterial group *Bacteroides*, two strains of *Bacteroidetes*, and *Escherichia coli*. Many of the same *Bacteroides* strains were also associated with expansion of the population of immune cells called regulatory T cells in the intestine.

These studies add to the storm of new knowledge about the mammalian gut microbial community, in terms of understanding the succession of species during colonization and teasing out effects of individual bacterial strains. This work also provides a new means for scientists to identify which resident gut microbes are helping or hindering their human hosts, in terms of key health indicators such as immune function, nutrient metabolism, and fat mass. These methods could be used in the future to identify probiotics or prebiotics—beneficial bacteria or the nutrients they rely on—to enhance human health and limit disease.

Seedorf H, Griffin NW, Ridaura VK,…Gordon JI. Bacteria from diverse habitats colonize and compete in the mouse gut. Cell 159: 253-266, 2014.

Faith JJ, Ahern PP, Ridaura VK, Cheng J, and Gordon JI. Identifying gut microbe-host phenotype relationships using combinatorial communities in gnotobiotic mice. Sci Transl Med 6: 220ra11, 2014.

Evaluating Treatments for Childhood Malnutrition Based on Changes in Gut Bacteria: A team of U.S. and Bangladeshi scientists discovered that children who are malnourished do not harbor gut bacteria typical for their age, even several months after receiving a nutritional intervention. The persistence of this "immature" collection of gut bacteria may be a reason why the children do not grow well even after receiving nutrient-dense food. Moderate or

severe forms of malnutrition affect a large number of children living in developing countries, such as Bangladesh. This malnutrition typically develops very early in life, between 3 and 24 months. Therapeutic interventions, such as the use of either a specially prepared, nutrient-dense processed food or indigenous foods high in calories and protein, have short-term, but not lasting, effects in terms of sustaining healthy growth and development. The community of microbes inhabiting the gut is known to play an important role in assisting their human host with many important functions, such as extracting nutrients from the diet and facilitating new blood vessel formation in the intestine. Scientists wondered whether malnutrition early in life might disrupt the establishment of a healthy, diverse microbial community, thereby limiting the impact of subsequent nutritional interventions. To test this idea, they identified the different microbial species present in fecal samples collected from children living in an impoverished urban area of Dhaka, Bangladesh. Using a technique that distinguishes among different types of bacteria, they were able to identify which bacterial species were present. They first used this method to characterize the microbial makeup of a group of children showing healthy growth and development for their age living in this part of the world. They confirmed this model of a healthy, or "mature," microbial community in another group of local, healthy children. Then, they compared them to severely malnourished children who were treated in a hospital in Dhaka for a few weeks with either a standard ready-to-use therapeutic food or a locally produced combination of rice and lentils with other nutrients. While both nutritional interventions improved growth of the malnourished children, they remained underweight and below normal height compared to healthy children. Fecal samples taken from the malnourished children before, during, and after treatment revealed a persistent immaturity in their gut microbial communities, such that they lacked bacterial species typically found in well-nourished children of a similar age. These findings suggest new strategies that might be used to improve the treatment of childhood malnutrition by bolstering the health of the gut microbial community, such as through more prolonged dietary interventions or development of targeted probiotics.

Subramanian S, Huq S, Yatsunenko T,…Gordon JI. Persistent gut microbiota immaturity in malnourished Bangladeshi children. Nature 510: 417-421, 2014.

UNDERSTANDING GUT-MICROBIOME INTERACTIONS

How a Species of Bacteria Makes Its Home in the Gut: Researchers have identified a group of microbial genes that allow a species of bacteria to take up residence in a certain area of a mouse's gut. In both mice and humans, the gastrointestinal tract, especially the colon, is home to hundreds of different species of bacteria that are important for digestive health. While several studies have examined how certain gut microbes can trigger or protect against the inflammation that leads to diseases such as Crohn's disease or ulcerative colitis, it is not clear how bacteria grow and survive in the turbulent environment of the gastrointestinal tract. It is also not certain how species of bacteria interact with each other within the gut—an important factor to consider when developing probiotic therapies with the intent of establishing a healthy gut microbial community.

Researchers attempted to answer these questions by examining how a species of bacteria found in large numbers in the gut of humans and other mammals, called *Bacteroides fragilis* (*B. fragilis*), colonizes the gastrointestinal tract in a mouse model. The scientists introduced *B. fragilis* into "germ-free" male and female mice raised under sterile conditions and allowed the bacteria to colonize the gut. When the scientists then introduced a similar yet different species of bacteria to these mice, both bacterial species were able to co-exist in the gut. However, when the scientists tried to give more *B. fragilis* to mice that already had this species, the newly introduced bacteria could not establish themselves. This led the scientists to conclude that *B. fragilis* prefers a certain limited area, or niche, in the gut, and once it saturates this area, then no more *B. fragilis* can settle there. To explore this idea, the scientists examined the linings of the guts in the mice. They found that *B. fragilis* preferred to grow within small pockets in the intestinal wall called crypts, which

may act as "rooms" that, once occupied, resist further colonization by additional *B. fragilis*. Using a genetic screen, the scientists were able to identify a set of genes that becomes activated when *B. fragilis* colonizes the gut. These genes are necessary for the bacteria to occupy the crypts and to exclude additional bacteria of the same species beyond a certain capacity. When these genes were deleted from the bacteria, the bacteria could no longer take up residence in the crypts or prevent other, normal *B. fragilis* from growing there. Bacteria missing these genes were also more susceptible to being cleared away by an antibiotic than wild-type bacteria, suggesting that the crypts may also serve a protective role for the microbes growing in them. This study provides insight into how certain species of bacteria may establish themselves and survive in the gut. Further research on how beneficial bacteria colonize the gut in humans may help in the design of probiotic or antibiotic therapies for treating digestive diseases.

Lee SM, Donaldson GP, Mikulski Z, Boyajian S, Ley K, and Mazmanian SK. Bacterial colonization factors control specificity and stability of the gut microbiota. Nature 501: 426-429, 2013.

Microbial Secretion Regulates Intestinal Immune Function: Research has uncovered one way in which beneficial microbes in the intestine support healthy immune function in their hosts—by releasing substances called sphingolipids that keep the activity of local immune cells in check. The "symbiotic" microbes in the human intestine, whose presence benefits both parties, include members of the *Bacteroidetes* phylum, such as *Bacteroides fragilis* (*B. fragilis*), which release unique molecules called sphingolipids. These molecules act on a type of host immune cell called the invariant natural killer T (iNKT) cell, the function of which is to quickly activate the immune system by releasing a flood of chemicals called cytokines. Researchers wondered whether sphingolipids produced by beneficial bacteria might protect against intestinal inflammation from an overactive immune system. They explored this idea in both male and female mouse models and in cells grown in laboratory culture. They raised mice to have either normal *B. fragilis* in their intestines or a mutated form of the bacteria that could not produce sphingolipids. Comparing these two groups of mice, those with normal *B. fragilis* had reduced numbers of iNKT cells. When these mice were later challenged with a chemical that causes colonic inflammation similar to human ulcerative colitis, those harboring the mutated bacterium developed more severe disease than mice colonized with the unaltered bacterium capable of releasing sphingolipids. Purifying the sphingolipids produced by these bacteria enabled the researchers to observe their direct effects on cells in culture: the sphingolipids dampened iNKT cell activation. When the researchers gave the colitis-inducing chemical to mice harboring the mutated bacterium and then treated these mice with purified sphingolipids at birth, they saw that the sphingolipids restored protection against colitis development later on in life. These studies show how early exposure to beneficial intestinal bacterial products can offer life-long protection against inappropriate immune activation and inflammation through regulating the activity of the host immune system. These bacterial products may be useful in treating autoimmune and allergic disorders that appear later in life.

An D, Oh SF, Olszak T,…Kasper DL. Sphingolipids from a symbiotic microbe regulate homeostasis of host intestinal natural killer T cells. Cell 156: 123-133, 2014.

Gut Protein Punches Holes in Bacteria: A protein produced by intestinal cells kills certain bacteria by creating small holes in the microbes' protective outer membranes. The human gut is home to trillions of bacteria that play an important role in digestive health. In some cases—such as inflammatory bowel disease—the body's immune system reacts inappropriately to these microbes, irritating the intestinal lining. One way the body avoids this unwanted response is to limit contact between bacteria and the intestinal wall by killing any bacteria that get too close to the intestinal lining. However, it was not certain how the body accomplishes this.

A team of scientists tackled this problem by investigating proteins secreted by intestinal cells that are able to kill bacteria. The researchers focused on a protein called "RegIIIα," which was previously found to be lethal to certain bacteria, although it was not certain how

the protein actually killed the microbes. Additionally, RegIIIα is produced by intestinal cells when bacteria come in close contact with the lining of the gut, which suggests that RegIIIα could be used as a defense against intruding microbes. While investigating how RegIIIα kills bacteria, the scientists found that adding it to bacteria allowed a traceable dye to diffuse into the bacterial cells, which meant RegIIIα had caused the bacteria's protective outer membrane to become leaky—something that can easily be fatal to most microbes. They saw the same leakiness when RegIIIα was added to artificial membranes made up of the same components that are in bacterial membranes. Other experiments showed that RegIIIα was creating tiny pores about one millionth of a millimeter in diameter in these membranes. The scientists used X-ray crystallography and electron microscopy to visualize the pores, and they found that each one was made up of six RegIIIα proteins that assemble to form a doughnut-shaped hole. This means that if a microbe gets too close to the intestinal wall, RegIIIα proteins produced by gut epithelial cells can insert themselves into the microbe, puncturing and killing it. The deadly effects of RegIIIα were not seen with all bacteria, however; some types of microbes have an extra chemical on their membrane surface that can protect them from this type of attack.

These findings show how the RegIIIα protein secreted by intestinal cells is part of an elaborate defense mechanism that helps create a bacteria-free buffer zone between gut bacteria and the intestinal wall, potentially preventing unwanted inflammatory reactions. Further research may shed light into how this system might break down during disease and, importantly, how it might be harnessed to prevent illness.

Mukherjee S, Zheng H, Derebe MG,…Hooper LV. Antibacterial membrane attack by a pore-forming intestinal C-type lectin. Nature 505: 103-107, 2014.

INVESTIGATING GUT INFLAMMATION

Origins of Gut Inflammation Found in Specific Cell Type: Scientists have discovered that processes taking place within a particular intestinal cell type called the Paneth cell are linked to the development of inflammation in a portion of the small intestine in mice. The resulting inflammation is similar to that seen in a form of human Crohn's disease. Crohn's disease, a form of inflammatory bowel disease that can affect the small or large intestines, is thought to result from an interplay between genetic susceptibility factors, such as those that affect immune function, and environmental factors, such as gut microbes. For example, one genetic mutation associated with Crohn's disease occurs in the *ATG16L1* gene, which is linked to an important process called "autophagy," or "self-eating." Cells use autophagy to reduce cellular stress by degrading and recycling extraneous or malfunctioning components. This genetic mutation also causes dysfunction in Paneth cells, which are known primarily for protecting against harmful gut microbes by secreting antimicrobial molecules. Why autophagy or Paneth cell dysfunction might be important in Crohn's disease, however, was unknown.

A team of researchers delved into how problems with processes such as autophagy occurring in intestinal cells might relate to the development of gut inflammation. Working with a series of mouse models genetically engineered to lack certain proteins important for autophagy and other processes in their intestinal cells, the team showed that turning off both the *Atg16L1* gene and a gene involved in monitoring proper protein structures resulted in reduced autophagy and increased stress in Paneth cells. Additionally, these animals exhibited a severe, spontaneous form of inflammation in the small intestine that closely mimicked cases of human Crohn's disease. This study sheds new light on the key cellular and genetic factors, and processes such as autophagy, that are involved in intestinal inflammation, particularly in Crohn's disease occurring within the small intestine. These findings offer important clues that may lead to better diagnosis and management of this form of inflammatory bowel disease in the future.

Adolph TE, Tomczak MF, Niederreiter L,…Blumberg RS. Paneth cells as a site of origin for intestinal inflammation. Nature 503: 272-276, 2013.

Studies Identify Factors Associated with Intestinal Inflammation: In an effort to understand the causes of inflammatory bowel disease, scientists have identified several genes and types of bacteria that are associated with these diseases in the human gut. Inflammatory bowel diseases (IBD) include conditions such as Crohn's disease and ulcerative colitis, which are characterized by symptoms such as diarrhea, intense abdominal pain, and weight loss. While the exact causes of IBD are not known, inflammation in the gut is believed to result from an inappropriate reaction of the cells lining the intestine to some of the trillions of bacteria that inhabit the digestive tract. Genetics also plays a large role in the development of IBD, as there have been over 160 areas of the human genome that have been identified to contain risk factors. Despite these known links, however, it is not clear which bacteria, and which specific genes, are important for the initiation and development of IBD.

To address these questions, a group of researchers set out to identify IBD-specific changes in the bacterial community in the human gut, along with changes in gene activity within the gut cells. They focused on the area of the small intestine closest to the colon, called the ileum, which is believed to be a primary site where Crohn's disease originates. The researchers took biopsies from ilea of male and female children and adolescents who had either Crohn's disease or ulcerative colitis. They then identified the bacteria in the samples and analyzed the genes that were activated in the human ileal cells. They compared the results to biopsies from individuals who did not have IBD. The researchers found that those with Crohn's disease or ulcerative colitis had higher levels of a type of bacteria called Proteobacteria and an increase in the activity of a gene called *DUOX2*. They also found that people with Crohn's disease had lower amounts of a type of bacteria called Firmicutes and lower activity of a gene called *APOA1*. This means people with IBD have a particular microbial and genetic "signature" that could provide targets for improved diagnosis and therapy. This signature could also be studied further to better understand these diseases.

Another group of researchers attempted to identify the bacteria associated with IBD by determining which bacteria are coated with a type of "antibody" or immune protein, called IgA, that the body produces to protect itself from foreign substances. IgA is present in the mucus layer covering the inside of the intestines, where it attaches to disease-causing bacteria and neutralizes them. First, the researchers used a model of colitis with female and male mice to determine if the degree of IgA coating can identify bacteria causing the disease. They found that the mice with colitis had more IgA-coated gut bacteria compared to normal mice, and the most highly coated bacteria were the species that were causing colitis in these animals. Next, the researchers analyzed human fecal samples and found that, like the mouse model, individuals with IBD had higher levels of IgA-coated gut bacteria than healthy controls. When germ-free mice with chemically induced colitis were colonized with bacteria from the human samples, only the bacterial species that had been highly coated with IgA caused severe intestinal inflammation and bleeding. The bacteria that had been poorly coated with IgA had no effect. This means the amount of IgA coating on gut bacteria may pinpoint which species are most likely to trigger an inflammatory response, and targeting those bacteria may be an effective strategy for treating IBD.

Together, these studies shed light on the complicated origins of IBD. Similar studies may identify more factors that are involved, which could lead to new diagnostic tools and treatments for these painful and debilitating diseases.

Haberman Y, Tickle TL, Dexheimer PJ,…Denson LA. Pediatric Crohn disease patients exhibit specific ileal transcriptome and microbiome signature. J Clin Invest 124: 3617-3633, 2014.

Palm NW, de Zoete MR, Cullen TW,…Flavell RA. Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. Cell 158: 1000-1010, 2014.

INSIGHTS INTO FUNCTIONAL BOWEL DISORDERS

New Role in Bowel Function Discovered for Familiar Protein: In a recent mouse study, scientists found that a well-known anti-cancer protein called

retinoblastoma 1, or RB1, plays a surprising role in gastrointestinal motility. Embedded in the lining of the mammalian gastrointestinal tract is a mesh-like system of nerves called the enteric nervous system. These nerves send signals to gut muscles to contract and relax in a synchronized fashion, pushing ingested food through the intestines and enabling stool to pass normally. Failure of the enteric nervous system to communicate with the intestinal muscles could lead to gastrointestinal motility disorders. For example, in people with Hirschsprung's disease, nerves are missing from portions of the intestine, so the gut muscles do not work properly, and the contents of the intestines become stuck. In many other gastrointestinal motility disorders, the enteric nervous system is intact, but for unknown reasons it is not functioning properly.

Working with a mouse model, researchers found that RB1 may be important for the proper function of the enteric nervous system. Because RB1 is best known for its critical role in preventing tumors from forming, the scientists initially intended to study RB1's importance in the development of skin cancer. However, when the researchers deleted RB1 in certain cells of mice, they found, unexpectedly, that the mice developed severe intestinal blockages that were similar in some ways to the bowel obstructions experienced by humans with Hirschsprung's disease. The researchers examined the intestines of the mice and found that the enteric nervous system was still present, but it was disorganized, and many nerve cells were much larger than those in the control mice. In particular, the nerve cells that produce nitric oxide, a factor important for muscle relaxation, were especially affected by the loss of RB1—these cells had large, irregular nuclei (the part of the cell that contains DNA) and were unable to divide. These affected nerve cells also produced too much nitric oxide, which inhibited muscular contractions and motility in the small intestines. This study suggests that the RB1 protein is important for proper function of the enteric nervous system in mice, and it may help to understand the causes of gastrointestinal motility disorders in humans.

Fu M, Landreville S, Agapova OA…Heuckeroth RO. Retinoblastoma protein prevents enteric nervous system defects and intestinal pseudo-obstruction. J Clin Invest 123: 5152-5164, 2013.

Molecular Interactions Offer Clues to Congenital Form of Diarrhea: Scientists have uncovered the molecular workings behind a rare but severe form of inherited diarrheal disease in newborns. The rare disease, known as microvillus inclusion disease (MVID), affects some individuals of European, Middle Eastern, and Navajo American Indian descent, resulting in chronic diarrhea in newborns for which there is no treatment apart from intravenous nutrition or intestinal transplant. This genetic disease is marked by reduced sodium absorption into intestinal cells thought to be caused by the loss of tiny, finger-like projections called microvilli on the inner surface of cells lining the intestine and by gaps between the cells. However, understanding of the molecular processes gone awry in this disease remained murky. Researchers used intestinal cells grown in laboratory culture and intestinal samples biopsied from Navajo patients with a particular form of MVID gene mutation to carry out their investigations of this disease. By examining the samples from patients, they identified defects in intestinal structure and organization that occur with this disease, and they then used the laboratory-grown intestinal cells to investigate the underlying molecular mechanisms. For these studies, they genetically altered the intestinal cells to reduce production of a protein called myosin Vb (MYO5B), mimicking the mutation found in the Navajo patients with MVID. MYO5B acts as a molecular "motor," helping move other proteins within the cell to where they need to go. In both the cultured cells and patient samples, the reduction in functional MYO5B resulted in a loss of the microvilli, altered the levels of proteins that are normally in the junctions between the cells, and misdirected several proteins within the cell. Two of these proteins, RAB8A and RAB11A, were shown to play an important role, working together with MYO5B to maintain the microvilli. The loss of interactions among these proteins led to changes to the microvilli and distribution of cellular contents, characteristic of

MVID. These changes in turn profoundly affected the absorption and transport of items such as sodium, which typically helps to balance the movement of other electrolytes and fluids in and out of cells. These studies shed light on the complex molecular interactions in intestinal cells that are derailed by the mutations causing MVID, leading to severe diarrhea. These molecules and their functions may provide targets in the future for treating this severe diarrheal disease in susceptible newborns.

Knowles BC, Roland JT, Krishnan M,...Shub MD. Myosin Vb uncoupling from RAB8A and RAB11A elicits microvillus inclusion disease. J Clin Invest 124: 2947-2962, 2014.

Endoscopic Procedure for Sphincter of Oddi Dysfunction Fails To Reduce Abdominal Pain: In a clinical trial to examine a procedure used in clinical practice with the intent of relieving pain after gallbladder removal, researchers have found that this procedure, which carries considerable risk, may not be effective. The gallbladder is often removed to treat conditions such as chronic gallstones, local inflammation, or pain that is suspected to originate in the gallbladder or bile ducts. Patients occasionally experience recurrent abdominal pain after this surgery, but in many cases the source of the pain is not clearly established. One suspect has been a condition called sphincter of Oddi dysfunction (SOD). Although not proven, it has been suggested that this condition is caused when the sphincter (or circular muscle) that allows bile and pancreatic juices to flow into the intestine does not relax properly. To remedy SOD, patients typically undergo a procedure called sphincterotomy, in which a tube with a small camera is inserted through the mouth and into the intestine, and the sphincter is cut open. Sometimes an additional procedure is carried out to measure pressure in the sphincter. However, the suggested benefits of these procedures are controversial, and they carry a substantial risk of significant complications, including pancreatitis or perforation of the bowel wall.

In an attempt to address the uncertainty surrounding the treatment of SOD, a study was conducted across seven clinical centers to see if sphincterotomy actually reduced pain following gallbladder surgery. The trial included over 200 participants who experienced recurrent abdominal pain after their gallbladders were removed. The participants underwent either sphincterotomy or a mock procedure (where the camera was inserted but the sphincter was not cut) to treat their suspected SOD. In addition, pressure was measured in the sphincter using a standardized method. While both groups of participants experienced a reduction of pain severity, sphincterotomy did not reduce abdominal pain compared to the mock procedure. Additionally, between 11 and 15 percent of the participants developed pancreatitis after these procedures, underscoring the risk of complications that may occur as a result of the invasive operations. Furthermore, the sphincter pressure measurements had no correlation with the outcomes, which calls into question the idea that high pressure in the sphincter is the cause of symptoms in these patients. The results of this trial suggest that sphincterotomy does not improve pain in cases of suspected SOD following gallbladder removal—information that could save patients from the burden of this unnecessary and risky procedure.

Cotton PB, Durkalski V, Romagnuolo J,...Robuck P. Effect of endoscopic sphincterotomy for suspected sphincter of Oddi dysfunction on pain-related disability following cholecystectomy: the EPISOD randomized clinical trial. JAMA 311: 2101-2109, 2014.

Complex Factors Influence Experience of Irritable Bowel Syndrome: Two analyses of data from a clinical study on irritable bowel syndrome (IBS) outcomes point to several key determinants of quality of life and feeling healthy in individuals affected by this syndrome. IBS is a type of functional gastrointestinal disorder marked by abdominal pain, as well as diarrhea, constipation, or both; it more frequently affects women than men. The NIDDK-sponsored Irritable Bowel Syndrome Outcome Study is a multi-center, placebo-controlled randomized clinical trial with the goal of determining whether self-administered cognitive behavioral therapy is as helpful as standard therapy with a therapist in reducing IBS symptoms and overall burden. In addition to this primary aim, researchers

have used the data generated by the study to understand the impact of specific factors on the experience of women and men living with IBS.

Recognizing the strong mind-body connection thought to underlie conditions such as IBS, researchers investigated how even the anticipatory fear of IBS symptoms might affect the quality of life experienced by people with moderate to severe IBS. They administered multiple surveys to 234 study participants (nearly 80 percent of whom were women), including those designed to measure fear of future gastrointestinal symptoms, IBS symptom severity, and quality of life. Then, they quantified and analyzed the responses statistically to determine associations between these self-reported measures. The fear of IBS symptoms had a large impact on reducing individuals' day-to-day quality of life, even more so than the symptoms themselves. This finding suggests that greater attention to the major role that fear of future IBS symptoms plays in quality of life for a person with this disorder can help health care providers provide more effective care.

Another analysis of data from the study focused on the relationship between several psychosocial or physical factors and how highly people with IBS rated their health—considered an accurate predictor of their future health outcomes, such as disability or health care usage. Participants rated their health on a scale ranging from poor to excellent and simultaneously completed surveys to measure a range of possible contributors, including their IBS symptom severity, quality of life, abdominal pain, fatigue, stress, depression, anxiety, negative interactions, and social support. Their responses were quantified and analyzed statistically to identify associations. The researchers found that factors such as stress, depression, and anxiety were associated with a perception of being in worse health in those with IBS. Surprisingly, as with the other analysis, the severity of IBS symptoms played a lesser role in participants' self-assessments of their overall health. This analysis captures some of the complexity that factors into how individuals with IBS perceive their own health. Physician awareness of this complexity could help to improve the doctor-patient relationship, as well as patient satisfaction and compliance with medical care.

Lackner JM, Gudleski GD, Ma CX, Dewanwala A, Naliboff B; Representing the IBSOS Outcome Study Research Group. Fear of GI symptoms has an important impact on quality of life in patients with moderate-to-severe IBS. Am J Gastroenterol 109: 1815-1823, 2014.

Lackner JM, Gudleski GD, Thakur ER, Stewart TJ, Iacobucci GJ, and Spiegel BMR; Representing the IBS Outcome Study Research Group. The impact of physical complaints, social environment, and psychological functioning on IBS patients' health perceptions: looking beyond GI symptom severity. Am J Gastroenterol 109: 224-233, 2014.

Chance and Prepared Minds Lead from Lab to New Drug Development

Science rarely moves in a straight line—like a good page-turner, the story of scientific discovery is often full of twists and turns, dead ends and red herrings, and then a sudden burst of insight, sometimes from an unexpected source. For all the painstakingly prepared proposals and long hours spent at the lab bench or clinic, scientists often speak of serendipity as playing an essential part in their tales of scientific discovery. And these discoveries, originally directed at a specific biomedical question, can at times inspire an answer to another, seemingly unrelated problem. One story with all of these elements of surprise is that of an unexpected scientific journey spanning more than 2 decades. It begins with a basic research discovery in adult intestinal cells and arrives those decades later at new opportunities for improved treatments against diseases at multiple sites throughout the human body, including two recently approved drugs for hemophilia.

From Failure, Fortune

On July 22nd, 1992, NIDDK grantee Dr. Richard Blumberg was working in his lab when his postdoctoral fellow came to express frustration over a failed negative control in one of his experiments. The fellow had been conducting experiments in an adult human intestinal cell line with immune molecules called antibodies, which attach to proteins on the cell surface. His negative control antibody was designed not to bind the cellular proteins that the experimental antibody was binding. But the control antibody had latched on instead to some unknown protein.

"Chance favors only the prepared mind," is a saying attributed to the microbiologist Louis Pasteur. When Dr. Blumberg looked at the result, in an instant his mind returned to his own postdoctoral training, working with a type of protein on cell surfaces called a major histocompatibility complex (MHC) class I molecule. MHC class I molecules are displayed on the cell surface to help the immune system distinguish healthy from infected cells. He experienced a flash of recognition—the control antibody was likely attaching itself to a related molecule known as the neonatal Fc receptor, or "FcRn." But, as the name of the molecule suggests, at the time it had only been found in newborn rodents, not in their adult cells, and the molecule had never been found in humans.

First discovered in the mid-1960s, FcRn was known to interact with a portion of the immunoglobulin G (IgG) antibody called the Fc domain, controlling transport of IgG across intestinal epithelial cell layers in early life in rodents. IgG is the most abundant type of antibody in the blood and extracellular spaces in internal tissues, including portions of the intestines, where it helps protect against infection. Newborn rodents receive IgG mainly from their mothers' milk *via* the aforementioned process, while in humans, IgG is transferred from mother to fetus across the placenta to confer protection.

In the years following that "failed" experiment in 1992, Dr. Blumberg's group and others confirmed that the "neonatal" Fc receptor was indeed a misnomer. The receptor continues to be produced into adulthood in a number of cell types throughout the human body. In the 1990s, they published their findings confirming FcRn in adult liver hepatocytes and intestinal epithelial cells, followed by reports in lung epithelial cells, endothelial cells that line blood vessels, and, most importantly, multiple types of cells that are involved in immunity.

They and others also uncovered a broad range of functions carried out by FcRn in humans, including carrying IgG across the placenta, transporting IgG back and forth across mucosal layers such as the intestinal and lung epithelial barriers, delivering IgG bound to a protein from an invading pathogen to alert local immune cells, and controlling the movement of IgG molecules in the circulation. The interaction between

FcRn and IgG was found to give the antibody greater stability by delaying its degradation within cells and recycling it back into the circulation. This explains why IgG lasts so much longer in the bloodstream than other proteins—for weeks rather than days or even minutes.

Translating Laboratory Successes into Clinical Solutions

For more than 2 decades, the NIDDK has supported Dr. Blumberg's research investigating and translating that important initial discovery of FcRn in adult human cells. A pilot grant from the NIDDK-supported Harvard Digestive Diseases Center enabled the lab's first experiments to pursue this finding in the early 1990s, collecting evidence confirming FcRn's presence and function in different human cell types. After that, the group had enough data assembled to successfully apply for an R01 grant awarded through the NIDDK in 1997, allowing them to delve more deeply into the immunology and cell biology of FcRn's functions in transporting IgG.

From that first moment of discovery in 1992, Dr. Blumberg recognized the translational potential of the FcRn-IgG system in adult humans. This system had the unique ability to move large molecules across mucosal layers in the intestine and lung, a property that would theoretically enable oral or inhaled delivery of drugs that would otherwise be deliverable only by injection. They started filing for a patent, which was issued in 1995.

The finding in 1996 by other groups of FcRn's role in recycling IgG in the bloodstream further deepened their interest in this system for improving drug delivery. By tethering large macromolecules, such as drugs, just to the Fc piece of IgG, which would then be transported through the body to their site of action and repeatedly recycled by the FcRn, they could achieve longer-acting drugs. An added benefit discovered by another group was that, because the body recognizes and tolerates the Fc portion of the IgG antibody, molecules fused to Fc were less likely to set off an adverse reaction by the immune system and thus would presumably be safer to use.

In 1999, Dr. Blumberg and others launched a new pharmaceutical company to pursue translation of this work. Along the way, Dr. Blumberg collaborated with many other "prepared minds" who collectively helped fuel further discoveries and move the science forward to translation in the clinic. They included his co-investigator on the R01 grant, Dr. Wayne Lencer, who brought expertise in cell biology; Dr. Blumberg's brother, businessman and physician Dr. Laurence Blumberg, who helped with the business plan and funding for their fledgling company; Dr. Tom Maniatis, a molecular biologist and cofounder of other pharmaceutical companies, who helped Dr. Blumberg and his colleagues to develop the new company; and others in academia and industry.

In 2004, Dr. Blumberg and his collaborators at the newly formed company published another important discovery. They were able to create a unique Fc fusion protein with erythropoietin (EPO), a naturally occurring hormone sometimes used to treat anemia, which could be delivered in aerosol form through a tube in the lungs of a pre-clinical animal model in non-human primates. They found that, by linking only one EPO molecule to two Fc domains, rather than the two molecules used in the past, the resulting fusion protein, which they called a "monomeric" Fc fusion protein, delivered by aerosol was longer-acting and more effective, similar to EPO injections in humans.

The team decided to focus their attention next on hemophilia, due to a pressing need for more longer-acting drugs. Adults and children with hemophilia A or B are deficient in a specific clotting factor in the blood, either Factor VIII or IX, respectively, which puts them at risk for bleeding episodes. However, replacing these factors was no easy matter. Due to their large size and short half-lives in the circulation, they were difficult to deliver in a form that was easy to use. Moreover, existing drugs required frequent intravenous infusions as needed, at least every few days, to prevent complications from bleeding episodes, such as severe bruising and bleeding into joints that sometimes leaves individuals crippled. This is especially problematic for children, limiting the use of these factors during one of the most vulnerable periods

of life. Further, these factors also sometimes elicited a harmful immune reaction in patients, though another group had recently shown how IgG-accompanied Factor VIII did not elicit such a response.

In 2007, the company that Dr. Blumberg helped found was sold to a larger pharmaceutical company, which used the Fc fusion technology and knowledge generated by Dr. Blumberg and colleagues to develop two long-acting Fc-fusion Factor VIII and Factor IX therapeutic agents for hemophilia A and B, respectively. Like the Fc-fusion EPO drug for anemia, these agents were designed with only one drug molecule attached to two Fc domains for greater staying power and effectiveness. The company performed clinical trials on these drugs, showing they were safe and effective. In 2014, these drugs were approved by the U.S. Food and Drug Administration (FDA), with Alprolix™ released as a hemophilia B treatment in March followed by Eloctate™ for hemophilia A in June. Eloctate™ allows patients with hemophilia A to go 3 to 5 days between infusions, while Alprolix™ extends the time between treatments even longer, up to 1 to 2 weeks.

Bright Horizons for Better Treatments

In addition to the hemophilia drugs based on work by Dr. Blumberg and colleagues, Fc-fusion proteins developed by other groups have been approved by the FDA since the 1990s for the treatment of other diseases, largely autoimmune in nature, such as rheumatoid arthritis and psoriasis. Antibody-based therapeutics that depend on FcRn-based biology have also shown promise against a host of other diseases, including inflammatory bowel disease, colorectal cancer, and even protection against infectious diseases such as HIV-AIDS. These versatile proteins might also be tested for other uses, including as an antidote for an adverse drug reaction and as a means to clear radioactive materials administered for imaging.

Although the hemophilia drugs based on Dr. Blumberg's and others' work are delivered by injection, Fc-fusion drugs have the potential for less-invasive delivery in the future based on their unique ability to interact with the FcRn and cross mucosal barriers like the lung and intestine. For example, patients might one day use an inhaler to deliver an Fc-fusion drug through the lung epithelial tissue to reach other disease sites throughout the body. This concept has been enabled by the team Dr. Blumberg and his colleagues assembled, as shown by successful completion of a phase I study of an inhaled Fc-fusion protein containing EPO. Researchers who have been inspired by Dr. Blumberg's work and with whom Dr. Blumberg has collaborated are also looking into using Fc fragment-coated nanoparticles as a vehicle for oral delivery of drugs that are currently administered by injection, such as insulin for diabetes, thereby improving patient comfort and compliance.

Research grants to Dr. Blumberg's group and others are continuing to support exploration of the basic biology of the Fc system and its yet-unknown discoveries, which could lead to additional clinical technologies and therapies. All in all, the future looks bright for one "failed" laboratory experiment to continue to yield fruits that benefit patients for years to come.

PANCREATITIS RESEARCH

Inflammatory Protein Linked to Pancreatitis: New research has found that a protein produced by pancreatic and immune cells has a role in the development of pancreatitis in mice. The pancreas is a small organ (6 inches long in humans) that is located behind the stomach and has many vital functions, including the generation of digestive enzymes. Typically, these harsh, powerful enzymes are inactive until they leave the pancreas and enter the digestive tract. In pancreatitis, however, the enzymes are activated prematurely, damaging the pancreas and triggering inflammation. This condition is extremely painful and can lead to serious complications such as organ failure. The progression of pancreatitis is very complicated, involving many interactions between the cells in the pancreas and the components of the immune system, and many of the factors involved are not known.

While investigating factors potentially involved in pancreatitis, a group of researchers focused on a protein called interleukin-33 (IL-33). IL-33 had not been clearly linked to pancreatitis previously but was known to be involved in several other inflammatory diseases. Using male mice as a model, the scientists found high levels of IL-33 in the pancreases of the mice when pancreatitis was induced. Much of the IL-33 appeared to originate from immune cells that had migrated into the pancreas during the onset of inflammation. To see if pancreatic cells themselves could produce IL-33 during inflammation, the pancreatic cells were removed from healthy mice and exposed to a protein that typically causes inflammation. Not only did the stimulated pancreatic cells produce IL-33, but they also reacted to IL-33 by secreting additional inflammatory proteins that attract immune cells. This means that the production of IL-33 by pancreatic cells could intensify the inflammation that occurs during pancreatitis. In support of this model, the scientists found that injection of IL-33 into healthy mice caused inflammation in the pancreas, including the migration of immune cells into the organ and the release of inflammatory factors into the bloodstream. This study implicates IL-33 in the development of pancreatitis, and it suggests that controlling IL-33 levels in the pancreas could be a plausible approach in treating this condition.

Kempuraj D, Twait EC, Williard DE, Yuan Z, Meyerholz DK, and Samuel I. The novel cytokine interleukin-33 activates acinar cell proinflammatory pathways and induces acute pancreatic inflammation in mice. PLoS ONE 8: e56866, 2013.

UNDERSTANDING AND TREATING LIVER INJURY

Animal Model with "Humanized" Liver Predicts Drug Toxicity in Human Livers: Scientists have enlisted a special type of mouse with human cells in its liver for a proof-of-concept study to predict which experimental drugs can cause liver failure and should thus not be tested in humans. As the primary spot for drug metabolism, the liver is particularly susceptible to injury from some drugs, which can result in liver failure, need for a transplant, and death. Animals such as mice are often used to test experimental drugs for any dangerous side effects before these drugs are tested in clinical trials. However, these animal models feature key physiological differences from humans that can cause a drug to be benign in the animals, yet harmful in humans. For example, in 1993, a clinical trial of a drug called fialuridine resulted in liver failure in 7 of the 15 participants, despite no indications the drug could cause liver problems when tested earlier in multiple animal models. In the recent study, a group of scientists sought a better way to test drugs for liver toxicity in the laboratory. To do this, they used "chimeric" mice whose own liver cells were mostly replaced with liver cells from human donors. Both male and female mice, as well as liver cells from both female and male donors, were used. The scientists proceeded to determine whether they could detect signs of drug-induced liver toxicity in the human, but not rodent, cells. Specifically, they tested whether this model could have been used to predict the human-specific liver failure caused by the drug fialuridine. After 4 days of treatment with the highest dose, the chimeric mice showed signs of liver toxicity, such as elevated liver enzymes, fatty liver, and cellular changes, while the control mice without any human cells did not. They then treated both the chimeric and

control mice with another drug known not to cause liver failure in humans; the lack of toxic effects showed the model could distinguish drugs that cause human liver failure from those that do not. This study demonstrates the utility of this kind of animal model with a humanized liver for screening experimental drugs. Use of these pre-clinical animal models could reduce the chances of exposing volunteers in clinical trials to experimental drugs that can cause acute liver failure.

Xu D, Nishimura T, Nishimura S,...Peltz G. Fialuridine induces acute liver failure in chimeric TK-NOG mice: a model for detecting hepatic drug toxicity prior to human testing. PLoS Med 11: e1001628, 2014.

Liver Regeneration Breakthrough Using Mature Human Cells: A team of scientists has succeeded in coaxing mature human cells to be reprogrammed into a type of liver cell that can repopulate the organ after liver failure in a mouse model. Researchers have been searching for cell-based alternatives to liver transplantation because of the limited supply of donor organs. It would be ideal to use cells from an individual's other healthy tissues to repopulate the diseased liver, but mature cells are usually terminally committed in their specific roles and cannot switch to performing the part of a liver cell. On the other hand, so-called induced pluripotent stem cells, created by reprogramming mature cells all the way back to a stem-cell-like state, have shown promise. These cells, however, have been unable to proliferate adequately *in vivo* and can also carry a risk of producing tumors.

Now researchers seem to have hit on a unique solution using cells that are not too stem-cell-like and also not too mature. Starting with mature cells called fibroblasts taken from humans, they induced the cells to regress somewhat by inserting genes for three factors produced by embryonic stem cells. They then added growth factors and other molecules to encourage the cells to take on a more liver-like persona. The cells then produced several proteins such as albumin that are distinctive for liver cells, took on a liver-cell-like shape, and displayed liver-associated functions such as storage of glycogen (a reserve form of glucose [sugar]), storage and uptake of fats, and production of urea. But the real test came when the scientists transplanted these liver-like cells into a mouse model of liver injury and failure. Over the ensuing months, the cells expanded to repopulate the liver and underwent further maturation, improving the survival of the mice. The transplanted cells also showed a lasting effect, synthesizing human albumin that was still detectable in the mouse's blood 9 months after transplant. Though further experiments are needed before this cell technology can be applied to humans, these experiments represent a major step towards using cells from a person's own body to heal their diseased liver. This technology could overcome the current challenges confronted by patients of long wait times for donor organs and life-long immunosuppressive drugs to prevent organ rejection.

Zhu S, Rezvani M, Harbell J,...Ding S. Mouse liver repopulation with hepatocytes generated from human fibroblasts. Nature 508: 93-97, 2014.

HEPATITIS C PREVENTION

Exposure to Low-level Hepatitis C Virus Does Not Protect Against Future Infection: Scientists at the NIDDK have turned conventional wisdom—that low-level exposure to hepatitis C virus (HCV) protects against subsequent, full-strength encounters with the virus—on its head, finding instead that, in an animal model, such exposures may put individuals at risk for future infection by suppressing immune function. Researchers had observed that some people resist developing hepatitis C despite coming into contact with repeated, low doses of the virus, such as through infected family members or injection drug use; these individuals often produce a type of immune cell, called a T cell, that responds specifically to HCV. This observation led to the assumption that their immune systems were primed to protect them against a future, full-blown HCV exposure, similar to how individuals who spontaneously clear an acute HCV infection are able to quickly clear any future infections. The NIDDK team set out to test this assumption. The scientists conducted their HCV study in chimpanzees, chosen for their similarity to humans in responding to HCV, which does not infect other animal models. They gave three

chimpanzees repeated infusions of plasma and blood cells taken from people who had antibodies to HCV, but only trace amounts of viral RNA—signifying a prior, low-level exposure—and who did not show signs of an active hepatitis C infection. The chimpanzees developed a similar profile to their human donor counterparts in that they remained free of measurable levels of circulating virus, but started producing T cells that target HCV. When the researchers then challenged the low HCV-exposed animals with a full dose of the virus, the prior low-dose exposures did not protect against a full-blown viral infection. Looking more closely at certain types of T cells produced by the pre-exposed chimpanzees, they saw that these cells were ineffective at responding to a full-dose HCV infection, compared to cells from chimpanzees who were either infected for the first time or re-infected after recovering from a full-blown infection. Upon further investigation, the researchers found boosted levels of another type of T cell, called the regulatory T cell, in the pre-exposed chimpanzees. These regulatory T cells appeared to be the cause of the immune suppression toward HCV in these animals. These observations from a small group of the most closely related species to humans—chimpanzees—now await confirmation in people exposed to repeated, low doses of HCV. However, they provide strong evidence that runs contrary to the belief that low-level HCV exposure protects individuals against acute hepatitis C. This finding is relevant to designing effective vaccination strategies in the future against HCV and other microbes to which humans can be repeatedly exposed, such as those that cause malaria, tuberculosis, and HIV/AIDS.

Park SH, Veerapu NS, Shin EC,...Rehermann B. Subinfectious hepatitis C virus exposures suppress T cell responses against subsequent acute infection. Nat Med 19: 1638-1642, 2013.

SCIENTIFIC PRESENTATION

The Light and Dark Sides of an Intestinal Heat Shock Protein

Dr. Eugene B. Chang

Dr. Eugene B. Chang is the Martin Boyer Professor of Medicine at the University of Chicago. A graduate of The Johns Hopkins University, he earned his medical degree from the University of Chicago School of Medicine, where he has been a member of the faculty since 1986. He presently has an NIDDK grant that supports training for post-doctoral researchers in metabolism and nutrition, and he serves as a co-director of the Pritzker School of Medicine Summer Research Program. His research focuses on host-microbial interactions in the intestines, particularly in defining signal pathways that are involved in maintaining intestinal stability. His studies are also aimed to better understand how perturbations of gut bacteria contribute to the development of digestive diseases, especially inflammatory bowel disease. He has defined several new mechanisms of action of probiotic organisms that are currently being developed as therapeutic agents. He joined the National Diabetes and Digestive and Kidney Diseases Advisory Council in 2014. At the Council's May 2014 meeting, Dr. Chang presented a lecture on the multiple roles of a heat shock protein in digestive diseases.

The gastrointestinal (GI) tract is one of the harshest environments in the body. The cells that line the stomach are continuously bathed in acids and digestive enzymes. At the other end of the tract, the cells that line the colon are exposed to millions of bacteria. These microbes must be kept in check to prevent inappropriate inflammatory reactions that could lead to inflammatory bowel disease (IBD)—a distressing and serious condition that causes persistent diarrhea, cramping abdominal pain, fever, and rectal bleeding. In some cases, the symptoms of IBD can only be alleviated by surgical removal of the colon, followed by a lifelong need for an external ostomy pouch to collect intestinal contents. In other cases, people with IBD may eventually develop colon cancer.

One focus of Dr. Chang's research is to gain further understanding of IBD by studying how the cells lining the GI tract respond—either appropriately or inappropriately—to the bacteria in the gut. He shared results from his lab that tell an interesting story of how a type of protein called a "heat shock protein" (HSP) can protect intestinal cells from the many dangers in the GI tract. But, as Dr. Chang explained, although a certain HSP can safeguard cells from injury, it may also have a darker, dangerous side.

Heat Shock Proteins as Guardians Against Stress

It is not surprising that HSPs are found in virtually every cell in the body, because they play an important role in protecting other proteins in the cell from many types of external stress. For example, when cells are exposed to high heat, such as during a bad fever, many proteins can lose their shapes and begin to unravel, impairing their ability to function properly. These defects in the proteins could lead to the death of the cell. However, if the temperature is raised gradually, the cells can respond by producing HSPs to rescue the damaged proteins. The HSPs interact with the impaired proteins and return

them to their original shape so that they can function normally and move to the proper part of the cell. In this way, the HSPs act as guides to allow proteins to regain (or retain) their natural, functional shape, and they help cells survive during environmental stress.

While some types of HSPs are "constitutive," or always present, others are "inducible" and only produced when the cell is exposed to stressful conditions, such as heat, infections, acids, or other factors that may cause protein damage. In fact, the amount of HSPs produced in times of cellular stress is so large that they may be 10 percent of the total protein in the cells.

Not surprisingly, these HSPs are highly induced in the turbulent environment of the gut. Dr. Chang presented evidence that HSP levels are particularly high in the stomach, where acids and digestive enzymes are plentiful, and in the colon, where the majority of gut bacteria live. The cells that have the highest amount of HSPs are also the ones that are in direct contact with these hazards.

Hsp70: The Intestinal Cell's Response to Gut Bacteria

There is ample evidence that intestinal cells produce HSPs in response to the exposure to gut bacteria. One type of HSP in particular—Hsp70—is produced in abundance in the area of the colon closest to the small intestine, and its presence tapers closer to the rectum. This correlates with the types of microbes that are present in those regions, Dr. Chang explained. On the other hand, the guts of germ-free mice—which do not have gut bacteria—do not contain the mouse form of Hsp70. This provides further evidence that Hsp70 production in intestinal cells is triggered by exposure of the cells to gut microbes.

Dr. Chang and his research team decided to closely examine this relationship between gut bacteria and Hsp70 by allowing gut microbes to colonize an area of a rat's gut that is typically not exposed to high levels of bacteria, and then determining whether this results in Hsp70 production. They chose the small intestine, which typically harbors far fewer bacteria than the colon. One reason for this difference is that intestinal contents tend to move much more slowly in the colon, and this sluggish motion allows bacterial colonization to take place. To see if bacterial colonization would cause Hsp70 to be produced in small intestinal cells, Dr. Chang's team decided to alter a portion of the small intestine to limit the movement of its contents, making it more like the colonic environment and encouraging bacterial growth. They accomplished this by performing surgery on male rats to create "blind loops" in their small intestines. A blind loop is a short branch of intestine with one end closed off to form a dead end. When the blind loop was oriented so that the dead end was at the bottom of the branch, intestinal bacteria became trapped there, and Hsp70 was produced in the cells within the loop. When the blind loop was oriented so that the dead end was at the top of the branch, the contents of the blind loop were pushed out by the regular muscle contractions of the intestine, and there was little to no production of Hsp70. In other words, this inducible HSP can be highly produced in portions of small intestine, but only under conditions that promote bacterial growth. This, said Dr. Chang, is a great example of how gut microbes affect the human body.

Dr. Chang next explored why Hsp70 production is triggered by gut bacteria. Because HSPs are known to have a vital role in protecting cells from stress, it is possible that they have a protective role in the gut, perhaps by helping the intestinal cells heal after they become damaged by inflammatory diseases like Crohn's disease or ulcerative colitis. To test this idea,

Dr. Chang's team gave mice a chemical called dextran sodium sulfate (DSS), which is used experimentally to cause colitis, or inflammation in the colon. In normal mice, DSS caused acute (brief) colitis that healed completely 10 to 14 days later. However, in mice that could not make Hsp70, the injury caused by DSS was more severe and led to a chronic (long-lasting) form of colitis that persisted even after withdrawal of DSS. The colons from the mice with this chronic colitis were similar to those in humans who have ulcerative colitis, a form of IBD that specifically affects the colon. This suggests that Hsp70 is important for returning the colon to a healthy state after it has been damaged—which, Dr. Chang explained, is the "good" side of Hsp70.

The Dark Side of Hsp70

One of the dangers of chronic colitis is that it may eventually lead to colon cancer. In humans, most cases of colon cancer—called "sporadic" colon cancer—first develop as polyps that protrude from the wall of the colon. The type of colon cancer that arises from colitis is different, however; it develops from multiple flat lesions instead of bulging polyps. When mice are exposed to DSS in combination with a cancer-promoting agent, they eventually develop cancerous polyps similar to those in sporadic colon cancer. However, when Hsp70 is absent in these mice, colitis leads to the development of flat tumors that are remarkably similar to the tumors in the human form of colon cancer that arises from colitis. This means Hsp70 determines what type of colon cancer will grow in mice with colitis.

But there is an even darker side to Hsp70, Dr. Chang explained. While Hsp70 is absent in the flat tumors of human IBD-associated colon cancer, the level of Hsp70 is extremely high in sporadic colon cancer. This means that Hsp70 may not just influence the type of cancer that grows, but could also promote the growth of sporadic tumors. To test this possibility, Dr. Chang's team used a mouse model of sporadic colon cancer that will spontaneously develop colon polyps. When the levels of Hsp70 were high in these mice, there was a drastic increase in the size and number of tumors; when Hsp70 was deleted from these mice, the tumors were smaller and less abundant. These results suggest that Hsp70—the same protein that helps intestinal cells survive damage from IBD—also contributes to sporadic tumor formation and progression in the colon.

BAGging Beta Catenin: How Hsp70 Encourages Tumor Growth

Dr. Chang next explored how Hsp70 may help drive the development of sporadic colon cancer. One possibility was that Hsp70 was somehow influencing the activity of a protein called beta catenin, which is a primary player in many cases of colon cancer. Beta catenin is usually found outside the cell's nucleus (the part of the cell that contains DNA), but when it is active, it will move into the nucleus, where it turns on genes that can help cancer cells grow and spread to other parts of the body. Not surprisingly, beta catenin is found primarily in the nuclei of tumor cells in cases of sporadic colon cancer. However, in mouse tumor cells that lack Hsp70, beta catenin is found primarily outside the nucleus. This means Hsp70 is somehow helping beta catenin move to the nucleus where it can encourage tumor cells to grow and invade other tissues.

The next step was to find out exactly how Hsp70 was directing beta catenin. One clue came when Dr. Chang's team discovered that Hsp70 actually associates with beta catenin and accompanies it into the nucleus. Additional experiments identified another molecule, called BAG-1, that also associates with Hsp70, resulting in an aggregate of Hsp70, BAG-1, and beta catenin. Importantly, BAG-1 contains a built-in signal that directs

it into the nucleus, taking Hsp70 and beta catenin with it. This suggests that, through its interactions with BAG-1, Hsp70 acts as a "chaperone" to usher beta catenin into the nucleus where it can activate genes that promote tumor growth and dissemination.

Future Directions

Dr. Chang closed by thanking his research team. His exploration of Hsp70 paints a fascinating yet disquieting picture of a protein that not only protects intestinal cells from stress, but also can, under certain conditions, contribute to the formation and growth of sporadic tumors in the colon. This study also illustrates the intricate relationships among colon cancer, gut bacteria, and digestive diseases such as inflammatory bowel disease. By knowing how diseases progress in the GI tract and identifying the factors that are involved—such as Hsp70—new therapies can be developed to treat or manage these conditions.

PATIENT PROFILE

Tarrie Barnes

Putting an End to a History of Hepatitis C

Tarrie Barnes

Tarrie Barnes was 12 or 13 years old when, on the way home from church, she and her siblings walked by the Baltimore hospital where her grandmother was staying. The image of her grandmother waving to her from the window holds a special place in Tarrie's memory. "It was a sunny day," she remembers. "And when I think of my grandmother, I think of happiness."

Although she was too young at the time to comprehend exactly why her grandmother was in the hospital, Tarrie would learn years later about the disease that eventually took her grandmother's life. "I just happened to be reading her death certificate, and it said 'cirrhosis of the liver,'" she recalls. "And I thought, well, my grandmother didn't drink, so why would it say 'cirrhosis'?" When Tarrie asked her grandmother's doctor about the death certificate, he told her that her grandmother's liver had succumbed to a disease called, at the time, "non-A, non-B hepatitis."

For Tarrie, the diagnosis was a premonition of her own future struggles with a silent yet debilitating and potentially fatal liver disease.

"Something Doesn't Look Right in Your Blood"

Tarrie, who is now 65, had been very close to her grandmother. As a child she would rather join her family at her grandparents' home on Saturdays instead of going to a park to play. At the house, festivities would begin: guitars, harmonicas, and food. Her grandmother was a first-rate cook—she would "dip her finger in something and make it taste good"—and would always greet Tarrie and her siblings by offering them something to eat. Her grandfather would proudly stand with his family and "put his fingers under his suspenders and bounce on his heels, and say, 'Look at what I started. I started all of this,'" Tarrie fondly reminisces. She treasures the memories of those Saturdays with her grandparents. "I just feel blessed that I was in the family I was in. And anytime we saw my grandmother smile, it made the day even nicer."

Tarrie's grandmother died in 1988. A year later, scientists published reports identifying a new virus, the hepatitis C virus, as the cause of non-A, non-B hepatitis.

Then, in 1990, after she had donated blood, Tarrie received a troubling letter from the American Red Cross. The letter, she remembers, essentially said: "Something doesn't look right in your blood." The hepatitis C virus—the same virus that had stricken Tarrie's grandmother—was suspected to spread through blood transfusions, so the Red Cross had begun to screen their supply for

infected blood. Also unsettling for Tarrie was that 15 years earlier, well before the screening had started, she herself had received a blood transfusion during surgery, meaning that she may have been exposed to the hepatitis C virus.

While the Red Cross letter stated that Tarrie could have viral hepatitis, it also mentioned the chance of a "false positive," which meant there was a possibility she wasn't infected even though she tested positive. (At this time, the screening methods were not as accurate as they would be a few years later.) Nevertheless, the Red Cross recommended that she have her blood checked, so Tarrie went to her doctor to get tested. The results came back negative. "I never thought anything else about it," remembers Tarrie. "I thought [the original Red Cross test] was a false positive." So Tarrie went back to her career at a telecommunications company and life with her husband and two children. But, at her doctor's recommendation, she stopped donating blood.

Tarrie was slowly getting tired more easily—something that, understandably, many people could experience without raising alarm. "Some days I would feel more tired than others....You don't realize that something is going on."

Nine years would pass. In the meantime, Tarrie started taking classes to fulfill her dream of becoming a teacher. But she also slowly began to experience symptoms that she casually attributed to aging, like many people would. She had occasional dizzy spells, sometimes to a point where she needed to hold on to her chair to keep the room from spinning. And sometimes she felt a pain in her side when she lifted something heavy. Tarrie didn't realize she had liver disease; she remembers thinking that "it was maybe my blood pressure." Most of all, she was slowly getting tired more easily—something that, understandably, many people could experience without raising alarm. "Some days I would feel more tired than others. Sometimes I couldn't do all that I wanted to do. I would get tired without knowing I was tired, because you're just used to it. You don't realize that something is going on."

It is common for people with hepatitis C not to realize that they have the disease. In fact, most people do not have any symptoms until the virus causes significant liver damage, which could ultimately result in the need for a liver transplant. Prior to the discovery of the virus and routine screening of the blood supply, many people acquired hepatitis C through a blood transfusion—the virus is most commonly transmitted by its introduction directly into the bloodstream. Once in the blood, the virus then infects cells in the liver, slowly killing them and causing scar tissue to form. Exposure to infected blood usually results in a chronic (long-lasting) infection because the body cannot get rid of the virus.

For Tarrie, the diagnosis did not come until she went to her doctor for a routine checkup in 1999. Her doctor told her that her liver test results were abnormal, and it was recommended that she see a liver specialist—the same specialist, coincidentally, who had treated her grandmother. Still not realizing she was sick, Tarrie's big shock came when the specialist walked into the examination room: "We're not going to talk about a liver transplant" were the first words out of his mouth. Taken aback, Tarrie began to realize that she could be dealing with something serious.

By 1999, there was a more accurate test for hepatitis C, and Tarrie tested positive. After a liver exam, she was diagnosed with advanced hepatitis, which means her

liver was damaged so badly by the virus that it was beginning to scar and lose functionality. If unchecked, the disease would ultimately cause her liver to fail.

Managing Life with Hepatitis C

Armed with advice from her liver specialist, and knowing how the disease affected her grandmother, Tarrie began her fight against hepatitis C. Her liver specialist encouraged her to join a hepatitis C support group that he supervised, and she began to learn as much as she could. "My mother believed in education and reading," says Tarrie. "She always made sure we read. I inherited that bug." The support group was a diverse assembly of people with hepatitis C who shared their experiences and learned from each other's struggles and successes. They discussed the symptoms they were having and how to deal with them. They talked about the changes in lifestyle they should adopt when dealing with a damaged liver, such as eating healthy and avoiding alcohol. When you have advanced hepatitis, you need to be careful, says Tarrie. "Anything you put in your body goes to your liver."

Tarrie eagerly soaked up knowledge about hepatitis C while giving encouragement to other members of the group. "By that time I was 51, and I had become more of a talker," she says. "I liked sharing. I liked learning about what was going on. And we did help each other....It was a good thing."

Tarrie's liver specialist also convinced her to undergo a 6-month clinical trial at the hospital in Baltimore. She went on medical leave from her job, because "I wasn't sure about what I would be facing," she recalls. Her support group had prepared her for potential side effects that would come along with anti-hepatitis medications. "I was told what I might experience would be similar to chemotherapy. You might lose your hair, or get chills or a fever. It affects each person in a different way."

The treatment ultimately wasn't successful for Tarrie, and her hepatitis remained at an advanced stage. But this was still only the beginning of her long, complicated battle with hepatitis C.

The First Trials at the NIDDK

Shortly after Tarrie's diagnosis, her daughter, then a biology major at Bowie State University, was selected for a research internship at the NIH. "She is so smart. She gets it from my mother, not me," Tarrie says glowingly of her daughter. "She loves biology."

Inspired in part by her mother's predicament, Tarrie's daughter began working in the laboratory with Dr. Theo Heller, a clinical investigator in the NIDDK Intramural Research Program, under the direction of Dr. T. Jake Liang, Chief of the NIDDK Liver Diseases Branch. She was a part of one of the first teams to successfully produce the hepatitis C virus in cultured cells, a major milestone that allowed scientists to study the life cycle of the virus more closely. She also told Dr. Heller about her mother, and he suggested that Tarrie participate in a clinical trial at the NIDDK. Dr. Heller "asked my daughter three questions about me," Tarrie remembers. "Did I join a study? Did I complete the study? Did I still have hepatitis C? The answer was 'Yes' to all three. From that, I qualified to go there."

"I enjoy going there," says Tarrie of her visits to the Clinical Center. "Everyone there at NIH has been so nice....It's like a big happy family."

PATIENT PROFILE

In 2001, Tarrie enrolled in her first NIDDK clinical trial at the NIH Clinical Center in Bethesda, Maryland. For 6 months, she took a combination of two antiviral drugs: interferon, which helps the body to defend itself against viruses, and ribavirin, which slows the replication of viruses. The treatment brought her virus levels down, and her liver began to show some signs of recovery. There were side effects, but for the most part they were manageable. "At the beginning I was in bed with chills and fever," recalls Tarrie. "Then I was just tired, but I didn't realize that the tiredness was probably more from the hepatitis."

By the time Tarrie completed the trial, the drugs had reduced the amount of virus in her blood, but they did not eliminate it. The virus continued to multiply over the next few years, and the condition of Tarrie's liver regressed. It didn't stop her from achieving her goal of becoming a teacher, however. In 2006, she graduated from Morgan State University and started teaching the first grade. But her health continued to decline, and she reluctantly decided to retire after a few years. "I didn't really have a choice," she remembers. "I could tell that confusion was starting to set in, and it was starting to interfere with my ability to teach. It was emotional … because I didn't want to leave."

Still, Tarrie was not discouraged, and she enrolled in another trial at the NIDDK, using a drug regimen similar to her last trial. However, this time one of the drugs was coated in lactose—and Tarrie didn't know she was lactose intolerant. "That trial was my worst," she recalls. "That really did me in. I was living in the bathroom." Determined nonetheless, she completed the grueling 6-month trial. But the treatment did not clear her of the virus—it soon rebounded again, and Tarrie was back at square one. Yet, she still held out hope.

Tarrie gives accolades to her faith and her family for helping her through the rough times. "Life has been an adventure," she says. "Faith has gotten me through a lot. And just having a loving family has made all the difference. It all started with my grandparents—letting us know to put God first, then family, and friends. As long as you have that love that connects you, you can get through anything."

One More Clinical Trial at the NIDDK: Saying Farewell to Hepatitis C

After three unsuccessful treatments for her hepatitis, Tarrie once again signed up for a clinical trial at the NIDDK. She was encouraged by breakthroughs in the understanding of the disease, and she had developed a close relationship with Dr. Heller. "He's fantastic. He told me they were always working on new and better medications. The more they learned about the virus, the better the medications they could get to help clear it."

The trial was, in fact, testing two new drugs called daclatasvir and asunaprevir that directly target specific components of the hepatitis C virus. Also, due to advances in the understanding of the virus, the staff at the NIH Clinical Center were able to identify the subtype that had infected Tarrie: it was called "genotype 1b." Because different genotypes of the hepatitis C virus can respond uniquely to different medications, knowing the genotype allows doctors to predict how successful a treatment will be. In Tarrie's case, the "b" was crucial. "I was excited when they told me that I was type b, because that meant I didn't have to take interferon [for this trial]," she explains.

PATIENT PROFILE

Tarrie was encouraged by breakthroughs in the understanding of the disease: "The more they learned about the virus, the better the medications they could get to help clear it."

Tarrie began the 6-month trial near the end of 2013. Three months after she completed the study, the virus could no longer be detected in her blood—the new drugs had worked. Tarrie was ecstatic. Moreover, the two pills she took had absolutely no negative side effects. "It was like heaven compared to the last study," laughs Tarrie. In fact, Tarrie was so thrilled by the results that she presented Dr. Heller with a challenge: if she remains clear of the virus, he will have to dance a jig for her. When Dr. Heller said he didn't know how to do the jig, Tarrie responded playfully, "Google it!"

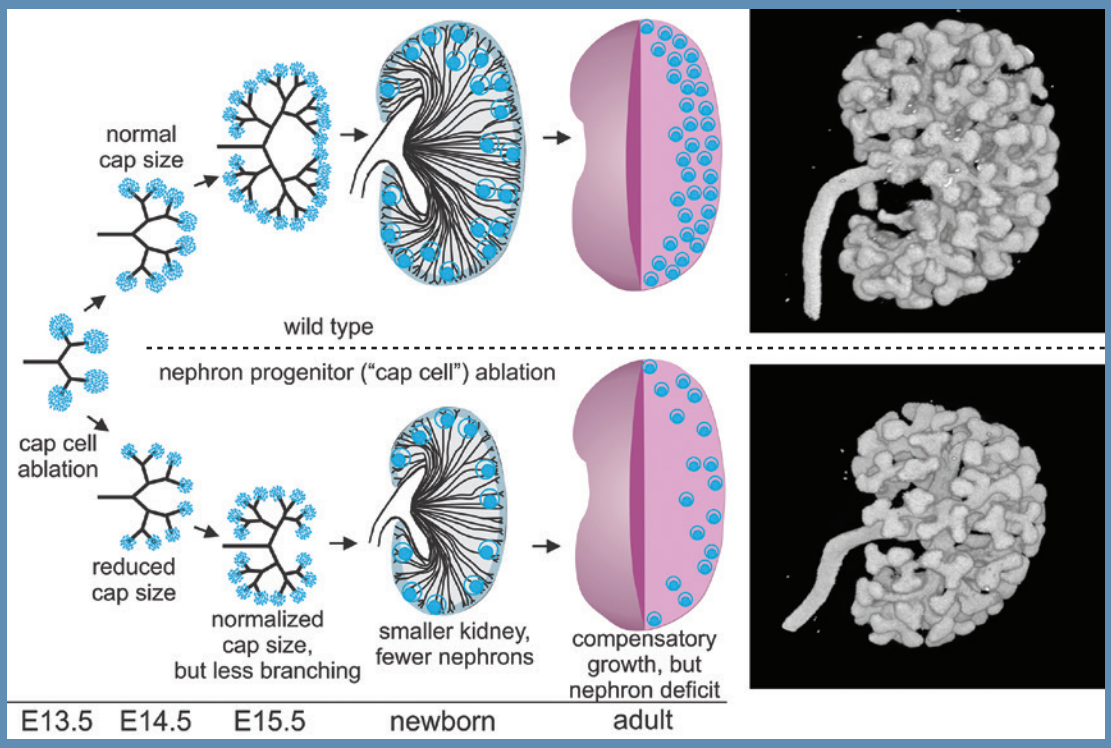
A New Chapter: Living Hepatitis-free

The hepatitis C virus has now been undetectable in Tarrie's blood for over 8 months, which means the odds of the virus recurring are very low. Her dizzy spells have become less frequent. In September 2014, she flew to Hawaii to visit her son, who is a naval officer, and to celebrate her 40th wedding anniversary with her husband. She still makes visits to the NIH to have her progress monitored. "I enjoy going there," says Tarrie. "Everyone at NIH has been so nice. The first or second time I went there, they already knew my name. I would even see doctors who I didn't know, but knew of my case, and they would say 'Hi, I heard you were doing well.' So, it's great. It's like a big happy family."

Not only has Tarrie's health improved, but her participation in the trials at the NIDDK also allowed her to contribute to the ongoing research on treatments for hepatitis C. "Those two pills, I think they can help a lot of folks," she says, referring to the two medications she took during her last NIDDK trial.

Tarrie holds a deep admiration for her grandmother, the woman who always made her happy. She would love the opportunity to continue to share the same happiness with her own grandchildren—in fact, she once told her church pastor that all she wanted to do was to live as long as her grandmother did.

Thanks to Tarrie's perseverance, along with a good dose of progress in medical research, she is well on her way.



Each human kidney contains about 1 million functional units that remove wastes from the blood, help control the body's fluid balance, and regulate electrolyte balance. New research in mice could help explain how kidneys generate the correct number of functional units, called nephrons, during development. (Left panel) Mice treated to have fewer nephron progenitor cells (bottom) during development have fewer nephrons at birth than normal mice (top)—a difference that persists into adulthood, even though overall kidney size is similar. (Right panel) Three dimensional renderings of the kidney internal structure during development show differences in size and extent of nephron branching between the normal (top) and progenitor-cell-limited (bottom) mice. As nephrons are not replaced after birth, having a lower nephron number at birth may cause kidney problems later in life. These new findings could contribute to efforts to understand and treat kidney disease.

Images courtesy of Dr. Frank Costantini, Columbia University Medical Center. Originally published in Cebrian et al*, Cell Rep 7(1):127-137 2014. Photo copyright 2014 by Cebrian,* et al*. and adapted and reprinted under a Creative Commons Attribution License (http://creativecommons.org/licenses/by-nc nd/3.0/legalcode).*

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 afflict 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 disorders 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 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 two 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, represents a life-threatening condition.

It has been estimated that more than 23 million Americans 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. As fibrosis is a common end point in several diseases, the NIDDK convened a meeting in 2014, "Targeting Fibrosis in Kidney, Bone Marrow, and Urological Diseases," to discuss ways to detect and measure fibrosis.

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 2012, over 636,000 patients received treatment for ESRD: nearly 450,000 received either hemodialysis or peritoneal dialysis and over 186,000 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. African Americans are nearly four times more likely to develop kidney failure than are non-Hispanic Whites.[2] American Indians and Alaska Natives and Hispanic and Latino Americans have twice the risk for kidney failure as do non-Hispanic Whites.[2] In recent years, scientists supported by the NIDDK have uncovered important genetic clues that may play a role in

[1] *Levey AS, et al. Ann Intern Med 150: 604-612, 2009.*

[2] *U.S. Renal Data System, USRDS 2014 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2014.*

health disparities related to kidney disease susceptibility and progression in minority populations.

The NIDDK supports a significant body of research aimed at understanding the biology underlying CKD. The NIDDK's chronic renal diseases program supports basic and clinical research on kidney development and disease, including the causes of kidney disease, 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 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. NDKEP also promotes the inclusion of estimates of kidney function as a part of routine blood testing and works 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 urological diseases and disorders such as benign prostatic hyperplasia, urinary incontinence, and urinary tract infections. Other disorders of the genitourinary tract, such as interstitial cystitis/painful bladder syndrome (IC/PBS)—also known as IC/bladder pain syndrome (BPS)—in women and men and chronic prostatitis/chronic pelvic pain syndrome in men, are also important components of the NIDDK's urology program. Additional areas of interest include research on treatments for kidney stones, such as shock-wave and laser lithotripsy to break up stones, and therapeutic approaches to inhibit their formation and growth.

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

NIDDK-supported basic and clinical research on IC/PBS is focused on elucidating the causes of these conditions, identifying "biomarkers" that will aid diagnosis, and improving treatment and interventions. Ongoing epidemiologic studies will help refine prevalence estimates and demographics. One example 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/PBS and to characterize the disease profiles in patients; this network was recently renewed for an additional 5 years.

Urinary incontinence is conservatively estimated to affect 13 million Americans, most of them women.[5] Many suffer in silence due to embarrassment and lack of knowledge about treatment options available.

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

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

[5] *Urological Diseases in America. NIDDK, NIH Publication Number 07-5512, 2007.*

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 provide new insights into non-surgical alternatives. As researchers continue to investigate treatment options, an equally important challenge is to improve strategies for assessing both the impact of urinary incontinence and other lower urinary tract symptoms in women and men and the effect of different diagnostic tools and interventions on patient outcomes; to address this challenge, the NIDDK launched and recently expanded 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 multiple other NIH Institutes and Centers, has launched a bladder health initiative to identify and characterize modifiable risk factors for lower urinary tract symptoms and urinary incontinence in women (see feature, "Paving the Way to Improved Bladder Health 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 chronic disease. NIDDK-supported research has recently identified mutations that cause a rare anemia called X-linked sideroblastic anemia.

The NIDDK is also keenly interested in the basic biology of stem cells, including adult hematopoietic (blood) stem cells, which are needed for bone marrow transplants and may have broader application in gene therapy research.

MEASURING RISK OF KIDNEY DISEASE AND PROGRESSION

Genetic Variation and Chronic Kidney Disease Progression: African Americans who have chronic kidney disease (CKD) and two copies of common variants in the *APOL1* gene are twice as likely to progress to kidney failure as those without these high-risk variants. Moreover, African Americans with the high-risk variants also tend to lose kidney function at twice the rate of those without the variant.

Previous studies have shown that African Americans with two copies of certain variants of the *APOL1* gene are at increased risk of developing kidney disease. Researchers attempted to further characterize the nature of this increased risk by analyzing data from people enrolled in two large studies of CKD: the Chronic Renal Insufficiency Cohort (CRIC) Study and the African American Study of Kidney Disease and Hypertension (AASK). The CRIC study is one of the largest and longest ongoing studies of CKD epidemiology in the United States; it is following both White and African American people with CKD, about half of whom also have diabetes. AASK was the largest and longest study of CKD in African Americans without diabetes whose CKD was attributed to high blood pressure. In the new analysis, the researchers found a correlation between the presence of high-risk variants of the *APOL1* gene and an increased risk of CKD progression among African Americans. This effect was seen regardless of whether patients maintained good blood pressure control or had diabetes.

This study builds on information learned over the past few years about how genetic factors can contribute to the increased risk of kidney disease in African Americans. These results have important implications for understanding the differences in kidney disease risk across populations. Moving forward, physicians may be able to make better choices about when to start screening for kidney disease and how to choose an appropriate therapy by identifying which patients have these gene variants and are therefore at increased risk of developing kidney disease and progressing to kidney failure.

Parsa A, Kao WHL, Xie D,...Appel LJ; AASK Study Investigators, CRIC Study Investigators. APOL1 *risk variants, race, and progression of chronic kidney disease. N Engl J Med 369: 2183-2196, 2013.*

Approximating Kidney Function Through Urine Analysis: The levels in urine of genetic material from a type of kidney cell correlate with loss of kidney function, according to a recent study. This finding offers the possibility of a new, noninvasive way to approximate the kidneys' filtering ability and monitor the progression of chronic kidney disease.

The podocyte is a cell that is a component of the glomerulus, the fundamental filtering apparatus in the kidney. It has been hypothesized that progressive loss of kidney function in many forms of kidney disease can be attributed, at least in part, to damage to the glomeruli and accompanying depletion of podocytes. As podocytes are lost, traces of them may be detectable in the urine. In the current study, investigators analyzed urine samples from over 350 adult and pediatric volunteers with kidney disease and compared them with urine samples from nearly 300 people without kidney disease. They observed a nearly 80-fold increase in podocyte-derived genetic material in patients who had glomerular disease (based on a previous biopsy) and who had progressive kidney disease. A second group of patients with Alport syndrome, a hereditary glomerular disease, exhibited a 23-fold increase in podocyte-derived genetic material. Interestingly, in people with autosomal dominant polycystic kidney disease, which does not feature glomerular injury, urine podocyte-derived genetic material was not increased. These observations suggest that the presence of podocyte genetic material in the urine may be a marker for glomerular injury and may provide a window into glomerular function.

These findings support the hypothesis that podocyte depletion is an important element of some forms of progressive kidney disease. Furthermore, measurement of urine levels of podocyte-derived genetic material may provide an easy, non-intrusive way to evaluate and monitor podocyte health in people with glomerular diseases.

Wickman L, Afshinnia F, Wang SQ,…Wiggins RC. Urine podocyte mRNAs, proteinuria, and progression in human glomerular diseases. J Am Soc Nephrol 24: 2081-2095, 2013.

Genetic Factors in Kidney and Cardiovascular Disease: Variants in the *APOL1* gene are associated with increased risk of kidney disease but not cardiovascular disease in African Americans with high blood pressure. Previous studies have shown that African Americans with two copies of certain variants of the *APOL1* gene are at increased risk of developing kidney disease from causes other than diabetes, but there have been conflicting data regarding the role of *APOL1* in cardiovascular disease.

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) is testing whether reducing 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).

Over 2,500 African American volunteers in the SPRINT study agreed to undergo genetic testing to allow researchers to examine their *APOL1* status as it related to their kidney function and risk of developing cardiovascular disease. Researchers found that study participants with two risk variants of the *APOL1* gene were more likely to have mild kidney disease, defined as diminished filtering capacity and/or protein in their urine, than people with a single risk variant or none. However, they were not more likely to have cardiovascular disease.

These data from the SPRINT study add to information learned over the past few years about the contribution of genetic factors to the increased risk of non-diabetic kidney disease in African Americans. The current investigation confirms that the presence of these variants is associated with mild kidney disease in this population but shows that they are not associated with cardiovascular disease. Although a key priority in treating high blood pressure is to reduce the risk of both

kidney and cardiovascular disease, more research is needed to identify the role played by *APOL1* variants in these two processes.

Langefeld CD, Divers J, Pajewski NM,...Freedman BI. Apolipoprotein L1 gene variants associate with prevalent kidney but not prevalent cardiovascular disease in the Systolic Blood Pressure Intervention Trial. Kidney Int 2014 Jul 16. doi: 10.1038/ki.2014.254.

KIDNEY DISEASE TREATMENT

Origin of Cells Involved in Kidney Repair Following Injury: A recent study in mice suggests repair to the kidney's proximal tubules following injury is mediated by proliferation of existing mature cells rather than by activation of stem cells.

After acute injury in the kidney, such as that caused by the loss and sudden restoration of blood flow, cells may be damaged and less able to filter blood, and some die. New cells in the proximal tubule appear to replace the impaired cells and restore the tubules' function; however, the origin of these new cells has been the topic of much debate. Some evidence suggests that they are derived from existing, mature cells in the proximal tubule that multiply; other evidence points to a population of adult stem cells in the kidney as the source of these cells.

To address this question, researchers using a mouse model inserted a gene into the DNA of mature cells of a specific type that are only found in the proximal tubule of the kidney to label these cells and any cells derived from them. They reasoned that if repair following injury were accomplished by an expansion of the number of mature cells, there would be an increase in the number of labeled cells. Alternatively, if repair were mediated by stem cells (which would not have this label), there would be no increase in labeled cells. The results of the experiment showed an increase in the number of labeled cells in the mouse kidney after kidney injury, and that a larger number of labeled cells was seen in more severely injured kidneys. Similar findings were seen in mouse kidney tubule cells that were grown in the laboratory. Interestingly, several genes associated with stem cells were "turned on" in the mature cells following injury.

This study provides evidence that repair following acute kidney injury occurs through a return of mature proximal tubule cells to a somewhat more stem cell-like state, followed by their proliferation.

Kusaba T, Lalli M, Kramann R, Kobayashi A, and Humphreys BD. Differentiated kidney epithelial cells repair injured proximal tubule. Proc Natl Acad Sci USA 111: 1527-1532, 2014.

Design Change May Improve Kidney Stone Treatment: New research has shown that changes to a medical machine called a lithotripter may improve kidney stone treatment. Kidney stones are one of the most common disorders of the urinary tract. Kidney stones can form when substances in the urine—such as calcium, oxalate, and phosphorus—become highly concentrated. A small stone may pass on its own, causing little or no pain. A larger stone may get stuck along the lower urinary tract and can block the flow of urine, causing severe pain and/or bleeding. The lower urinary tract is primarily made up of the ureters (two tubes connecting the kidneys to the bladder), bladder, and urethra (tube that carries urine from the bladder to the outside). One treatment option for a person with a larger stone, or one that blocks urine flow and causes great pain, is noninvasive shock wave lithotripsy. In shock wave lithotripsy, the lithotripter generates shock waves that pass through the person's body to break the kidney stone into smaller pieces to pass more readily through the urinary tract. The lens component of the lithotripter serves to focus the shock wave at the selected target (stone). Although current lithotripters are more powerful and more reliable than previous iterations of the machine, their treatment effectiveness is reduced.

NIDDK-supported researchers have recently determined that the more powerful lithotripters shift the shock wave off-target, which contributes to efficiency loss. In addition, this same group of investigators made modifications to the lens while keeping all other

aspects of the lithotripter the same. By introducing a groove around the outer portion of the lens, the researchers were able to redirect the shock wave to its proper target when tested in an animal model. The newly designed lithotripter pulverized 89 percent of the stones to sufficient size for passage through the urinary tract compared to 54 percent of the stones by the current generation of lithotripter. Moreover, it is anticipated that the newly designed lithotripter will cause less damage to surrounding tissue. If future research shows similar benefits in people with kidney stones, this newly designed lens may be adaptable by other manufacturers to improve their lithotripters currently in medical practice.

Neisius A, Smith NB, Sankin G,...Zhong P. Improving the lens design and performance of a contemporary electromagnetic shock wave lithotripter. Proc Natl Acad Sci USA 111:E1167-E1175, 2014.

Treatment with Two Antibiotics Dramatically Reduces Risk of Urinary Tract Infections in Children with Vesicoureteral Reflux: Long-term use of a drug combination can reduce the risk of recurrent urinary tract infection by up to 80 percent in children with vesicoureteral reflux (VUR). In children with this condition, developmental abnormalities in one or both ureters—the tubes connecting the kidneys to the bladder—allow urine to flow back from the bladder into the ureters, and sometimes into the kidneys. As a result, children with VUR are more likely to have recurrent urinary tract infections (UTIs), which can increase their risk of kidney scarring and the potential for kidney failure.

For decades, doctors have treated children who have VUR with a small daily dose of the antibiotics trimethoprim and sulfamethoxazole (TMP/SMZ), often for years, with the hope of preventing recurrent UTIs and kidney damage. Although this approach seemed logical, there was no conclusive evidence that it provided long-term benefits. The Randomized Intervention for Children with Vesicoureteral Reflux (RIVUR) study examined the use of this drug combination in a well-defined population of over 600 young children with VUR whose ages ranged from 2 months to 6 years over a 2-year period. RIVUR found that the risk of recurrent infection was reduced by 50 percent in children with VUR who received TMP/SMZ. Children with VUR and bladder and bowel dysfunction saw an even greater reduction, up to an 80 percent lower risk of recurrent infections.

While TMP/SMZ significantly reduced the incidence of recurrent UTIs during the trial, the number of children who developed kidney scarring did not decrease in the group receiving the antibiotics. The researchers suggest this may be due to parents' heightened vigilance for UTI symptoms and early treatment in the trial and because most of the children were enrolled after their first infection rather than after multiple infections, when more scarring might occur.

Further analysis of data from the RIVUR trial may provide insight into other factors that could reduce susceptibility to recurrent UTIs and kidney scarring. For now, though, the RIVUR study has demonstrated that treatment with TMP/SMZ offers the possibility of fewer infections for children with VUR, which may provide an opportunity for many of them to outgrow reflux as their bodies develop and mature.

The RIVUR Trial Investigators. Antimicrobial prophylaxis for children with vesicoureteral reflux. N Engl J Med 370: 2367-2376, 2014.

KIDNEY FORMATION AND FUNCTION

Disruption of Kidney Development Has Life-long Consequences: Depletion of a subset of cells in the developing mouse kidney resulted in fewer filtering units, a deficit that persisted throughout life. This study provides important information regarding one of the factors that contributes to the kidneys' ability to remove waste products.

Nephrons are the basic functional unit of the kidney; they consist of various cells and structures that work together to filter waste products and excess fluid from the blood. Nephrons arise through an interaction between progenitor cells and ureteric buds as the kidneys

form. The researchers used genetic manipulation to generate mice in which they could selectively eliminate progenitor cells at a specific stage of development while leaving the surrounding cells unharmed, resulting in a 40 percent reduction in the number of progenitor cells. In response, there was a corresponding decrease in the rate at which the ureteric buds grew and branched. This adjustment allowed the developing kidney to maintain the proper ratio of progenitor cells to ureteric buds, but at the cost of sharply limiting the overall number of nephrons in the kidney.

There is no evidence for the growth of new nephrons in humans after birth, and nephrons that are lost due to disease or injury cannot be replaced. A fuller understanding of kidney development may provide clues as to possible strategies to grow new nephrons in kidneys, which could allow lost kidney function to be restored.

Cebrian C, Asai N, D'Agati V, and Costantini F. The number of fetal nephron progenitor cells limits ureteric branching and adult nephron endowment. Cell Reports 78: 127-137, 2014.

Chloride Concentrations Help Regulate Salt Metabolism by Influencing a Key Enzyme: A direct interaction between chloride and the enzyme WNK1 plays an important role in the kidneys' regulation of salt levels and blood pressure.

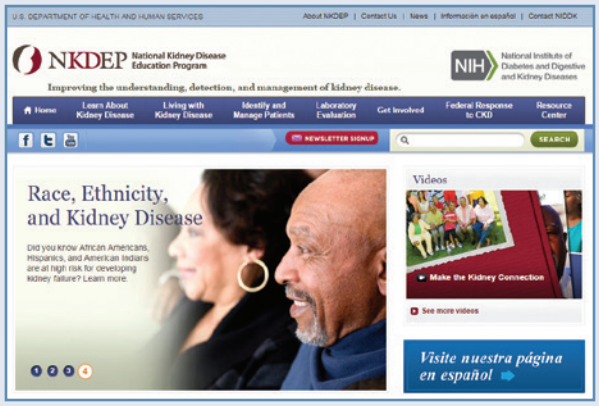
Cell-membrane-spanning co-transporter proteins regulate the levels of sodium, potassium, and chloride. In the kidney tubules, the return of these salts from the urine to the blood helps to maintain proper salt balance, fluid retention, and blood pressure. The activity of these co-transporters is closely associated with the levels of chloride inside of cells. Because of this correlation, researchers have hypothesized that these co-transporters might be under the control of a chloride-sensing mechanism. Researchers have now identified the regulatory enzyme WNK1 as one such regulator.

When chloride levels within the cell fall, WNK1 sets in motion a signaling cascade that leads to the activation of the sodium/potassium/chloride co-transporters and a corresponding increase in intracellular concentrations of these ions. Conversely, when chloride levels rise, WNK1's ability to activate the co-transporters is inhibited. Mutations to WNK1 that rendered it less able to bind chloride removed this inhibition, validating chloride as a modulator of WNK1 activity.

In humans, mutations in the WNK family of enzymes are associated with hyperaldosteronism II, an inherited disorder characterized by impaired salt secretion in the kidney that results in hypertension and elevated levels of potassium and chloride in the blood. A better understanding of the role of WNKs in salt metabolism may allow for the development of novel, targeted treatment strategies for hypertension and other diseases.

Piala AT, Moon TM, Akella R, He H, Cobb MH, and Goldsmith EJ. Chloride sensing by WNK1 involves inhibition of autophosphorylation. Sci Signal 7: ra41, 2014.

The NIDDK's National Kidney Disease Education Program Expands Its Outreach and Professional Education Initiatives



Educating those who are living with or at risk for chronic kidney disease (CKD) is a critical part of care. Providing straightforward, comprehensive information by a multidisciplinary team of health care professionals can encourage self-management and support informed decision-making, ultimately leading to improved outcomes.

In addition to a broad range of educational brochures and fact sheets, the NIDDK's National Kidney Disease Education Program (NKDEP) develops resources to help health care providers and educators present kidney health information to the public. NKDEP's newest resources include training programs for diabetes educators, community health workers, and pharmacists and a guide for primary care clinicians.

Providing kidney health resources for diabetes educators. Diabetes is the leading cause of CKD. Diabetes educators play a crucial role both in supporting early detection and slowing progression of CKD. NKDEP has developed a training program for diabetes educators, for which the American Association of Diabetes Educators provides continuing education credits. This program focuses on identification of kidney disease, slowing progression, addressing complications, and educating people with CKD.

Reaching out to the Hispanic community about kidney disease. NKDEP's *Riñones, Tesoros* ("Kidneys, Treasures") initiative aims to address the growing burden of kidney disease among Hispanics. The initiative's resources include Spanish-language educational materials, broadcast interviews, and an online newsletter, as well as a bilingual educational program and materials for use by community health workers.

Supporting CKD management in primary care. To help health care professionals manage CKD in their practices, NKDEP developed *Making Sense of CKD: A Concise Guide for Managing Chronic Kidney Disease in the Primary Care Setting*. The guide emphasizes the most important considerations for evaluating and managing CKD, including identifying and slowing disease progression among those at highest risk for progression to kidney failure.

Educating pharmacists about drug-induced acute kidney injury. Drug-induced acute kidney injury is common and preventable. To help address this problem, NKDEP has developed a training program for pharmacists on advising those at high risk for acute kidney injury about safe use of some commonly used medications. The University of Kentucky provides continuing education credit for this training.

Established in 2000, NKDEP aims to improve early detection of CKD, facilitate identification of patients at greatest risk for progression to kidney failure, promote evidence-based interventions to slow progression of CKD, and support the coordination of federal responses to CKD. To learn more about NKDEP and its resources, please visit www.nkdep.nih.gov

URINARY TRACT INFECTIONS: DIAGNOSIS AND HOST DEFENSES

Evaluating the Predictive Value of Urine Tests for Urinary Tract Infections: Results from a new study could help refine approaches to diagnosing and treating uncomplicated urinary tract infections (UTIs) in healthy, premenopausal women. UTIs are very common and occur most often in women. The majority of UTIs are caused by the bacterium *Escherichia coli* (*E. coli*), but a number of other microbes can infect the bladder, as well. To help diagnose a woman who has UTI symptoms, a health care provider may ask her to provide a urine sample, which she collects into a sterile container while in the midst of voiding her bladder. Once collected, the urine can be tested for the presence of certain microbes—often by seeing what types and amounts of microbes will grow in a laboratory setting. However, these so-called "clean catch" midstream urine samples can be contaminated by microbes living near the opening through which urine leaves the body, potentially confusing the results. Also, there is some uncertainty about how well the values for microbial growth used for diagnosing UTIs from these samples actually reflect the presence or burden of UTI-causing microbes in the bladder itself.

To determine how well midstream void samples predict causative agents in UTIs, researchers recruited 202 healthy, premenopausal women with symptoms of acute UTI. They obtained two urine samples from each woman, one from the bladder (by using a catheter inserted into the bladder *via* the urethra, the tube that carries urine from the bladder to outside the body), the other from a midstream void, and then compared the presence and burden of microbes in these paired samples. The researchers found that UTI-causing microbes grew in 142 specimens of bladder urine and 157 specimens of voided urine. The majority (65 percent) of women with UTI symptoms had *E. coli* in their urine, and there was a strong correlation between voided and bladder urine, even at *E. coli* growth values below those currently used to diagnose UTI. In contrast, they found that presence in midstream urine samples did not accurately predict bladder infection by two other, commonly detected microbes thought to cause UTIs. These and other results suggest that testing of midstream void samples is best used for detecting and diagnosing the most common cause of UTIs, *E. coli*. This new information can help guide health care providers who treat UTIs. In addition, the fact that bladder urine samples from over a quarter of women with UTI symptoms did not yield any microbes under standard laboratory growth conditions serves as a reminder that there may be other microbes not identified by the tests, and/or non-infectious conditions with the same symptoms as UTIs.

Hooton TM, Roberts PL, Cox ME, and Stapleton AE. Voided midstream urine culture and acute cystitis in premenopausal women. N Engl J Med 369: 1883-1891, 2013.

Kidney Cell Response to Urinary Tract Infection Helps Halt Bacterial Growth: Researchers have identified a new bodily defense mechanism deployed in the fight against urinary tract infections (UTIs). UTIs are very common and affect more women than men. The leading cause of UTIs is exposure to uropathogenic *E. coli* bacteria, also referred to as UPEC. While antibiotics resolve many infections, recurrence is common, and antibiotic resistance is rising; a better understanding of the natural course of UTIs could help lead to new treatments. In addition to flushing bacteria and infected bladder cells out of the urinary tract through urination, the body employs innate defenses, such as production of antimicrobial molecules, to stymie UTIs. In a new study, scientists investigated a suspected defense molecule called lipocalin 2 (LCN2). It is known that LCN2 can arrest the growth of *E. coli* and certain other bacteria by limiting their access to the essential nutrient iron. The researchers found that levels of LCN2 were very high in the urine of patients suffering from UTIs and fell as the UTIs resolved. Through experiments in female mice, they confirmed that urine levels of LCN2 rose in response to infection with UPEC, and that mice lacking LCN2 cleared UTIs much more slowly. By tagging both mouse cells and UPEC with light-emitting molecules and other visually detectable markers, the researchers were able to track the primary source of LCN2 during an infection to cells in the kidney called α-intercalated cells. Mice lacking these kidney cells were less well able to

suppress UTIs. These specialized kidney cells help maintain the acid pH of urine; when mice were exposed to UPEC, these cells quickly acidified their urine even further, which appears to be yet another defense against bacterial infection. While the exact mechanism has yet to be determined, evidence from this study suggests that contact with molecules produced by UPEC and/or with the bacteria themselves stimulates these kidney cells both to produce the LCN2 defense molecule and to acidify the urine within hours of infection. Discovery of this acute response that effectively targets the most common cause of UTIs expands the role played by the kidney in innate defense against such infections, and could also provide new insight into why diseases that damage the kidney's α-intercalated cells leave patients more susceptible to UTIs.

Paragas N, Kulkarni R, Werth M…Barasch J. α–intercalated cells defend the urinary system from bacterial infection. J Clin Invest 124: 2963-2976, 2014.

INSIGHTS INTO INTERSTITIAL CYSTITIS/PAINFUL BLADDER SYNDROME

Brain Region Changes Associated with Symptoms in Women with Interstitial Cystitis: A new study suggests that enlargement of certain brain regions is associated with symptoms in women with interstitial cystitis. Interstitial cystitis (IC), also called IC/painful bladder syndrome (PBS), is a urologic chronic pelvic pain syndrome whose symptoms include urinary urgency, frequency, and pelvic pain. The causes and risk factors for IC are not well understood, and there is no fully effective treatment. Researchers in the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network are using a variety of scientific approaches to better understand IC and similar pain syndromes and lay the groundwork for development of clinical interventions to prevent and treat these conditions. An important aspect of chronic pain studies is the emerging understanding that different parts of the brain may become altered in a way that augments and/or maintains the experience of pain. Network researchers used a sophisticated brain imaging technique to determine whether there are differences in the volume of gray matter—the core component of brain tissue—between women with IC and those without, and whether those differences align with symptoms. Comparing results from 33 women with IC and 33 women without IC, the team found several brain regions in which women with IC had a significantly greater volume of gray matter, three of which are involved in pain processing. Further, the scientists found that greater gray matter volume in one pain-processing brain region, called the right S1, was associated with greater symptoms of overall pain, urinary urgency, and anxiety reported by women with IC on the day of the brain scan. This is the first study to show regional brain differences between women with IC and healthy counterparts. Future studies may help researchers understand the relationship between altered brain regions and pain sensitivity in IC patients, as well as the impact of other variables, such as co-occurring pain conditions and gender, on these brain changes.

Kairys AE, Schmidt-Wilcke T, Puiu T,…Harris RE. Increased brain gray matter in the primary somatosensory cortex is associated with increased pain and mood disturbance in interstitial cystitis/painful bladder syndrome patients. J Urol 193: 131-137, 2015.

Paving the Way to Improved Bladder Health in Women

Daily life can be full of endless possibilities and joys, from spending time with friends and family to pursuing one's own work and interests. Urine leakage, bladder infections and pain, and day to day management of bladder problems, as well their personal and fiscal costs, can hinder full participation in these activities and also potentially increase risk for other serious health conditions. The NIDDK is pursuing a new endeavor focused on prevention of conditions affecting the bladder and lower urinary tract in women, with the larger goal of improving overall health.

Framing the Problem: LUTS, Bladder Health, and Impact

A variety of problems can affect the bladder and the urethra, the tube that carries urine from the bladder to outside the body. These include urinary incontinence (UI), urinary tract infections (UTIs), overactive bladder, and interstitial cystitis/painful bladder syndrome (IC/PBS). Researchers and health care providers use the term lower urinary tract symptoms, or LUTS, as an umbrella term to include all symptoms associated with any type of lower urinary tract dysfunction or condition.

Women bear a disproportionate share of the burden of LUTS compared to men. Some LUTS conditions, such as UI, increase in prevalence with age, while others, such as UTIs, can strike in youth or young adulthood, and become recurrent. Thus, LUTS are important across the lifespan. For some conditions, such as IC/PBS, the risk factors for onset and progression are still under study; for others, some risk factors have been identified—for example, childbirth is among several factors known to increase a woman's risk of developing UI.

Many millions of Americans suffer from LUTS. The yearly costs of management and treatment have been estimated at over 20 billion dollars;[1,2] this estimate does not include costs from lost productivity. Bladder conditions are also intertwined in complex ways with other serious, chronic, and costly health conditions. For example, obesity is another risk factor for development of UI. However, women who develop bladder leakage may then reduce their physical activity, exacerbating risk of weight gain and potentially increasing risk for the metabolic disease, type 2 diabetes. Diabetes, in turn, increases risk for development of UTIs. LUTS also increase risk of depression, which can also exacerbate obesity and other conditions and contribute to reduced work productivity. In older women with UI, the potential for fractures and falls, including life-threatening hip fractures, also increases. The impact of LUTS is thus not restricted to bladder issues and symptoms, but is also felt in its negative effects on overall health.

An enormous challenge with LUTS is that much of what is known about underlying conditions comes from people who have sought treatment. Many women choose to tolerate these conditions for extended periods of time before seeking care. Reasons that have been given range from embarrassment and stigma to thinking that these conditions are a normal part of aging. In addition to extended suffering, later care-seeking limits some of the insights researchers and health care providers can gain about the causes, triggers, or inciting events for onset of symptoms.

Moreover, researchers and health care providers do not have a good handle on what is "normal," *i.e.*, what is the baseline for a healthy bladder across the lifespan? Instead, much of the focus has been on helping girls and women who are symptomatic and who seek treatment to address their symptoms as best as possible, with therapies that include medication, behavioral modifications, biofeedback, and surgery. An additional complexity in LUTS is that symptoms do not always indicate dysfunction, dysfunction does not necessarily cause symptoms (at least initially), and the connection between healthy bladder habits and presence or absence of symptoms is unknown.

Striking a New Path: Prevention

Until recently, the primary research and health care focus for LUTS has been on treating the problem. Now, the NIDDK is spearheading an effort to expand the research picture to include prevention and bring it to the forefront. Several factors have led the NIDDK to pursue this new research direction: as with obesity, research has suggested that many LUTS risk factors may be modifiable, and prevention possible. Further, the enormous burden of LUTS, the relationship of LUTS to other diseases and conditions, and the escalating costs anticipated—especially with an aging U.S. population—strongly suggest the value of trying to prevent onset or worsening of these conditions. At the same time, there is a lot to learn about what constitutes bladder health and dysfunction, and what approaches may be feasible in prevention. Several other NIH Institutes and Offices with interests in this area are helping to develop this new program focus, including the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, the National Institute on Aging, the National Institute of Nursing Research, the NIH Office of Research on Women's Health, the NIH Office of Behavioral and Social Sciences Research, and the NIH Office of Disease Prevention.

To help inform this new effort, in 2013 and 2014 the NIDDK organized several meetings with a spectrum of experts from the NIH and the external scientific and health care communities to obtain diverse opinions on research needs in women's urologic health overall, and specifically in the area of prevention research. This series of meetings culminated in a large scientific workshop on May 3-4, 2014, "Path to Prevention of Lower Urinary Tract Symptoms (LUTS) in Women: Bladder Health." This meeting gathered researchers, health advocates, policy makers, NIH scientific staff, and health care providers to discuss gaps in what is known and to provide input on research questions, strategies, and future directions to take as the new program moves forward. In particular, the NIDDK was seeking input on:

- Risk factor assessment and prioritization in age groups across the lifespan
- Construction of a population survey to obtain information about what women and girls know and do about their bladder and LUTS, which would then be used to develop research ideas to improve bladder health in women and girls
- Stakeholder groups relevant to research on bladder health and their potential roles in research and implementation of findings
- Taking action for public health awareness and engagement—who should be involved (delivery and audience), what activities and messages should be pursued, and how and when this should be done
- Clinical prevention intervention studies and designs in bladder and pelvic health to consider for the future

Experts were drawn from across multiple fields—not just urology and urogynecology, but also pediatrics, epidemiology, prevention research, behavioral science, physical therapy, nursing, aging, patient education, and other disciplines. Participants contributed a wealth of information from a variety of perspectives, and identified a broad spectrum of areas in which we have insufficient knowledge to determine the ultimate impact on bladder or overall health, or which can be considered as possible points of intervention to consider in research—for example, voiding behaviors and habits, work and school environments, and health beliefs.[3]

The input the NIDDK received at this meeting helped inform the development of a Request for Applications (RFA) on "Prevention of Lower Urinary Tract Symptoms in Women: Bladder Health Clinical Centers (PLUS-CCs)" (RFA DK14-004, now published). The purpose of this initiative—the next key step in program development—is to form a clinical research consortium focused on developing an evidence base for normal or healthy bladder function and identifying behavioral and other risk factors for conditions associated with LUTS. It is expected that this information will provide a foundation for future, prevention-focused intervention studies for LUTS in girls and women. It is anticipated that awards to establish this consortium will be made in late Fiscal Year 2015.

Leveraging What We Know: Summit on Urinary Incontinence

At the same time as the NIDDK is expanding its women's urologic health research program to include and emphasize prevention, the Institute has led efforts to assess what has been learned from NIH-supported trials of treatments for UI—a highly prevalent LUTS condition—in order to benefit patients. In March 2014, the NIDDK hosted a highly productive meeting of investigators from several completed NIH-supported clinical trials of treatments for UI to understand better how the results of the trials have been adapted into practice; to identify key messages that could be transmitted to patients not only by urology care specialists, but more broadly by other health care providers; and to elicit opinions on unmet needs in UI research that could help stimulate future studies on identification, diagnosis, and treatment.[4]

Notably, the NIDDK also supports a Symptoms of Lower Urinary Tract Dysfunction Research Network (LURN). The goals for this cooperative research network are to develop tools for better measurement of patient experiences of LUTS and to better define the phenotypes of men and women with symptoms of lower urinary tract dysfunction—*i.e.*, to determine what anatomical, physiological and psychological characteristics these individuals have that contribute to underlying functional changes that accompany LUTS. Characterization of patients with LUTS will enable the researchers to understand the underlying pathophysiology and such information could help identify specific subgroups of people suffering with LUTS by virtue of shared characteristics and thereby help to refine diagnoses and improve treatments. Currently, investigators at six sites are working on developing self-reported measures for LUTS and ways to assess other, non-urologic factors contributing to LUTS. Parallel efforts are ongoing to establish a large cohort of people with LUTS and to conduct neuroimaging studies and sensory testing in people with urinary urgency, frequency, and incontinence.

Looking to the Future

The new focus for the women's urologic health research program at the NIDDK is a long-term effort to cover gaps in knowledge, move toward primary and secondary prevention of LUTS in women, and develop evidence-based public health messages about bladder health. While the new efforts are targeted to women due to the disproportionate burden of LUTS conditions on women, LUTS do affect both men and women. It is anticipated that, as more is learned, prevention research efforts will expand to include LUTS in men.

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[1] *Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am J Med 113 Suppl 1A:5S-13S, 2002.*

[2] *Hu TW, Wagner TH, Bentkover JD, Leblanc K, Zhou SZ, and Hunt T. Costs of urinary incontinence and overactive bladder in the United States: a comparative study. Urology 63: 461-465, 2004.*

[3] *A summary of the "Path to Prevention" meeting is available on the NIDDK website at www.niddk.nih.gov/news/events-calendar/Pages/LUTS.aspx#tab-minutes*

[4] *A summary of the "Summit on Urinary Incontinence Clinical Research in Women" is available on the NIDDK website at www.niddk.nih.gov/news/events-calendar/Pages/summit-urinary-incontinence-clinical-research-women.aspx#tab-minutes*

UNDERSTANDING AND TREATING HEMATOLOGICAL DISEASES

Newly Identified Mutations Cause Rare Anemia: Recent research has identified previously unknown mutations in the *ALAS2* gene that cause X-linked sideroblastic anemia. X-linked sideroblastic anemia is an inherited disorder that prevents developing red blood cells (erythroblasts) from making enough hemoglobin, which is the protein that carries oxygen in the blood. Approximately two-thirds of X-linked sideroblastic anemia cases are due to mutations in the *ALAS2* gene. The known *ALAS2* gene mutations result in a change in an enzyme called erythroid ALA-synthase, which plays a critical role in the production of heme (a component of the hemoglobin protein). Thus, *ALAS2* mutations impair the activity of erythroid ALA-synthase, disrupting normal heme production and preventing erythroblasts from making enough hemoglobin. When not incorporated into heme, iron builds up in the erythroblasts in the bone marrow, and these abnormally iron-loaded cells are called "ring" sideroblasts. The symptoms of X-linked sideroblastic anemia result from a combination of reduced hemoglobin and the abnormal excess of iron. Ranging from mild to severe, the common features include fatigue, dizziness, a rapid heartbeat, pale skin, and an enlarged liver and spleen. Over time, severe medical problems such as heart disease and liver damage can result from the buildup of excess iron in these organs.

While studying the DNA from five families having a preponderance of X-linked sideroblastic anemia, investigators identified previously unknown mutations in the *ALAS2* gene. These mutations were discovered in a part of the gene called the enhancer—an element that helps "turn on" the *ALAS2* gene. Specifically, any of several changes in the enhancer sequence were sufficient to diminish *ALAS2* gene activation. This study's characterization of a new set of mutations may help diagnose those having X-linked sideroblastic anemia, who can then be treated.

Campagna DR, de Bie CI, Schmitz-Abe K,…Fleming MD. X-linked sideroblastic anemia due to ALAS2 *intron 1 enhancer element GATA-binding site mutations. Am J Hematol 89: 315-319, 2014.*

Potential New Treatment Option for Adults with Sickle Cell Disease: A new blood stem-cell transplant regimen effectively reversed sickle cell disease in 26 of 30 adult participants and allowed them to achieve stable mixed donor chimerism, a condition in which a person has two genetically distinct cell types in the blood. Also of importance, 15 of the 30 adults were able to stop taking immunosuppressant medications 1 year after transplantation. In sickle cell disease, sickle-shaped red blood cells block blood flow. The blockage can cause severe pain, organ damage, and stroke. Mature red blood cells arise from stem cells. Transplantation of blood stem cells, obtained from bone marrow or another source such as umbilical cord blood, has been used to cure children with severe disease. However, the medical procedures used for preparing patients for transplantation have thus far been too toxic to be used in adults.

In this new regimen, instead of using chemotherapy to destroy the person's bone marrow before infusing donor stem cells—as in the standard, prohibitively toxic procedure—the researchers used a low dose of radiation combined with two immunosuppressive drugs. This type of procedure is referred to as "non-myeloablative," meaning that it does not destroy a person's own bone marrow. Rather, it is thought to create "space" for the donor stem cells to successfully engraft. After undergoing the non-myeloablative procedure, the participants, who all had severe sickle cell disease, were infused with peripheral blood stem cells from healthy sibling donors. The researchers reported that the partial stem cell transplantation regimen effectively reversed sickle cell disease in the majority of adult participants, and half were able to stop immunosuppressant medications. These medications are typically given to transplant recipients to prevent the immune system from rejecting the transplanted donor cells and to prevent donor cells from attacking the recipient (graft-versus-host disease). However, immunosuppressant medications reduce immune system strength and can cause serious side effects such as infection and joint swelling. The researchers

reported that no graft-versus-host disease has been detected in patients after stopping immunosuppression medications at a median follow-up of 3.4 years.

This study represents an important advance to make a potentially transformative treatment available to a wider range of people, especially those who could not tolerate a standard stem cell transplant or long-term use of immunosuppressant medications.

Hsieh MM, Fitzhugh CD, Weitzel RP,…Tisdale JF. Nonmyeloablative HLA-matched sibling allogeneic hematopoietic stem cell transplantation for severe sickle cell phenotype. JAMA 312: 48-56, 2014.

ADVANCES IN BLOOD CELL BIOLOGY

Gaining Insight into Blood Stem Cell Generation: A recent study has illuminated the importance of a cellular protein called GATA2 in the generation and survival of blood (hematopoietic) stem cells in mice. Hematopoietic stem cells (HSCs) give rise to all the types of blood cells, including red blood cells, but the molecular mechanisms regulating the generative process are not completely understood. In contrast to HSCs, hematopoietic progenitor cells (HPCs) are relatively immature cells that are precursors to a fully mature (differentiated) cell of the same tissue type but that have only a limited capacity to differentiate into more than one cell type as HSCs do. As cells and tissues develop in the embryo, the process by which they take on specific characteristics involves cellular proteins called transcription factors, which regulate whether genes are "turned on" or "turned off." Consistent with a potential role in HSC generation, the transcription factor GATA2 is known to be present in cells implicated in the formation of hematopoietic tissue in the developing mouse embryo, but its function in the generation of HSCs is unknown.

Using a "conditional knockout" strategy, investigators sought to examine what, if any, role GATA2 plays in HSC generation. Through this genetic engineering approach, scientists can produce mice that lack a specific gene only under certain conditions—a so-called conditional knockout. This approach contributes valuable information about the normal function of a gene and its encoded product, such as GATA2, by allowing scientists to observe the consequences of its absence at specific times or developmental stages, and/or in particular tissues. The collective findings from this study indicate that GATA2 is required for HSC generation and HPC formation during embryonic development. In addition, GATA2 is required for survival of HSCs and HPCs in the developing embryo. Thus, these results highlight a unique role for GATA2 function in mouse embryonic hematopoiesis and should inform future regenerative approaches designed to treat blood diseases.

de Pater E, Kaimakis P, Vink CS,…Dzierzak E. Gata2 is required for HSC generation and survival. J Exp Med 210: 2843-2850, 2013.

New Insight into the Mouse Blood Stem Cell Aging Process: Recent research identified changes in blood (hematopoietic) stem cells (HSCs) that may contribute to age-associated loss of function. The HSC, a type of adult stem cell, holds great promise for future biomedical applications because of its ability to self-renew and develop into any kind of blood cell. However, previous research has shown that as the HSC ages, its capacity to develop into different types of blood cells diminishes. The mechanism(s) responsible for this change is not well defined.

New research has provided insight into the HSC's aging process by systematically evaluating the gene expression (whether genes are turned "off" or "on") in cells from both young and old male mice. For this study, the researchers used several techniques, including a process called transcriptome analysis, to determine the extent to which genes are on or off depending on the age of the HSCs. They also studied chemical modifications along the genome that affect gene expression. The genome is made up of DNA, a long, winding molecule that contains the instructions, in the form of genes, needed to build and maintain cells. For these instructions to be carried out, DNA must be transcribed into corresponding molecules of RNA, referred to as transcripts. A transcriptome is a collection of all the transcripts present in a given cell.

Often researchers can count the number of different types of transcripts in the transcriptome to determine the level of activity of different genes, also called gene expression, in a certain cell or tissue type. In humans and other multi-cell creatures, nearly every cell contains the same genes, but different cells show different patterns of gene expression. These differences in gene expression are responsible for the many different properties and behaviors of various cells and tissues as they experience health, normal aging, and disease. Cells also make various modifications along the genome, such as adding chemical "markers" to their DNA. Called "epigenetic" changes, these modifications affect gene expression.

A new analysis of mouse HSC transcriptomes and various epigenetic markers identified many genes that were expressed differently by old and young mice. Researchers found, for instance, that genes regulated by a growth factor called TGF-β showed differences in expression between young and old HSC cells. This finding suggests that there is less signaling by TGF-β in older cells. Previous research has shown that TGF-β helps control the growth and proliferation of cells, the process by which cells mature to carry out specific functions (differentiation), cell movement, and the self-destruction of cells. In addition, the researchers confirmed and extended previous studies by identifying epigenetic changes in young versus old HSCs that are consistent with the aging HSCs' inability to develop into other types of blood cells.

Taken together, this study provides a comprehensive analysis of the genomic properties of young and old mouse HSCs and suggests how changes in the stem cell during aging promotes self-renewal and hinders HSCs' ability to transition into other types of blood cells. This research provides new insight into the aging process and may be the basis for future treatments for aging-related disorders.

Sun D, Luo M, Jeong M,…Goodell MA. Epigenomic profiling of young and aged HSCs reveals concerted changes during aging that reinforce self-renewal. Cell Stem Cell 14: 673-688, 2014.

Getting Heme into Hemoglobin: A recent investigation determined that a gene called *Tmem14c* is required for heme production within the mouse red blood cell. Heme is an iron-containing molecule that is important for many biological processes. Heme combines with globin proteins to form hemoglobin, which carries oxygen in red blood cells from the lungs to the rest of the body. Notably, there is virtually no "free" heme in the human body due to the potent heme-scavenging system in the blood. Within cells, heme levels are maintained by a balance of heme production, degradation, and distribution. The mechanisms of heme production and degradation have been intensively studied over several decades. However, the critical steps and location within the red blood cell where heme production takes place are largely unknown.

Building on their previous finding that initially identified the *Tmem14c* gene as having a role in heme production in the mitochondrial compartment of the red blood cell, researchers mechanistically dissected the functional role of this gene using biochemical, molecular biology, cell biology, pharmacologic, and genetic methods. The *Tmem14c* gene was found to be "turned on" in mouse embryonic tissues such as the yolk sac, liver, bone marrow, liver, spleen, and blood vessels—yolk sac, liver, and bone marrow play a role in red blood cell production in the embryo. The investigators also reported that the TMEM14C protein was produced in embryonic liver. To learn more about its possible function, the researchers determined that the protein is found associated with mitochondria, the home of cellular energy production. The researchers then examined blood cells derived from mouse embryonic stem cells that had been modified to no longer contain a functional *Tmem14c* gene. The resulting *Tmem14c* deficiency caused a decrease in the percentage of hemoglobin-containing red blood cells. In mice, *Tmem14c* deficiency (resulting from the combination of both copies of the *Tmem14c* gene being non-functional) was found to be lethal in at the embryonic stage, evidence that this gene is essential for normal development. In contrast, mice with one

copy of the non-functional gene were viable and fertile and had normal levels of red blood cells.

To examine its potential role in heme production, the ability of embryonic liver tissue to produce heme was assessed in the absence or presence of a functional *Tmem14c* gene. The investigators reported a buildup of pre-heme molecules in the absence but not presence of *Tmem14c*—indicating that a functioning gene is required for complete heme production.

This study provides valuable insight into the role of *Tmem14c* in heme production and hemoglobin-containing red blood cells. Future studies may determine whether the human version of this gene contributes to fewer red blood cells than normal (anemia) in people with various blood disorders.

Yien YY, Robledo RF, Schultz IJ,…Paw BH. TMEM14C is required for erythroid mitochondrial heme metabolism. J Clin Invest 124: 4294-4304, 2014.

STORY OF DISCOVERY

FGF-23 Steps into the Spotlight as a Key Player in Phosphate Metabolism and Kidney Disease

While the kidneys are perhaps best known for their role in cleansing the blood, another one of their important functions is to regulate the levels of various salts and minerals in the blood. One of the consequences of chronic kidney disease (CKD) is that, in addition to a diminished ability to filter waste, the kidneys are less well able to maintain the balance of salts and minerals, which can in turn have wide-ranging impacts on overall health. One important pathway under the influence of the kidney involves the regulation of phosphate. A recently characterized member of the family of fibroblast growth factors (FGFs), FGF-23, appears to play an important role in regulating the metabolism of phosphate, and may play a previously unappreciated role in the initiation and progression of CKD. Evidence supporting this hypothesis includes data from studies of animals, individuals with CKD and kidney failure, and people with acute kidney injury, suggesting that this factor may represent a broadly acting regulator of phosphate metabolism that plays a key role in kidney function.

The Kidneys and Calcium and Phosphate Metabolism

Proper kidney function is essential for life; people whose kidneys have failed must undergo dialysis or receive a kidney transplant to survive. The supply of donor organs is much smaller than the need for them, so most people with kidney failure rely on dialysis to survive. While dialysis provides a life-saving form of kidney replacement for people whose kidneys have failed, most people with CKD will die before they progress to kidney failure, with cardiovascular disease the most common cause of death.

In addition to its well-known and critical role in waste removal, the kidney plays an important role in the regulation of the balance of calcium and phosphate, two elements that are key to maintaining normal bone metabolism, cardiovascular function, and many cellular signaling processes. Calcium is the most abundant mineral in the human body. Ninety-nine percent of it is found in bone, much of it bound with phosphate; the remaining 1 percent circulates in the blood, where it plays an important role in metabolism. While approximately 10 percent of a person's bone mass is degraded and rebuilt each year, circulating levels of calcium and phosphate are held relatively constant through a complex regulatory system.

An early sign of impaired kidney function is often a small decrease in levels of calcium circulating in the blood coupled with a small increase in circulating phosphate. In response to these changes, the parathyroid glands (located in the neck) secrete parathyroid hormone (PTH). This hormone acts on the bones, kidneys, and gut to produce an increase in circulating calcium and a decrease in circulating phosphate. As CKD progresses, calcium and phosphate levels continue to skew, and PTH levels rise further in an attempt to restore balance of these two important elements. Unfortunately, chronically elevated levels of PTH can result in devastating bone loss as the body turns to the skeleton as a source of calcium. This condition led to the concept in the early 1970s of the so-called "trade-off hypothesis," which proposed that a ratcheting-up of PTH in people with CKD, and its attendant consequences, was the "trade-off" the body made as it tried to maintain

close-to-normal mineral balance. This hypothesis grew out of research supported by the NIH's National Institute of Arthritis, Metabolism, and Digestive Diseases, which in 1986 would become the National Institute of Diabetes and Digestive and Kidney Diseases and the National Institute of Arthritis and Musculoskeletal and Skin Diseases.

Although modern therapies can address the problem of bone loss in people with CKD through dietary modification and drugs, there is significant evidence that long-term exposure to elevated levels of phosphate in the blood is in and of itself a risk factor for cardiovascular disease in these individuals. These risks include mineral deposits in the blood vessels and heart that can cause tissues to stiffen. Because of this, for many years there has been a keen interest in better understanding phosphate metabolism in its own right. While PTH is recognized as one of the key regulators of phosphate, there had long been a suspicion among many researchers that PTH alone could not explain all of the intricacies of phosphate metabolism, and that some other factor or factors must be playing a role in this process. In fact, this hypothetical factor was even given a name—"phosphatonin."

FGF-23 and Phosphate Metabolism

Much of current knowledge about calcium and phosphate metabolism grew out of fundamental research conducted over the past 40 years by researchers studying the regulation of cell growth. The cornerstone of this knowledge comes from studies of fibroblasts, cells comprising much of the tissue that provides physical and biochemical support to other tissues. Because they are relatively easy to grow in the laboratory setting, they have long been a favored experimental model for the study of the factors that influence cell growth. In the early 1970s, NIH-supported researchers showed that small amounts of a mixture of proteins isolated from pituitary glands in the brain could stimulate the growth of fibroblasts in culture. In the mid-1980s, scientists identified two distinct growth-stimulating proteins in this mixture, which they termed "acidic" and "basic" FGFs based on their chemical properties. Nearly 30 years later, over 20 additional members of the FGF family have been identified. They play roles in biological functions as diverse as embryonic development, cell differentiation, nerve cell survival, wound repair, and tumor growth.

The twenty-third member of the FGF family was identified in 2000. FGF-23 is primarily produced by bone cells. It has been shown to have an impact on mineral and salt metabolism. Initial studies of FGF-23's physiological role in humans occurred in the context of several varied rare diseases, including hypophosphatemia (abnormally low levels of phosphate in the blood), rickets (defective mineralization of bones), and osteomalacia (softening of the bones). All of these conditions are characterized by low phosphate levels and bone loss. These diseases were ultimately found to be associated with elevated levels of FGF-23. In one, the FGF-23 protein was normal, but simply overproduced. In another, a mutation in the *FGF-23* gene rendered the protein resistant to degradation. These findings represented some of the first studies to suggest a broad, direct role for FGF-23 in phosphate metabolism and disease, and that unregulated FGF-23 signaling might result in dangerous disruptions in phosphate levels that could have wide-ranging physiologic consequences.

Much of the knowledge about the molecular function of FGF-23 comes from studies conducted in mice in the early 2000s. Either the injection of FGF-23 or the implantation of cells producing high levels of FGF-23

results in markedly diminished phosphate levels in the blood and elevated levels of phosphate in the urine. Mice genetically engineered to produce high levels of FGF-23 show similar characteristics, as well as more widespread problems such as bone deformation. Conversely, mice engineered to lack the *FGF-23* gene display elevated phosphate levels in the blood and have calcium deposits in many organs, abnormal bone mineralization, and a shortened lifespan. Detailed mechanistic studies in mice supported by the NIH demonstrated that FGF-23 promotes secretion of phosphate by the kidney and increases calcium absorption in the gut. Interestingly, many of these functions are the same as those that had been attributed to the hypothetical "phosphatonin." While PTH retained an important role in the regulation of calcium and phosphate, it soon became clear that this picture was incomplete, and that FGF-23 was a key player in phosphate regulation.

FGF-23 and Kidney Disease

As FGF-23's central role in phosphate metabolism was being characterized through studies of animal models and rare human diseases, NIDDK-supported researchers began to extend and expand studies of FGF-23, especially in individuals in whom proper phosphate metabolism was compromised, such as people with CKD. They found that in early stages of kidney disease, FGF-23 levels increase as kidney function declines. In fact, FGF-23 levels begin to rise before clinically significant changes in calcium, phosphate, and PTH are detectable in the blood. It was proposed that a small, initial rise in circulating phosphate levels very early in kidney disease may trigger an increase in FGF-23 as the body prompts the kidneys to excrete the excess phosphate. This increase in FGF-23 levels seems to precede the previously observed increase in PTH levels in CKD patients.

FGF-23 and PTH reduce the activity of phosphate transporters in the kidney, leading to diminished phosphate reabsorption and increased phosphate excretion in the urine. As CKD progresses and circulating phosphate levels rise, FGF-23 levels in the blood gradually increase to try to restore mineral balance. As kidney function further declines, more FGF-23 is produced in response to subsequent increases in serum phosphate concentrations. One NIH-supported study indicated that by the time patients reach kidney failure, FGF-23 levels can be up to 1,000-fold higher than those seen in healthy people.

While increases in FGF-23 levels are associated with a decline in kidney function, it is not clear whether FGF-23 plays a direct, causative role in this progression. Nevertheless, several lines of evidence suggest that measuring circulating levels of FGF-23 in patients with early-stage kidney disease could yield valuable information regarding their prognosis. One study of over 200 people with nondiabetic kidney disease found that increased FGF-23 levels correlated with risk of kidney disease progression, and that this risk was related to the levels of FGF-23 in the blood. Among individuals who are starting dialysis, elevated FGF-23 levels are associated with an increased risk of death both during the first year and over the first 2 years. The ability to identify and stratify patients with CKD based on their initial levels of FGF-23 would provide valuable information to physicians, suggesting that individuals at greater risk of progression due to elevated levels of FGF-23 might benefit from more aggressive care.

Premature death from all causes, and from cardiovascular disease in particular, is higher in people with CKD than in healthy adults. In fact, individuals with CKD are much more likely to die than to survive long enough to progress to kidney failure. Cardiovascular disease is the leading cause of death

in people with kidney disease, and abnormally high levels of FGF-23 are associated with increased risk of cardiovascular disease in patients with CKD. Research supported by the NIDDK and other NIH components has shown that elevated FGF-23 levels are associated with an enlarged heart, which indicates that this muscle is working harder than it should to pump blood throughout the body. Support for the notion that these changes are a consequence of higher FGF-23 levels comes from NIDDK-supported experiments conducted in animals. Mice that received injections of FGF-23 developed enlarged left ventricles, suggesting that FGF-23 may actually cause this form of cardiovascular disease rather than simply be a byproduct of it.

FGF-23 and Acute Kidney Injury

In contrast to CKD, which usually progresses slowly over time, acute kidney injury (AKI), also called acute renal failure, is characterized by a relatively rapid loss of kidney function, usually over a period of several hours or days. The resulting inability to excrete waste products and maintain fluid and salt balance poses urgent health problems for patients and their physicians. AKI may arise from a number of causes, such as sepsis (a serious, whole-body inflammatory reaction caused by infection), decreased blood pressure, or kidney damage from drugs or other toxins. Even though most people with AKI will regain some degree of kidney function, many do not, and this medical condition is associated with high in-hospital mortality rates. There is no effective drug therapy to reverse AKI. The goal of treatment—which may include dialysis along with other approaches—is to prevent fluid and waste from building up in the body while waiting for the kidneys to resume functioning.

The first suggestion that FGF-23 might play a role in AKI came in 2010. An individual was admitted to the hospital and diagnosed with AKI. Analysis of his urine showed that his FGF-23 levels were more than six times higher than normal. A subsequent NIH-supported study of 12 people with AKI found significantly higher than normal FGF-23 levels. The degree of the elevation correlated with severity of AKI and those with particularly high FGF-23 levels were more likely to die. Another NIDDK-supported study of 30 people with AKI found similar results. Those with elevated FGF-23 levels were more likely to require dialysis and die. More recently, an NIH-supported analysis of data collected from more than 3,000 people over the age of 65 as part of a study of risk factors for cardiovascular disease found that higher FGF-23 levels were associated with greater risk of hospitalization for AKI over a 10-year period.

For over 40 years, researchers and physicians have thought of AKI as a condition with causes and consequences distinct from those of CKD. However, more recent analyses have suggested that both conditions, rather than being two distinct phenomena, may in fact lie on a continuum. This hypothesis proposes that these two conditions differ not so much in their fundamental nature as they do in the speed with which they emerge. Both are characterized by a loss of kidney function and each is a risk factor for the other: people who have experienced a bout of sudden AKI and who recover are at increased risk of developing CKD in the future; reciprocally, individuals with slow-developing CKD are at increased risk of AKI as their disease progresses. Furthermore, both AKI and CKD place individuals at higher risk of the subsequent development of cardiovascular disease, kidney failure, and premature death. Evidence that FGF-23 might be involved in AKI as well as in CKD, either as a marker for disease severity or prognosis or as an active contributor to the disease process, further strengthens the argument that this factor plays a key role in the maintenance of normal kidney function.

STORY OF DISCOVERY

FGF-23: Biomarker, or More?

In recent years, there has been much enthusiasm regarding the potential benefits of biomarkers, which are molecules that can be easily detected and measured that may be indicators of an underlying condition that is otherwise difficult to evaluate. There seems to be fairly strong evidence that FGF-23 represents, at the very least, a potentially valuable biomarker for kidney disease initiation, prognosis, and progression. If FGF-23 can be validated as a biomarker for kidney disease—either in CKD, AKI, or both—increases in its levels could enable physicians to detect CKD early in the course of the disease using a simple blood test before overt symptoms appear and before irreversible organ damage occurs. Increased levels of FGF-23 could also allow doctors to more accurately assess the prognosis of patients with AKI. Alternatively, FGF-23 levels and trends over time could allow physicians to predict a given person's likely clinical path. Those with more dire prognoses could be treated earlier or receive more aggressive therapy, allowing a more personalized approach to treatment. Indeed, a recent study conducted as part of the NIDDK-supported Chronic Kidney Disease Biomarkers Consortium reviewed the records of more than 13,000 healthy volunteers who enrolled in a clinical study between 1990 and 1992, and found that people with higher levels of FGF-23 when they first entered the study had an increased risk of kidney failure over the subsequent 20 years. This correlation was seen across all people, regardless of their age, race, and kidney function at the time that they enrolled.

More research is needed to elucidate the ways in which FGF-23 exerts its multiple effects. If FGF-23 can be conclusively linked to specific disease mechanisms as a causative agent in CKD progression or its complications—rather than merely an indicator of it—this protein's impact could be quite profound. Studies in mice suggest that elevated levels of FGF-23 may play a direct role in the development and progression of CKD rather than simply be a by-product of the disease. Pilot studies in humans are currently testing medications to lower circulating levels of FGF-23 and phosphate in patients with moderate CKD in the hopes of developing strategies to lower the risk of complications. Approaches that inhibit FGF-23 action might prevent, lessen, or slow damage to the kidneys and vasculature of patients with CKD. From these studies, new insights into CKD initiation and progression may be found, new drug targets may be identified, and new treatment approaches may be developed.

Scientists have progressed a long way from a time when they felt the need to propose a hypothetical "phosphatonin" to explain the complex regulation of phosphate metabolism, to the current day, when they are characterizing the role of FGF-23 in these key physiological processes.

SCIENTIFIC PRESENTATION

Sex Differences in Kidney Disease

Dr. Sharon Anderson

Dr. Sharon Anderson is the Interim Chair of the Department of Medicine at the Oregon Health and Science University (OHSU). Her research interests include the role of high blood pressure in kidney disease and mechanisms of progression of chronic kidney disease, including diabetic nephropathy, the aging kidney, and polycystic kidney disease. She is an active physician, researcher, and educator.

Dr. Anderson received her M.D. from Louisiana State University Medical Center. After internal medicine residency training at OHSU, she completed her clinical nephrology training at the Beth Israel Deaconess Medical Center and her research training at the Brigham and Women's Hospital, Harvard Medical School. She is a past President of the American Society of Nephrology, and was the first woman to lead the Society when she assumed the position in 2009.

At the September 2014 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Anderson reviewed current knowledge regarding the ways in which kidney disease can affect males and females differently, and shared the results of recent studies of these differences.

Males and females differ in anatomy, but there are important physiological differences between the sexes as well. One of the more striking ones is the observation that certain diseases are much less severe in women than in men. Studies have suggested that this may arise, to some degree, as a consequence of a beneficial effect of the female sex hormone estrogen, which is present in much higher levels in women; or to a negative effect of testosterone, the male sex hormone. Previous data indicate that estrogen may have a protective effect in the eye, brain, bones, and, possibly, the cardiovascular system. However, as recently as 2002, there was very little information about whether estrogen may play a role in kidney disease in humans.

Sex differences in chronic kidney disease have been long recognized. As long ago as the early 1950s, it was known that male rats placed on a high-protein diet developed increasing levels of protein in their urine—a sign of kidney damage—over their lifespan. However, female rats placed on the same diet did not show similar signs of kidney damage. Further experiments suggested that this difference was related, at least in part, to the rats' respective sex hormones. Male rats on the high-protein that had been castrated surgically—and thus no longer produced biologically significant levels of the male sex hormone testosterone—had much lower levels of protein in their urine when placed on the high-protein diet. This protective effect was reversed if the castrated rats were given injections of testosterone. Similarly, female rats placed on the high-protein diet that were given testosterone had urine protein levels that were similar to those of normal male rats. This led to the notion that testosterone might have a detrimental effect on kidney health, and/or that estrogen might have a beneficial, protective effect.

Dr. Anderson noted that chronic kidney disease arising from several causes—including IgA nephropathy, membranous glomerulopathy, focal and segmental glomerulosclerosis, polycystic kidney disease, and

kidney disease of unknown origin—progresses faster in men than in women. The notable exception to this pattern is seen in women with diabetic kidney disease, whose kidney disease progresses at a rate similar to that seen in men. Why this is so is not clear, but it may relate to the fact that women with this form of kidney disease tend to be older and post-menopausal, and therefore they produce much lower levels of estrogen.

Sex Differences in Animal Models of Polycystic Kidney Disease

Dr. Anderson turned to experiments that she and her colleagues have conducted in a rat model of polycystic kidney disease (PKD). Autosomal dominant PKD (henceforth simply "PKD") is the most common genetic cause of chronic kidney failure. PKD is characterized by the growth of numerous fluid-filled cysts in the kidneys. Over time, growth of these cysts results in enlarged kidneys in which normal tissue is displaced and kidney function is impaired, sometimes quite severely. The first rat model of PKD was identified in 1989 and characterized in 1994. The Han:SPRD rat line was discovered by happenstance; a spontaneous mutation in a line of rats produced animals that developed cystic kidney disease that closely resembled PKD in humans. This rat model replicates much of the disease's natural history and has become a valuable model system to study PKD.

In the Han:SPRD rat model, estrogen appears protective against cystic kidney disease, with males developing more and larger cysts than females. Male rats with cystic disease exhibit diminished filtering capacity in their kidneys compared to normal rats, and this can be partly reversed by removal of endogenous testosterone by castration. Males also showed higher blood pressure and poorer values for other markers of kidney function. In contrast, female rats with cystic disease have filtering capacity that is normal, but show a modest loss of this after their ovaries are removed.

The researchers next turned their efforts to trying to understand whether estrogen-like hormones also exerted a protective effect. Estrogen in the body can be converted to alternate forms that can have different effects on cells. Two such molecules are 2-methoxyestradiol (2-ME) and 2-hydroxyestradiol (2-OHE), which appear to share many of the beneficial effects of estrogen but may be safer for clinical use. These estrogen metabolites have a protective effect on the kidneys in some models, so the scientists were curious as to whether the compounds would slow the growth of cysts and/or preserve kidney function in male rats with cystic kidney disease.

When these two compounds were tested in male Han:SPRD rats, the results were surprising. In the study, the volume of the kidney occupied by cysts was greatest in the untreated male rats and slightly lower in those that had been castrated, as previously shown. Treatment with 2-ME showed a reduction similar to castration, but treatment with 2-OHE reduced cyst volume significantly. Similar results were seen when kidney filtering capacity was measured, with 2-OHE showing a larger beneficial effect than either castration or 2-ME. The mechanism through which this effect is mediated is not known, but these results suggest that some estrogen metabolites may represent a novel, safe intervention to slow progression of PKD in males.

Patients with PKD often develop cysts in their livers, as well. In contrast to what is seen in the kidneys, however, where females have less pronounced cyst growth, the opposite is seen in the liver. The number and size of liver cysts in people with PKD is associated not only with age and severity of kidney cysts but also with female sex and number of pregnancies,

suggesting that exposure to estrogen might promote cyst growth in the liver while inhibiting it in the kidneys.

Sex Differences in Acute Kidney Injury

Dr. Anderson next spoke about a possible role for sex hormones in acute kidney injury (AKI). This condition (also called "acute renal failure") is a serious medical condition characterized by a relatively rapid loss of kidney function, usually over a period of several hours or days. It is a relatively common complication among hospitalized patients. Even though a significant fraction of patients with acute kidney injury will regain kidney function, many do not, and this medical condition is associated with high in-hospital mortality rates among the critically ill. AKI is more common in older patients and non-White patients; rates are also higher in men than in women.

Of all of the cases of AKI during hospitalization, surgical patients undergoing heart surgery are at particular risk. There are multiple animal models to study AKI, but a colleague of Dr. Anderson's used one that had not previously been used to study this condition. In this model, cardiac arrest is induced in a mouse through the injection of potassium chloride. With the circulation of blood halted, cells and tissues become starved for oxygen and begin to die. Ten minutes later, drugs are administered and chest compressions are begun to restart the heart. In the AKI study using this model, 24 hours after they recovered, the animals' kidneys were assayed for function and structural damage.

The results suggested that estrogen protected against AKI in mice that underwent cardiac arrest: kidney damage was much less severe in female mice than in males. Further support for this conclusion was seen in experiments in which female mice that had had their ovaries removed showed more severe kidney damage than those that had not following cardiac arrest, but injection of estrogen to such mice prior to the experiment averted kidney damage. This finding supports the contention that estrogen can protect the kidneys when their supply of oxygen is interrupted.

Mechanism of Estrogen Protection

The effect of estrogen on cells is mediated in general through at least two known estrogen receptors (ER), ERα and ERβ. ERα has been linked to rapid cellular responses that do not require gene activation; ERβ is more closely associated with responses to vascular and likely other tissue injury that do require gene activation. ERα receptors are the predominant form of the receptor found in the female kidneys, and ERβ receptors are the main form in the male kidney. However, the protective effect of estrogen in the cardiac arrest AKI mouse model did not depend on the presence of either of these estrogen receptors, as female mice lacking either the ERα or the ERβ genes still were protected from most of the AKI damage by estrogen. Moreover, treatment with a drug that blocks the binding of estrogen to both ERs did not reverse the protective effect of estrogen. Estrogen also did not appear to work through another known receptor, GPR30. These observations suggest that the protective effect of estrogen in kidneys might be mediated by a yet unknown pathway, perhaps through the direct interaction between the hormone and other cellular molecules.

Sex Differences in Biological Research and Implications for Medical Care

Dr. Anderson closed by noting that the vast majority of medical research in the past was conducted in cells or animals that were either male or whose sex was not reported. The recent observations

about sex differences in kidney disease underscore the importance of efforts to move toward a more personalized way of practicing medicine. She recommended that future studies should analyze data for differences based not only on sex and gender, but also on age, race/ethnicity, and changes that occur over time—and combinations of these factors. As more is learned, physicians may develop therapeutic approaches for kidney disease based on a patient's individual profile, perhaps choosing early interventions in those at risk of developing disease; or selecting more aggressive treatments in those at risk of rapid progression; and tailoring the treatment to the patient's unique genetic, environmental, and behavioral situation. Using this approach, researchers and doctors will be able to do a better job of deploying the tools they have today while developing better treatments for tomorrow.

On May 14, 2014, Janine A. Clayton, M.D., Director of the NIH Office of Research on Women's Health (ORWH) and Francis S. Collins, M.D., Ph.D., Director of the National Institutes of Health, published a commentary in the journal Nature *entitled "Policy: NIH to balance sex in cell and animal studies." Drs. Clayton and Collins announced that the NIH plans to address the issue of sex and gender inclusion across biomedical research through oversight, review, and policy, as well as through collaboration with various stakeholders. For details and updates about this issue and NIH efforts, please see the ORWH webpage "Studying Sex to Strengthen Science (S4)" at http://orwh.od.nih.gov/sexinscience/*

PATIENT PROFILE

For Capt. John "Jack" Sautter, An Unexpected Diagnosis Becomes a Call to Action

After Discovering That He Had Polycystic Kidney Disease, a Marine Seeks a Better Future for Himself and Others

Jack Sautter and daughter Katherine

There are many ways to describe John Sautter. First of all, call him Jack. Jack is a Captain in the U.S. Marines and a third generation veteran. He is a lawyer, working as a prosecutor in the Marine Corps. He holds a Ph.D. in political science. He is also a proud son, brother, husband, and father. Until a little over 7 years ago, Jack was also the picture of perfect health, or so he thought.

In one day, though, his life changed forever; he was diagnosed with polycystic kidney disease (PKD), a disease that has been traveling in his family for at least four generations. Rather than simply accept the diagnosis as an insurmountable obstacle, Jack approached this new challenge the same way he had approached every other one he'd faced: he searched for ways to address it. One path he chose was to volunteer for an NIDDK-supported clinical trial of treatment options for people with PKD and, in doing so, try to build a better life not only for himself but for all people—both now and in the future—with the disease.

Diagnosis

On a crisp fall day in 2007, a then 29-year old Jack enjoyed a spirited rugby game with some friends from law school, followed by a backyard barbeque. He returned home, tired from the evening's activities, and tumbled into bed. When he woke the next morning, his foot was in so much pain that he could barely walk. "I thought I'd broken my foot," playing rugby the previous day, he says. He wasn't particularly worried, though, as he'd injured the same foot playing football years earlier. Still, he thought it best to have it checked out and headed off to the local hospital's emergency room.

"I thought I'd broken my foot," playing rugby the previous day, Jack says.... "I did not go to the hospital thinking that I was going to be diagnosed with PKD."

After a brief physical exam and evaluation, the pain in Jack's foot was diagnosed as gout, which surprised him. Gout is caused by the deposition of crystals

of uric acid in the joints—usually in the extremities, commonly in the toes—which causes inflammation and sometimes quite severe pain. These crystals form as a consequence of elevated levels of uric acid in the blood. Usually, uric acid remains dissolved in the blood and is filtered and excreted by the kidneys. High levels of circulating uric acid are sometimes, but not always, an indication of underlying kidney problems.

As the emergency room staff asked questions about Jack's medical history, he told them that several members of his family, including his father, had been confirmed to have PKD, and several other relatives were thought to have had it. "The doctors rolled in an ultrasound machine and held it up to my kidneys…and I could see the cysts, right there." This unexpected news was traumatic and scary, and his initial reaction was one of "panic and fear." He adds, "I did *not* go to the hospital thinking that I was going to be diagnosed with PKD." In that instant, Jack went from a self-described "carefree, young, rugby-playing Marine officer who could do anything, accomplish anything" to someone whose "life was inexorably changed."

There was another reason why this diagnosis was particularly unsettling for Jack. Cardiovascular disease is often seen in people who have longstanding PKD, and it had been just 3 years since his father had passed away after a heart attack. He was 56 years old when he died.

Polycystic Kidney Disease

PKD is a genetic disorder characterized by the growth of numerous fluid-filled cysts in the kidneys. There are two main forms of PKD. The most common is autosomal dominant PKD, which is the kind in Jack's family. Symptoms usually develop between the ages of 30 and 40, but they can begin earlier, even in childhood. In the United States, about 600,000[1] people were estimated to have PKD in 2000, and cystic disease is the fourth leading cause of kidney failure.

In most cases of autosomal dominant PKD (henceforth referred to simply as "PKD"), the slow progression of cyst growth can go unnoticed for many years. Many people with early-stage PKD have no symptoms, and their physical condition appears normal. The cysts, which can number in the thousands, can profoundly enlarge the kidneys while replacing much of their normal structure, resulting in reduced kidney function and potentially leading to kidney failure and a host of other health problems. Jack says that, when his father was in his 50s, his kidneys were estimated to be the size of small footballs. "You could actually see his kidneys bulging out of his sides," he adds.

Many people with PKD experience a decline in their kidney function as the cysts grow, and about one-half of them progress to kidney failure and require dialysis to live. High blood pressure is another common health problem for people with PKD. In most people with PKD, high blood pressure appears by age 20 or 30; it can lead to serious cardiovascular complications such as heart attack or stroke, both of which contributed to Jack's father's death. Other complications of PKD include urinary tract infections, blood in the urine, and kidney stones.

Jack Sautter Takes Action

Fortunately for Jack, tests at the hospital revealed that his kidney function was normal, suggesting that his PKD had been detected before serious damage had occurred. After the shock of his diagnosis had passed, "I started doing what everybody does in the modern age: I jumped onto the Internet and started reading everything I could." He scoured the websites

of hospitals, news organizations, and even blogs in an effort to learn as much as he could about the disease.

In November of 2007, Jack found a set of slides on the website of Tufts University School of Medicine. The presentation was from a conference that the school had hosted on PKD; these particular slides talked about a clinical trial called HALT-PKD. He thought, "Wow, this is really fascinating!" At the time, Jack was attending law school in Vermont; Tufts is located in nearby Boston, Massachusetts. When he discovered that Tufts was one of the sites participating in the trial, he located contact information for the university's HALT-PKD site and picked up the phone. Shortly after that conversation, he had enrolled in the study.

The HALT PKD Trial

Launched in 2002, the HALT-PKD trial enrolled two groups of volunteers based on their kidney function: those with relatively healthy kidneys were enrolled into "Study A," and those with more advanced disease and diminished kidney function were enrolled into "Study B." Jack entered "Study A," which recruited 558 volunteers with early-stage PKD at seven medical centers around the country. This study tested whether drugs that target the renin-angiotensin system—an important regulator of blood pressure and fluid balance—could slow the progression of the disease.

In the HALT-PKD Study A, the volunteers were given oral medications aimed at lowering their blood pressure, which is expressed as a higher number "over" a lower number. Half were assigned to a group with the goal of achieving "standard" blood pressure, defined as between 120 to 130 over 70 to 80. The other half targeted a lower blood pressure, between 95 to 110 over 60 to 75; while lower than the "standard" target, this is still within the normal range. Within each group, the participants were started on either a single drug or two drugs to reach their goal. (Those receiving a single drug received a placebo pill as their second "medication.") Jack was randomly assigned to the low blood pressure group. "I was hoping to be assigned to this group," he says. "I thought that perhaps I could benefit from the study by having lower blood pressure," although at the time he signed up he had no way of knowing what the outcome of the trial might be.

Jack was an enthusiastic volunteer in the HALT-PKD study. Even a 7-month deployment to Helmand province in southern Afghanistan from October 2011 to May 2012 could not prevent him from continuing to participate.

Throughout the trial, Jack and his fellow study participants had regular check-ups, provided blood samples every 6 months, and underwent magnetic resonance imaging (MRI) of their kidneys to monitor cyst growth several times. Jack was an enthusiastic volunteer. Even a 7-month deployment to Helmand province in southern Afghanistan from October 2011 to May 2012 could not prevent him from continuing to participate. He asked his physician in the United States to forward his medical records to the unit's battalion surgeon, who arranged for Jack to visit the local combat hospital. There, he was able to provide blood samples that were analyzed on site; the results were forwarded to the HALT-PKD investigators at Tufts. When Jack returned home, he continued his participation in HALT-PKD until the trial's end in 2014.

Data collection in HALT-PKD ended in the late summer of 2014. The investigators spent several months analyzing the data, and the results of the trial were

announced at the annual scientific meeting of the American Society of Nephrology in November 2014 (see below for more information). Jack is "really looking forward" to learning the results, because they may benefit not only him personally, but also his family members and the larger numbers of people with PKD.

PKD and the Sautter Family: Past, Present, and Future

PKD has a long history in the Sautter family. Jack's great-grandmother, a member of the first generation of the family to be born in this country, is thought to have had PKD, although she was never formally diagnosed. His grandfather, an Army pilot in World War II, had PKD. His father, a 20-year Army veteran, had it. Jack is acutely aware of the toll exacted by advanced PKD, as he had helped care for his father during the last years of his life. Speaking of him, Jack says, "A lot of things that slowed him down and made life more difficult for him were related to PKD." These experiences made Jack acutely aware that PKD affects not only the people with the disease themselves, but their families and caregivers as well.

After a tour in Okinawa, Japan and Hawaii, Jack is currently stationed at Camp Pendleton in California, where he lives with his wife and 2-year-old daughter. When he reflects on his participation in the HALT-PKD trial, he knows that, even if the findings from the trial do not directly help him in the near term, his participation in this research might help other people with PKD in the future. While he says that one motivation for entering the trial was that it might benefit him, he is quick to add, "Like in all things, our decisions are not as simple as just one motive; there are lots of motives…. It wouldn't shock me if my daughter has PKD." And, while he hopes that she stays healthy, if she is someday diagnosed, "hopefully, she will benefit from this research." He adds, "That was definitely on my mind when I was trying to become a part of HALT-PKD and continuing to do the study."

The NIDDK and the PKD Foundation sponsored the HALT-PKD trials, which consisted of two treatment trials for autosomal dominant polycystic kidney disease. HALT-PKD was the largest and longest study of treatments for this condition. The results were initially reported at the annual scientific meeting of the American Society of Nephrology in November 2014, and were published as this document was going to press. More information about the study outcomes is at www.nih.gov/news/health/nov2014/niddk-17.htm

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[1] *Grantham JJ, Nair V, Winklhofer F. Cystic diseases of the kidney. In: Brenner BM, ed. Brenner & Rector's The Kidney. Vol. 2. 6th ed. Philadelphia: WB Saunders Company; 2000: 1699-1730.*

Funding Trends and Support of Core Values

NIDDK's core values emphasize maintaining a strong investigator-initiated R01 program, preserving a stable pool of talented new investigators, supporting key clinical studies and trials, and continuing strong support of training and career development programs, consistent with the vision of NIDDK Director, Dr. Griffin P. Rodgers (see Director's Message).

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

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 and posted these data on this site. The data shown here were more recently updated to include Fiscal Year 2014. The NIDDK will continue to update these charts as new data become available.

NIDDK FUNDING OUTCOMES FOR FISCAL YEAR (FY) 2014 AND HISTORICAL APPLICATION AND FUNDING TRENDS

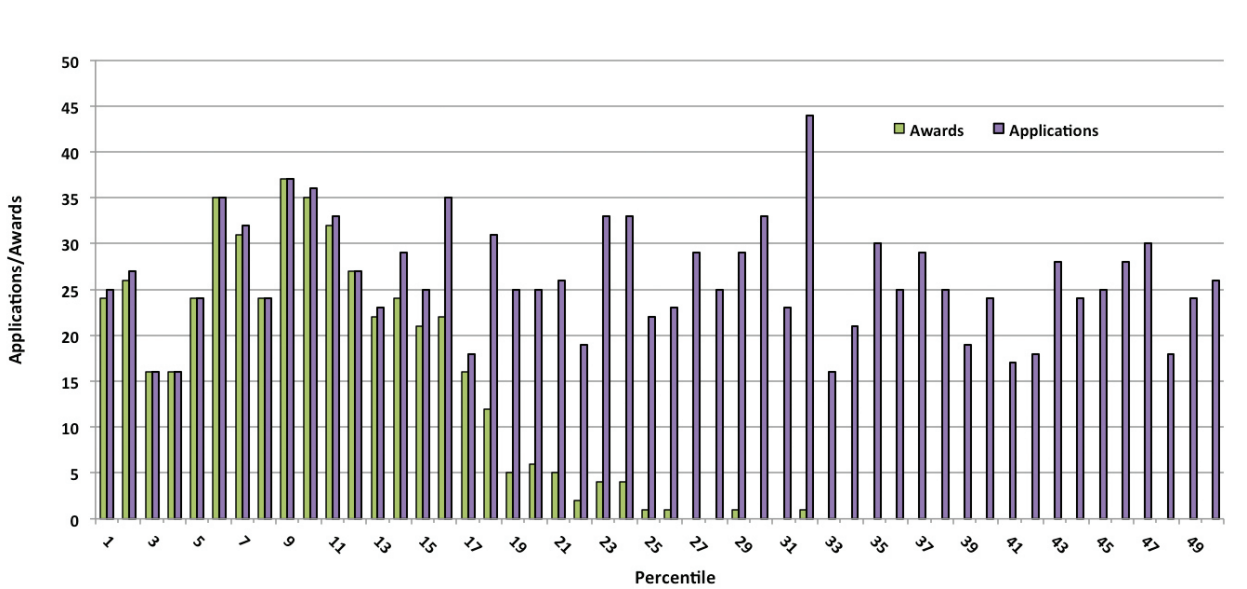
With the exception of Figure 8 (which includes initiative data), the data in the following charts exclude initiatives (*i.e.*, Requests for Applications), grants funded through the *Special Statutory Funding Program for Type 1 Diabetes Research*, and funds appropriated through the American Recovery and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

GRANT ACTIVITIES

Code	Title	Code	Title
K01	Research Scientist Development Award – Research and Training	R13	Conference
K08	Clinical Investigator Award (CIA)	R15	Academic Research Enhancement Awards (AREA)
K23	Mentored Patient-Oriented Research Career Development Award	R18	Research Demonstration and Dissemination Project
K24	Midcareer Investigator Award in Patient-Oriented Research	R21	Exploratory/Developmental Grant
K25	Mentored Quantitative Research Career Development Award	R24	Resource-Related Research Project
K99	Career Transition Award	R34	Planning Grant
P01	Research Program Project	R37	Method to Extend Research in Time (MERIT) Award
R00	Research Transition Award	SBIR/STTR	Small Business Innovation Research Grants/ Small Business Technology Transfer Grant
R01	Research Project	T32	Institutional National Research Service Award
R03	Small Research Grant		

FIGURE 1

FIGURE 1: NUMBER OF NIDDK COMPETING R01 APPLICATIONS SCORING WITHIN THE TOP 50TH PERCENTILE AND NUMBER OF NIDDK COMPETING R01 APPLICATIONS FUNDED IN FY 2014.

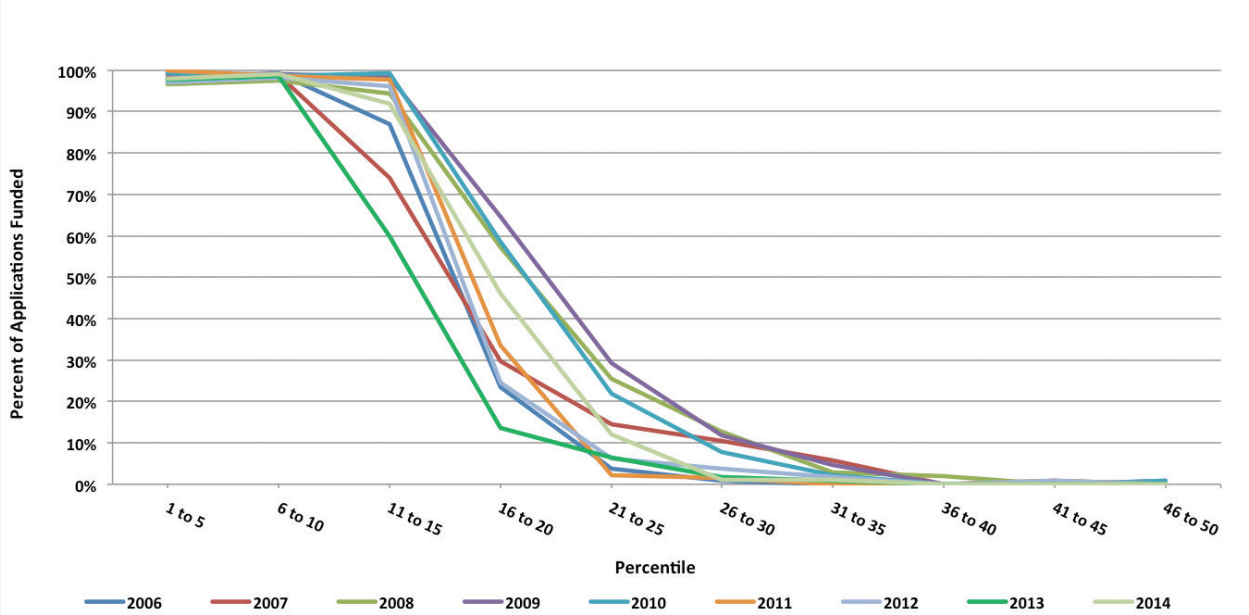


Note: "Applications" shown in the chart above include all applications that scored 50th percentile or better. Unscored applications, scored applications with no percentiles, and applications scoring above the 50th percentile are not shown (46 percent [n=1,137] of the applications received were unscored or scored above the 50th percentile). No unscored applications were funded in FY 2014.

The NIDDK nominal payline in FY 2014 for the vast majority of R01 applications was the 13th percentile for established investigators and the 18th percentile for Early Stage New Investigators. 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 closely adheres to its payline but does exercise some programmatic discretion to reach for a limited number of especially innovative or programmatically important applications.

FIGURE 2

FIGURE 2: NIDDK COMPETING R01 APPLICATION FUNDING CURVES FOR FY 2006-2014.



To generate the data for Figure 2, applications were placed into "percentile bins" as follows: Bin 1-5 includes all applications with percentile scores from 0.1 to 5.0, Bin 6-10 includes applications with percentile scores from 5.1 to 10.0, *etc*. Only applications that scored 50th percentile or better were included in the analysis.

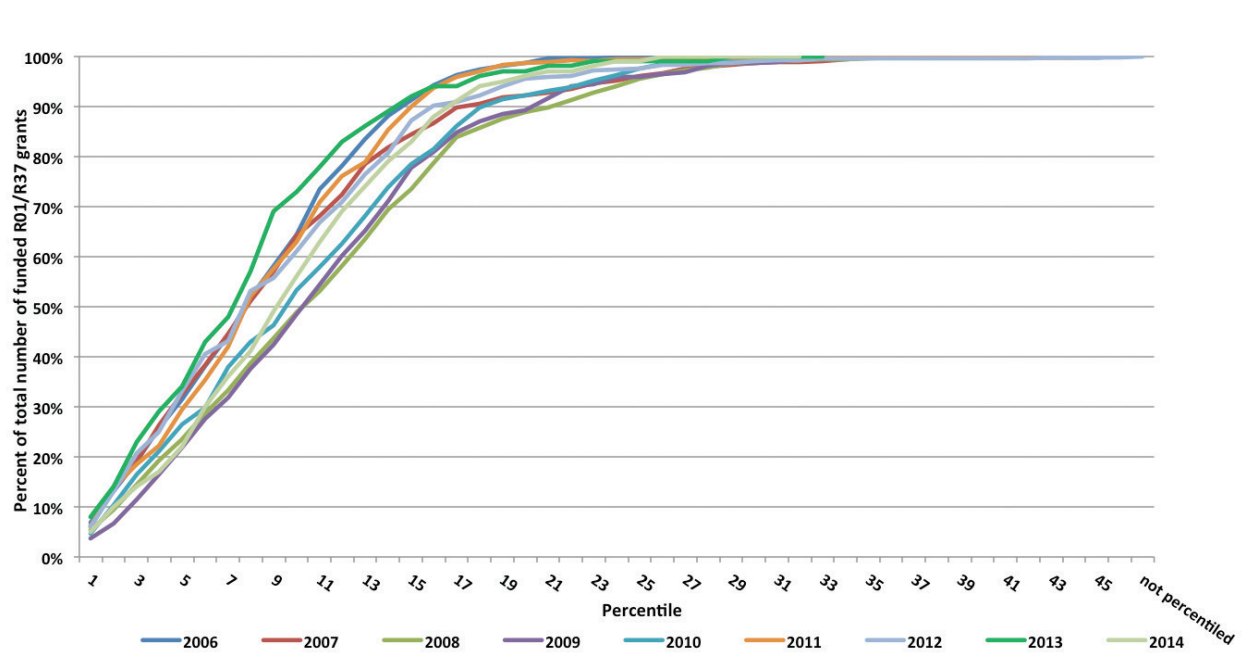
The data demonstrate steep deflections in the percentage of applications funded at the nominal payline for each year. Paylines for the years included in Figure 2 are shown in the table to the right.

Note: In FY 2012, the NIH and the NIDDK began focusing on Early Stage Investigators (ESIs; see definition of and benefits conveyed to ESIs on the NIH "New and Early Stage Investigator Policies" webpage at *http://grants.nih.gov/grants/new_investigators/index.htm*), which is a subset of New Investigators (see also Figures 11 and 12).

Fiscal Year	General Payline	New Investigator*/ Early Stage Investigator Payline**
2006	14	16*
2007	13	15*
2008	17	19*
2009	17	19*
2010	17	19*
2011	15	17*
2012	13	18**
2013	13	18**
2014	13	18**

FIGURE 3

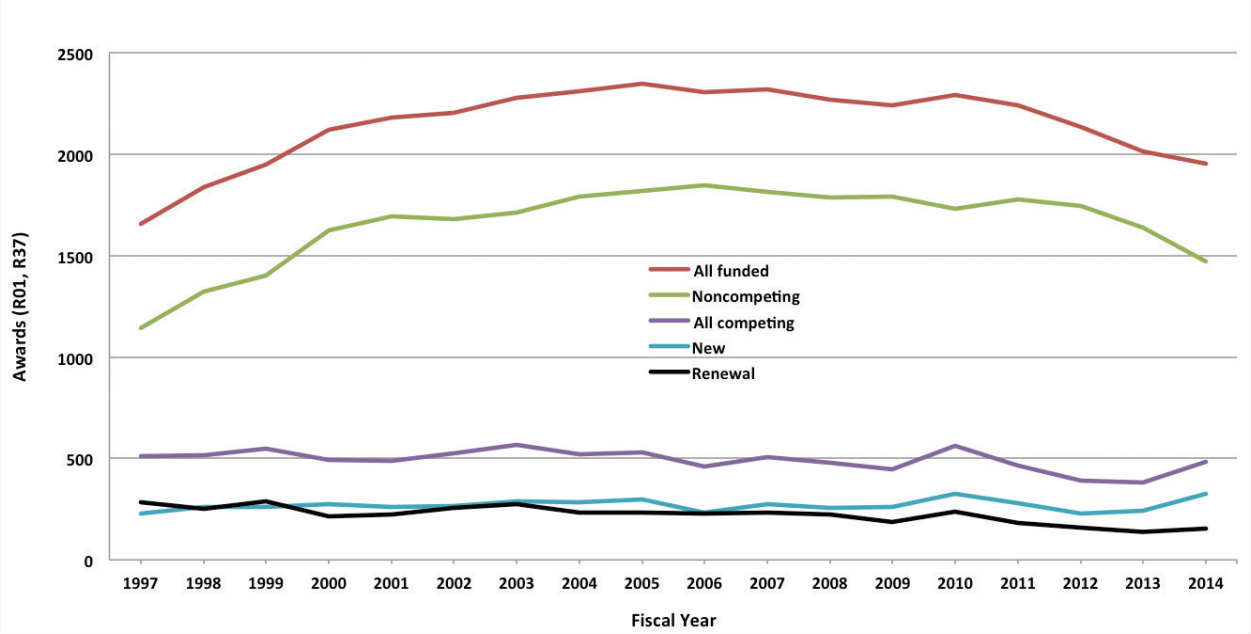
FIGURE 3: CUMULATIVE PERCENTAGE OF R01 AWARDS ACROSS PERCENTILES (FY 2006–2014).



Only funded applications are considered in the data set charted in Figure 3. Percentile bin size equals one percentile and there is no overlap between bins. Percentiles with decimal places were summed into the next highest integral percentile as follows: 0.1 to 0.9 was summed into 1, 1.1 to 1.9 was summed into 2, *etc*. These cumulative funding data again demonstrate that the vast majority of applications funded by NIDDK fall within the payline but that NIDDK does exercise some programmatic discretion to reach for a limited number of especially innovative or programmatically important applications.

FIGURE 4

FIGURE 4: NUMBER OF NIDDK R01 AND R37 GRANTS (COMPETING AND NONCOMPETING) FUNDED IN FY 1997-2014.



Overall, the total number of R01 grants funded by the NIDDK has increased 18 percent since FY 1997. The major portion of this increase occurred during the years of the NIH budget doubling (FYs 1998-2003). Since FY 2005, there has been a decline in the number of grants funded. In general, about half of the competing grants funded by the NIDDK are new (Type 1) awards and half are competing renewals. However, in the past six FYs, competing renewal awards have lagged behind new awards, and the separation between new and competing renewal awards was somewhat more pronounced in FY 2014.

FIGURE 5

FIGURE 5: NUMBER OF COMPETING NIDDK R01 APPLICATIONS (INCLUDING REVISIONS) RECEIVED FOR FUNDING CONSIDERATION IN FY 1998-2014.

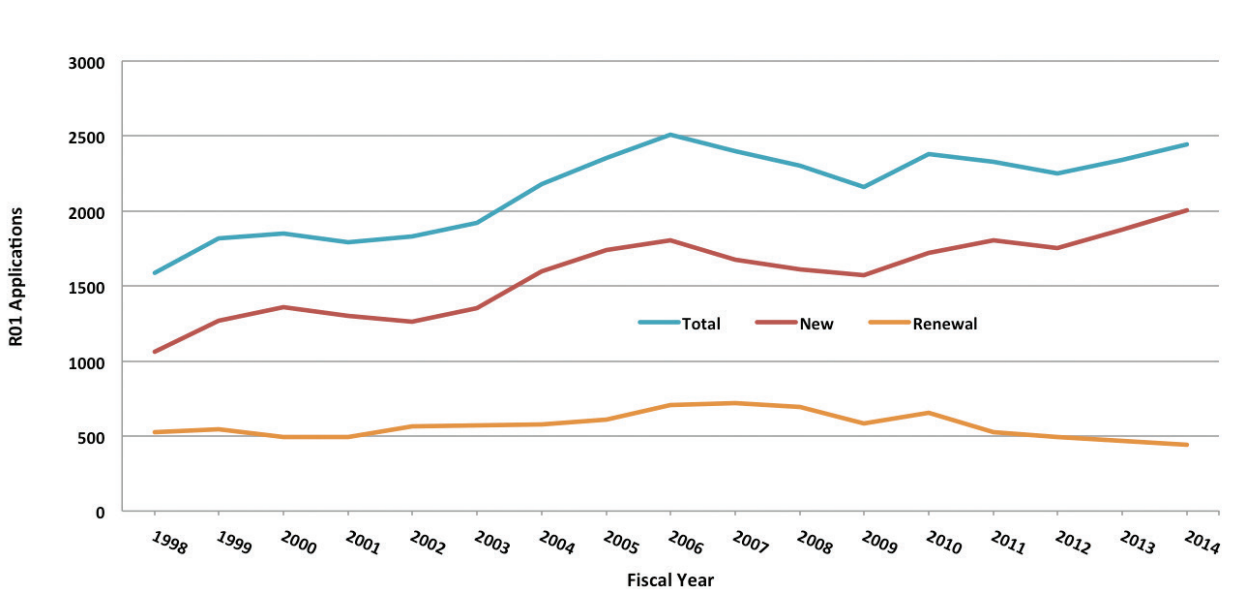


Figure 5 shows a substantial increase in the number of competing R01 applications received by the NIDDK between FY 1998 and 2006. However, the numbers of competing applications have declined slightly since FY 2006. Much of the observed increase between FY 1998 and 2006 was primarily due to new (Type 1) applications. Submission rates for competing renewal applications fluctuated somewhat between FY 1998 and 2014, but since FY 2007 numbers of renewal applications have waned. It should be noted that only one amendment and resubmission of an application was allowed after January 25, 2009. The full implementation of the NIH policy eliminating a second amended application is coincident with a rise in "New" competing R01 applications in FY 2010.

FIGURE 6

FIGURE 6: OVERALL NIDDK EXPENDITURES (INCLUDES DIRECT AND FACILITIES AND ADMINISTRATIVE COSTS) ON R01 AWARDS (COMPETING AND NON-COMPETING) IN FY 1995-2014.

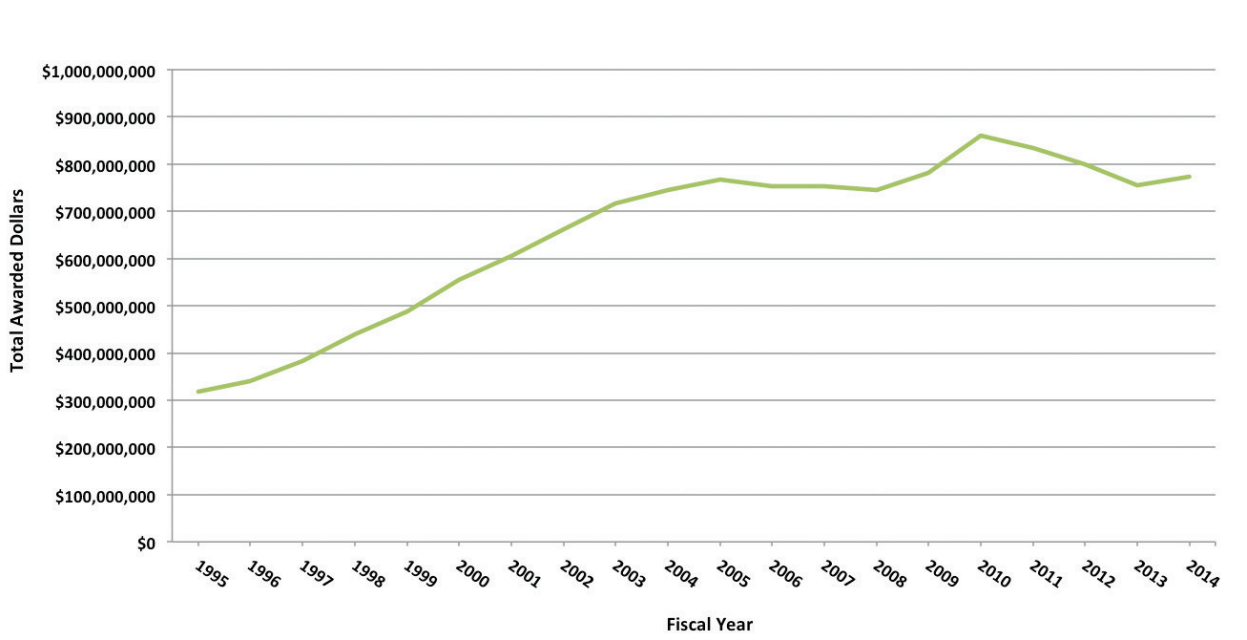


Figure 6 shows that NIDDK expenditures on R01 grants have increased markedly (143 percent) since FY 1995. This is because the NIDDK is funding a larger number of these awards (Figure 4) and also because the median cost of an R01 has increased substantially (Figure 7).

FIGURE 7

FIGURE 7: MEDIAN TOTAL COSTS (INCLUDES DIRECT AND FACILITIES AND ADMINISTRATIVE COSTS) OF NIDDK R01 GRANTS (COMPETING AND NON-COMPETING) IN FY 1995-2014.

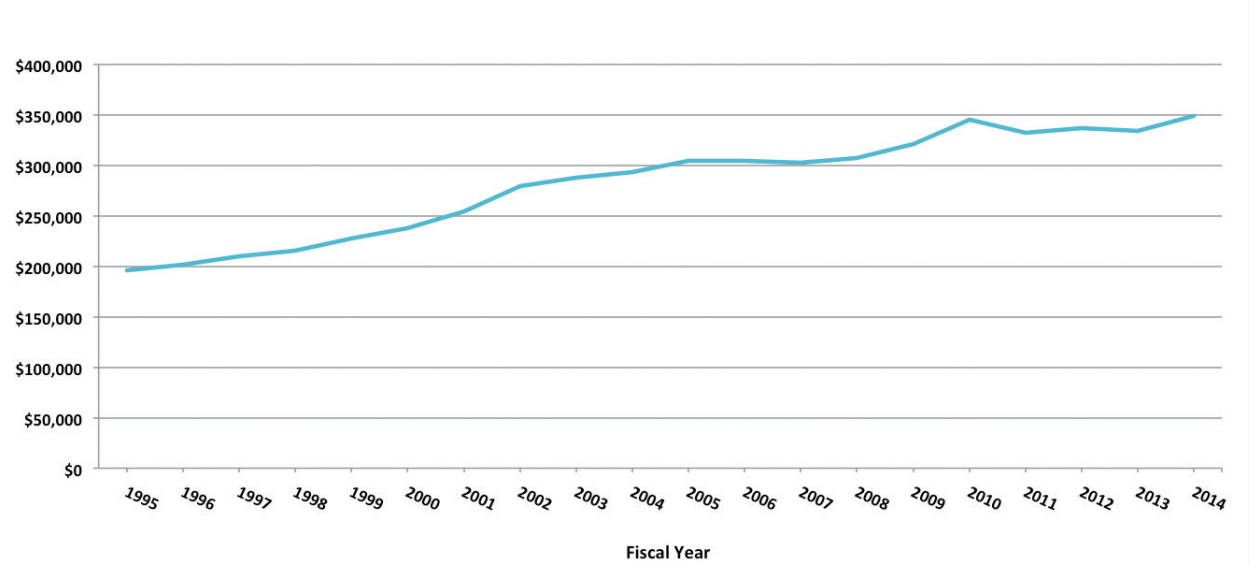


Figure 7 illustrates that the median cost of R01 awards has increased approximately 78 percent since 1995, although there was a slight decrease in FY 2011 compared to FY 2010. In the past ten years (FYs 2005-2014), the number of grants receiving $500,000 or more in total costs has gone from 5 percent of the total number of awards to 17 percent of the total awarded R01s. The number of grants receiving $250,000 or less in total costs has declined from 20 percent of the total awards to 7 percent.

FIGURE 8

FIGURE 8: NIDDK EXTRAMURAL RESEARCH FUNDING BY CATEGORY (COMPETING AND NON-COMPETING).

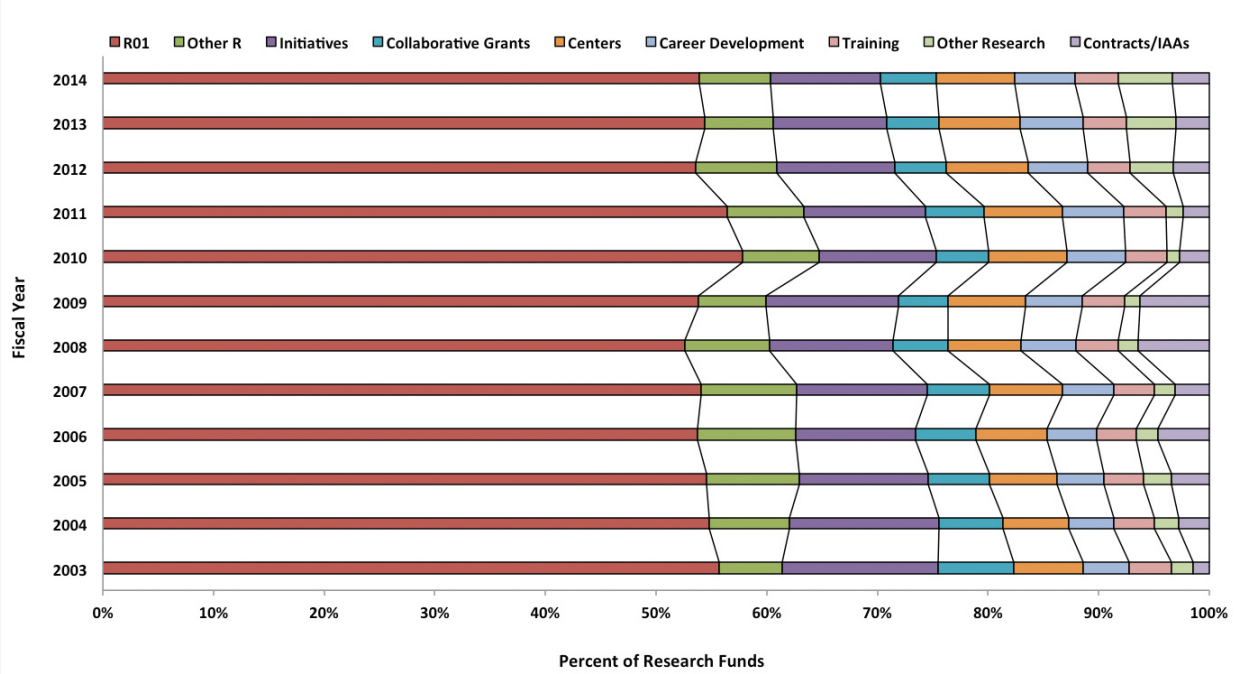


Figure 8 shows that relative funding levels of most NIDDK extramural research categories have remained fairly stable since FY 2003. These data were presented to the NIDDK's Advisory Council in May 2012 in the context of the NIDDK's core values. The NIDDK core values emphasize maintaining a strong investigator-initiated R01 program, preserving a stable pool of talented new investigators, supporting key clinical studies and trials (support is generally represented in the Initiatives and Contracts categories), and continuing strong support of training and career development programs. Figures 9-12 illustrate other examples of how the NIDDK's portfolio has reflected NIDDK core values over time.

NIDDK Portfolio Categories:

- **R01** – 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 applications submitted to NIDDK RFAs and R24s
- **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 categories above
- **Contracts and Interagency Agreements (IAAs)** – Includes some large clinical studies

FIGURE 9

FIGURE 9: MAINTAINING A STABLE POOL OF NIDDK INVESTIGATORS: NUMBER OF INVESTIGATORS SUPPORTED BY AT LEAST ONE R01.

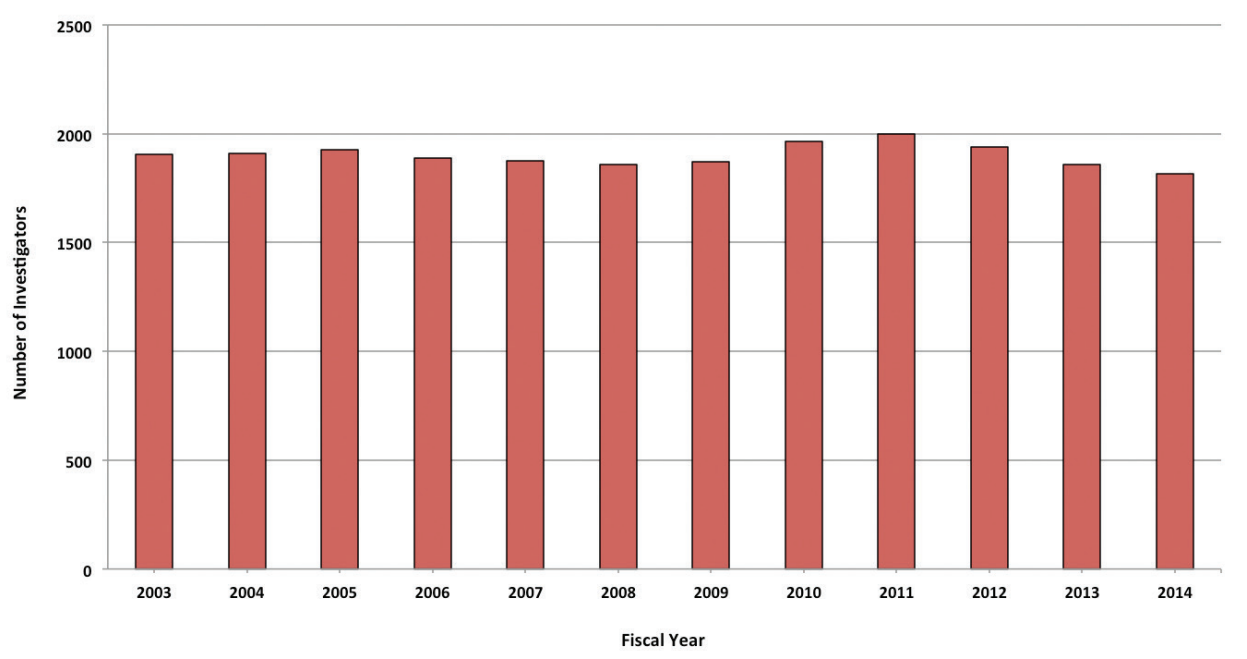


Figure 9 shows that the number of Principal Investigators (PIs) supported by at least one R01 remained relatively stable between FY 2003 and 2009. In FYs 2010 and 2011, there were increases in the numbers of PIs supported with an NIDDK R01. It should be noted that in FY 2008 the NIH, for the first time, began making multiple principal investigator R01 awards to support team science projects. The observed increases in numbers of PIs supported by the NIDDK in FYs 2010 and 2011 are largely attributable to multiple principal investigator R01 awards. The subsequent declines in FYs 2012, 2013, and 2014 are likely due in large part to paylines that became more stringent (*i.e.*, after FY 2011) and inflationary pressures in the context of flat or declining budgets.

FIGURE 10

FIGURE 10: PRESERVING A STABLE POOL OF NEW INVESTIGATORS: NUMBER OF NIDDK NEW INVESTIGATOR R01 APPLICATIONS AND AWARDS.

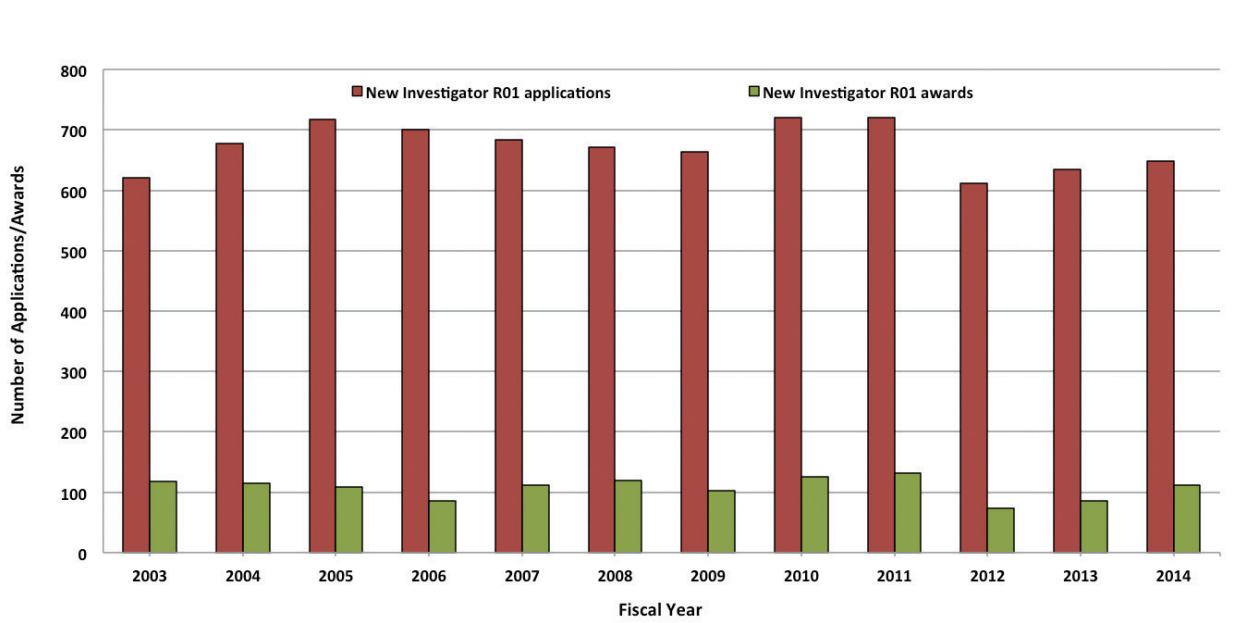
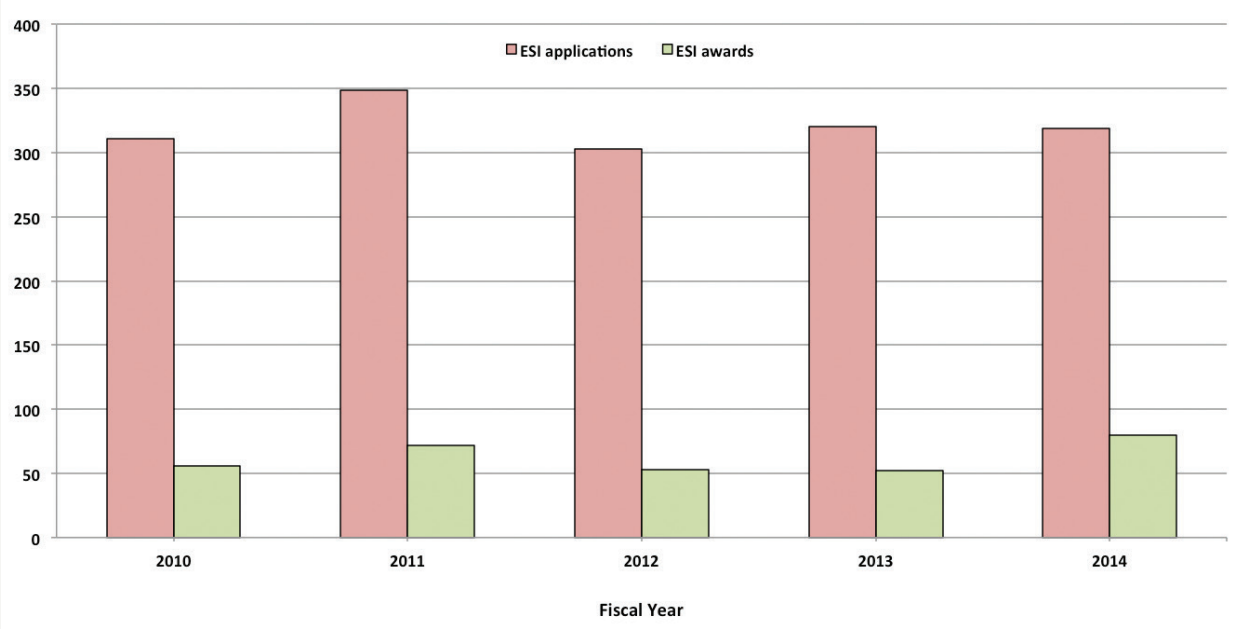


Figure 10 shows that while application rates for New Investigators have remained fairly high, there was a deceleration in the number of New Investigator awards between FY 2003 and 2006. Starting in FY 2007, the NIH and NIDDK established new policies focused on New Investigators, and these policies appear effective in mitigating downward pressures on New Investigator awards. The decrease in number of New Investigator awards in FY 2012 reflects a decrease in the number of applications from New Investigators that year. Numbers of New Investigator applications and awards recovered somewhat in FY 2013 and again in FY 2014. In addition, in FY 2012 the NIH and NIDDK began focusing on Early Stage Investigators (ESIs; see definition of and benefits conveyed to ESIs on the NIH "New and Early Stage Investigator Policies" webpage at *http://grants.nih.gov/grants/new_investigators/index.htm*), which is a subset of New Investigators (see table associated with Figure 2 and Figures 11 and 12). It should be noted that these data count applications and awards, not persons.

FIGURE 11

FIGURE 11: PRESERVING A STABLE POOL OF NEW INVESTIGATORS: NUMBER OF NIDDK EARLY STAGE INVESTIGATOR (ESI) R01 APPLICATIONS AND AWARDS.



Comparison of Figure 10 and 11 shows that while the subset of ESI applications fell in FY 2012 essentially in proportion to the total drop in New Investigator applications, the proportional drop in number of awards to ESIs was not as great. This is attributable in part to the NIDDK's differential payline for ESI applications (see the "Resources for New NIDDK Investigators" webpage at *http://grants.nih.gov/grants/new_investigators/index.htm*). The number of ESI awards in FY 2013 was essentially flat compared with the number of ESI awards in FY 2012, and the number of ESI awards in FY 2014 increased approximately 54 percent compared to FY 2013 ESI award numbers.

FIGURE 12

FIGURE 12: PRESERVING A STABLE POOL OF NEW INVESTIGATORS: PERCENT OF NEW INVESTIGATOR APPLICATIONS AND AWARDS THAT ARE ESI.

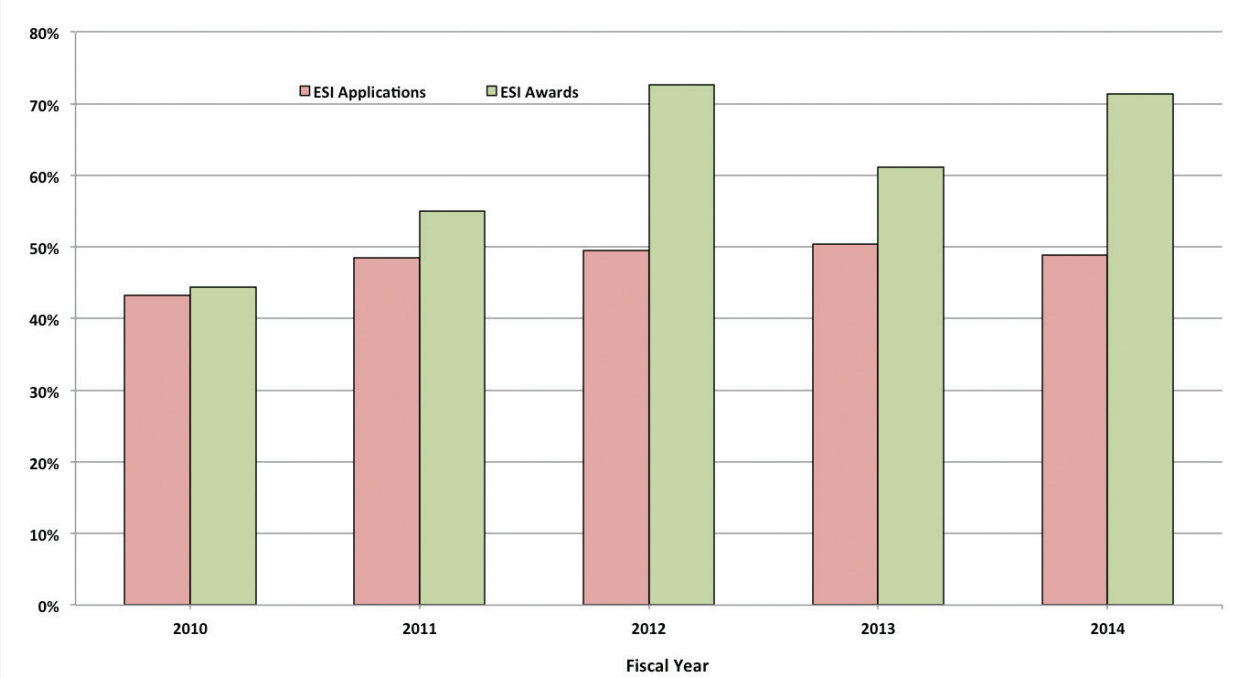


Figure 12 demonstrates that the NIDDK's differential payline for ESIs in FYs 2012 to 2014 (see the "Resources for New NIDDK Investigators" webpage at *http://grants.nih.gov/grants/new_investigators/index.htm*) has been effective in enhancing ESI representation among New Investigator awards.

FIGURE 13

FIGURE 13: SUPPORT PIVOTAL CLINICAL STUDIES AND TRIALS: NIDDK HUMAN SUBJECTS RESEARCH FUNDING AS A PROPORTION OF ALL EXTRAMURAL RESEARCH FUNDING.

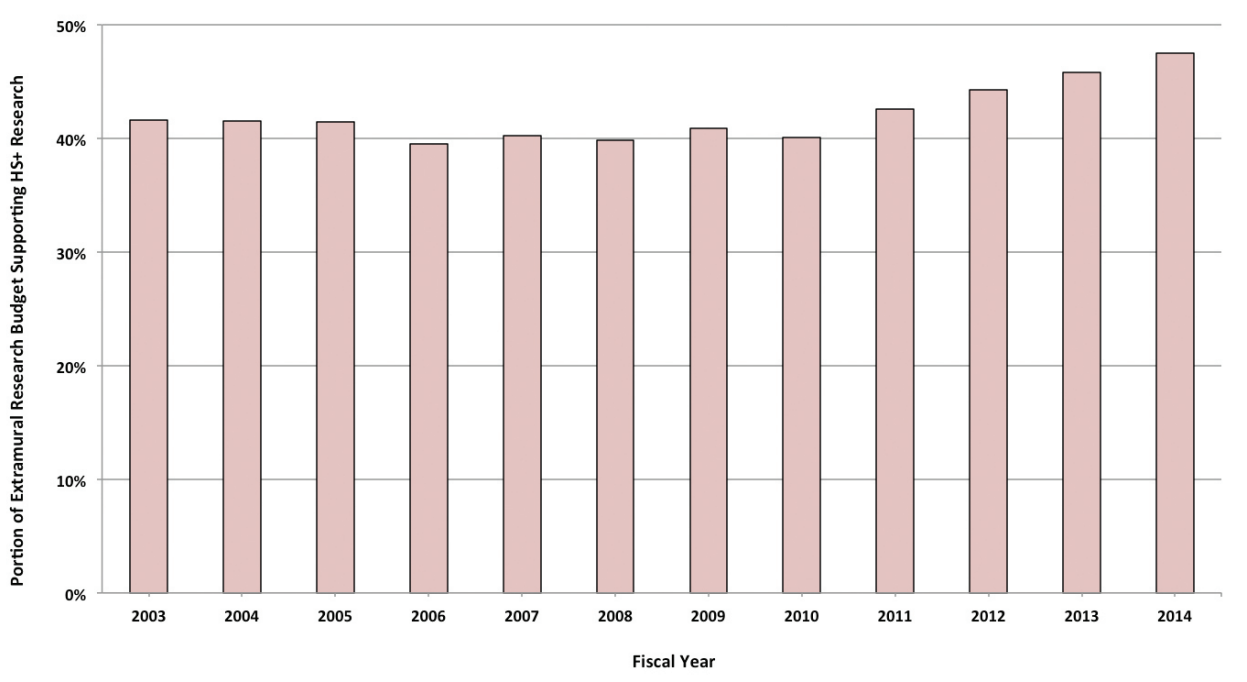


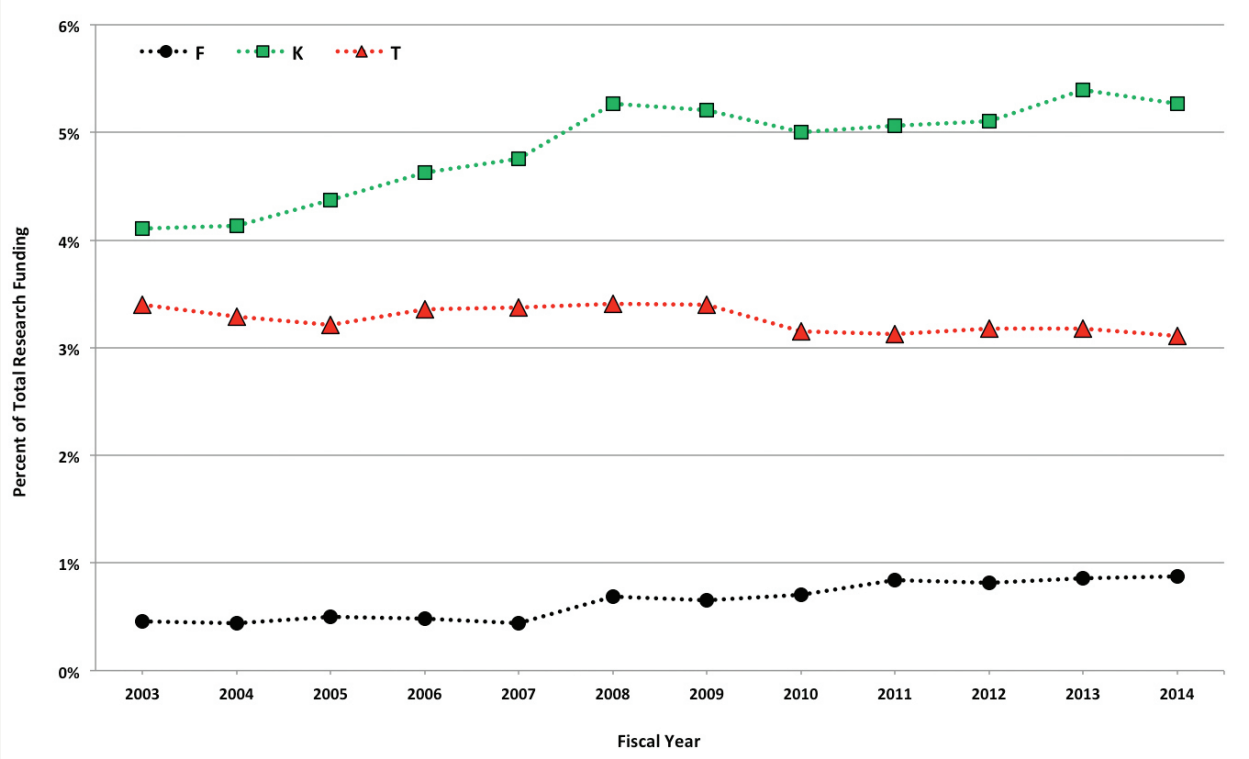
Figure 13 demonstrates that the NIDDK commits a substantial proportion of its research funding to the support of clinical research involving human subjects. For the purpose of this analysis, we used the definition described in Kotchen *et al*, *JAMA* 2004 Feb; 291(7):836-43 and included all studies coded as using Human Subjects (HS+).

FIGURES 14A TO 14D

THE NIDDK IS COMMITTED TO TRAINING THE NEXT GENERATION.

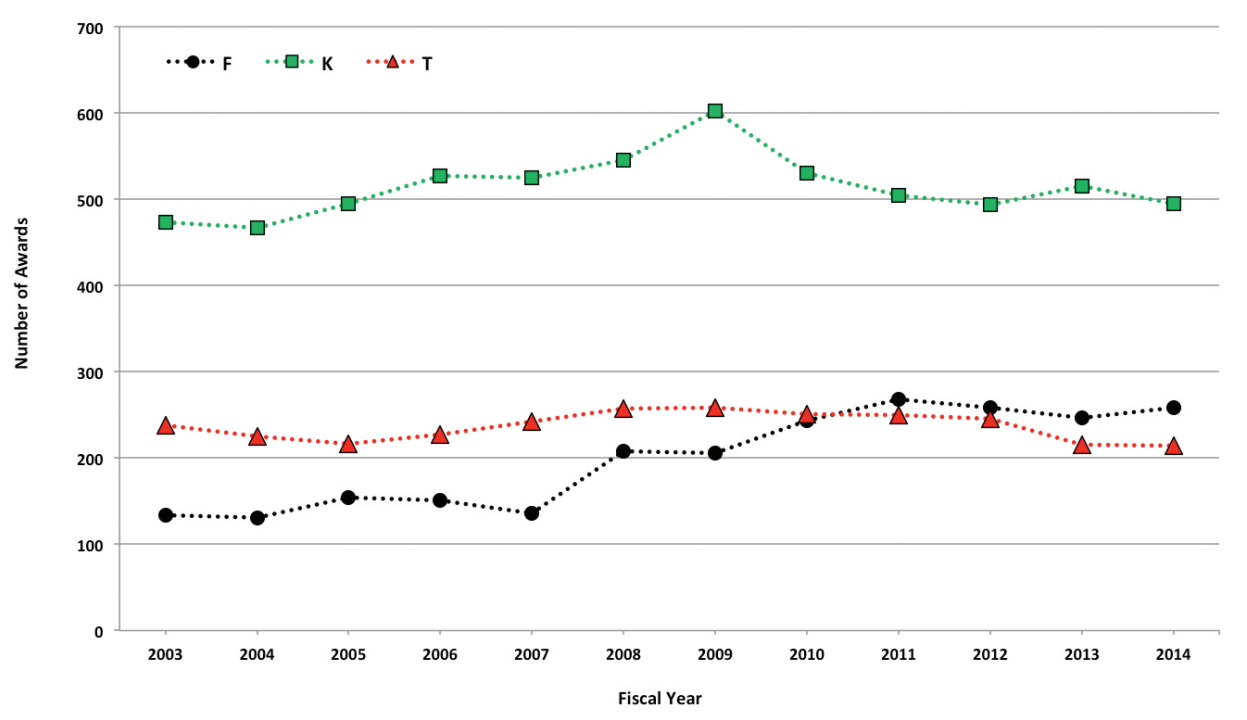
Figures 14A to 14D demonstrate that the NIDDK's commitment to training and developing the careers of the next generation of scientists remains strong. Figure 14A shows that overall support of training and career development programs has increased since FY 2003. By design, there has been a slight deceleration in the number of T awards, but at the same time the number of F awards has increased. Figures 14B and 14D illustrate that the numbers of NIDDK T awards and associated training slots have remained relatively stable. Figure 14C shows that while the numbers of NIDDK K08 (Mentored Clinical Scientist Development Award) awards decreased from FY 2003 to 2014, the numbers of K01 (Mentored Research Scientist Development Award) and K23 (Mentored Patient-Oriented Research Career Development Award) awards have increased. The NIDDK will continue to monitor carefully its training and career development programs to ensure appropriate balance.

FIGURE 14A: NIDDK FELLOWSHIP (F), CAREER DEVELOPMENT (K), AND TRAINING (T) AWARDS AS A PERCENT OF TOTAL RESEARCH FUNDING.



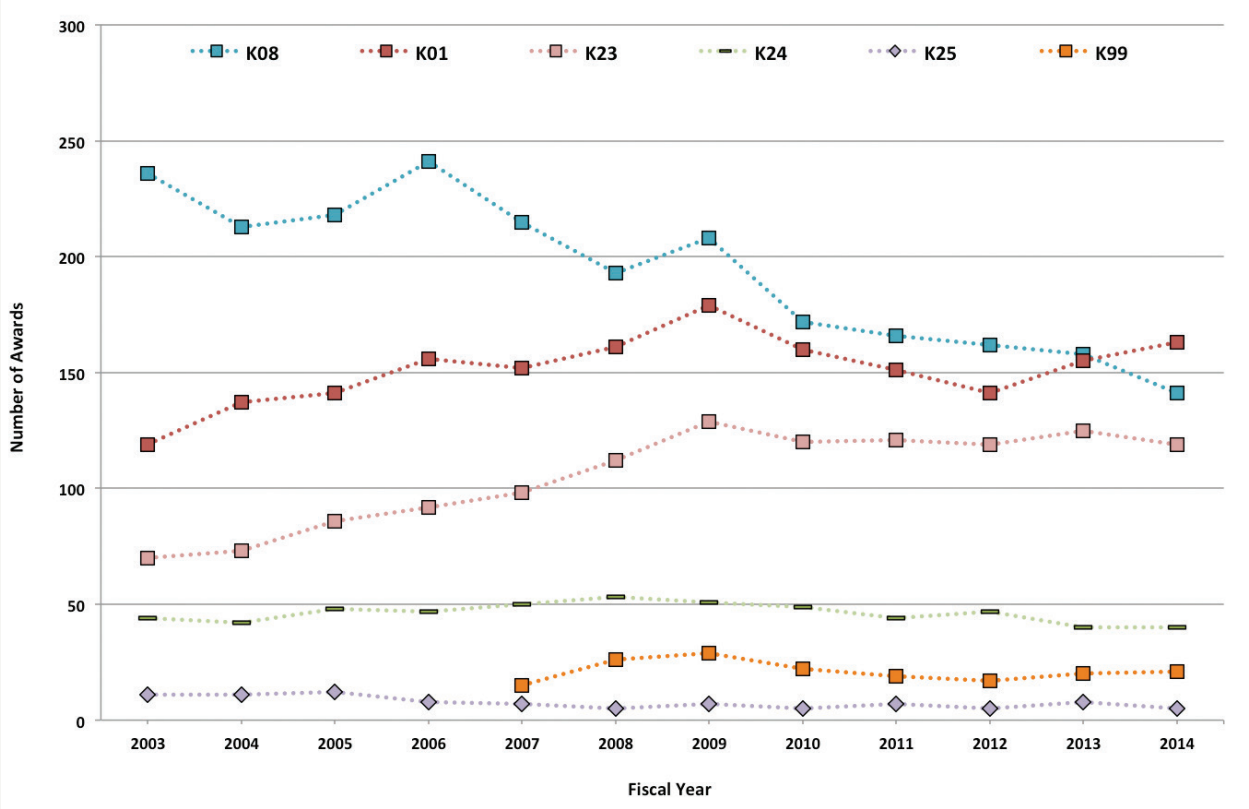
FIGURES 14A TO 14D

FIGURE 14B: NUMBER OF NIDDK FELLOWSHIP (F), CAREER DEVELOPMENT (K), AND TRAINING (T) AWARDS BY FISCAL YEAR.



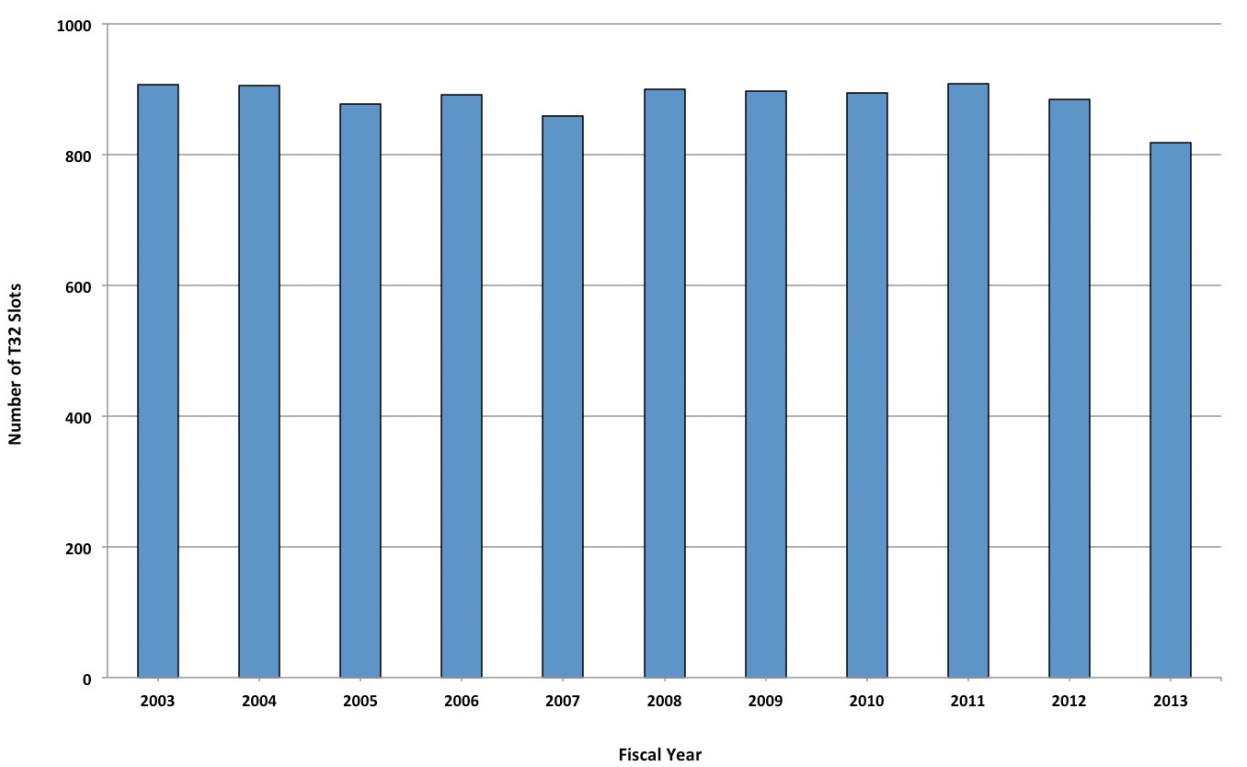
FIGURES 14A TO 14D

FIGURE 14C: NUMBER OF NIDDK CAREER DEVELOPMENT (K) AWARDS BY ACTIVITY AND FISCAL YEAR.



FIGURES 14A TO 14D

FIGURE 14D: NUMBER OF NIDDK TRAINING (T32) AWARD SLOTS BY FISCAL YEAR.



Note: Numbers of training slots are reported at the end of the training budget year (not at the time of award). Therefore, unlike the previous charts, FY 2014 data are not included here.

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NIDDK Funding Trends and Support of Core Values

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